Kidney and Blood Pressure Regulation

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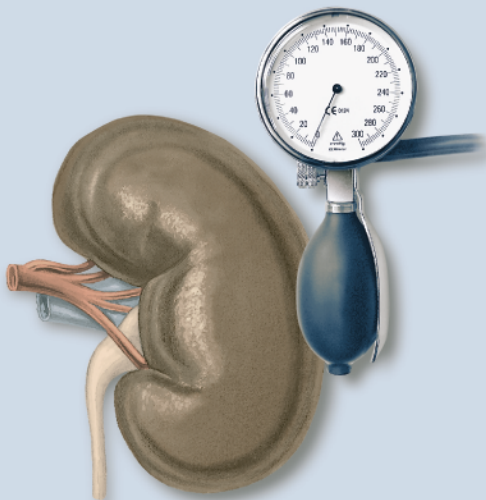
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Contributions to Nephrology

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Kidney and Blood Pressure Regulation

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Contributions to Nephrology

(Founded 1975 by Geoffrey M. Berlyne)

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Preface

This book attempts to integrate the progress in the physiological aspects of blood pressure regulation mechanisms related to the kidney. With this in mind, we tried to collect a series of original contributions from leading experts in the field. The continuous evolution of techniques and biomedical aspects has overall led to significant progress; however, in recent years, major concerns have been expressed about gene target research. Therefore, molecules of different origin and nature are investigated from various aspects and a number of particles are hardly integrated. In contrast to these developments in molecular biology, many large-scale clinical trials investigating the effects of antihypertensive drugs on cardiovascular events have been and are at present being carried out, and these results are not always consistent. Between these two extremes – gene-targeted science and large-scale clinical trials – the exact mechanisms behind the pathophysiological process of renal disease have been investigated. It is likely that a combination of metabolic and hemodynamic abnormalities explain the progression of renal diseases. Clearly, mechanisms related to the response to blood pressure elevation are important as is the possibility that the metabolic and hemodynamic pathway is inhibited. This has been a greater challenge than we originally envisaged, not least of all because there has recently been an explosion of interest in blood pressure regulation in the kidney. This challenge has been admirably met by the international panel of authors who agreed to contribute to this book. Their contributions are outstanding.

We acknowledge that the wisdom is theirs and the mistakes are ours. Needless to say, this book does not provide all of the answers to the clinical as well as basic challenges faced by those specialists who work in this field of

hypertension and the kidney, but we hope it does provide a solid foundation from which to move forward and tackle one of the most important relations between blood pressure regulation and the kidney. Obviously, much work still needs to be done and one of the intentions of this book is to stimulate further research in this area where so many subdisciplines of medical science are involved – from the extremes of genetic and molecular biology to clinical and pharmacological research trials.

We wish to express our appreciation to our many associates and colleagues who, in their particular fields, have helped us with constructive criticism and helpful suggestions. This book could not have been produced without the dedicated help of our co-workers in the editorial offices of the individual editions. Finally, we continue to be indebted to the staff of Karger Publishers.

Hiromichi Suzuki
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An Overview of Blood Pressure Regulation Associated with the Kidney

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The kidney is involved in the maintenance of peripheral vascular resistance through the action of angiotensin II (Ang II), which is the final product of the renin-angiotensin system (RAS) and participates in the volume control of cardiac output by regulating urinary salt and water excretion. Since it is well known that arterial pressure is equal to cardiac output multiplied by total peripheral resistance, the kidney is indispensable for regulation of blood pressure. When the blood pressure rises above normal, the kidneys excrete increased quantities of fluid, and progressive loss of this fluid causes blood pressure to return toward normal. Conversely, when the blood pressure falls below normal, the kidneys retain fluid, and the pressure normalized. Neurohormonal and possibly other factors limit urine sodium excretion, thereby expanding extracellular fluid volume or requiring higher renal perfusion pressure to permit sodium excretion adequate to prevent extracellular volume expansion. When the kidney is injured by any cause, it leads to a physiological changes that are responsible for progressive hypertensive renal diseases [1]. For example, patients with a strong family history of hypertension who undergo heminephrectomy for any reason become hypertensive [2].

The key factor is the regulation of renin. Excess of sodium intake decreases renin synthesis and secretion in the juxtaglomerular cells, and conversely, reduction of sodium intake increases renin synthesis and secretion. Blood volume and cardiac output are affected by the vasoconstrictor substance, Ang II, which is derived from angiotensin I (Ang I) by the action of angiotensin-converting enzyme (ACE); this occurs mainly in the lungs where circulating
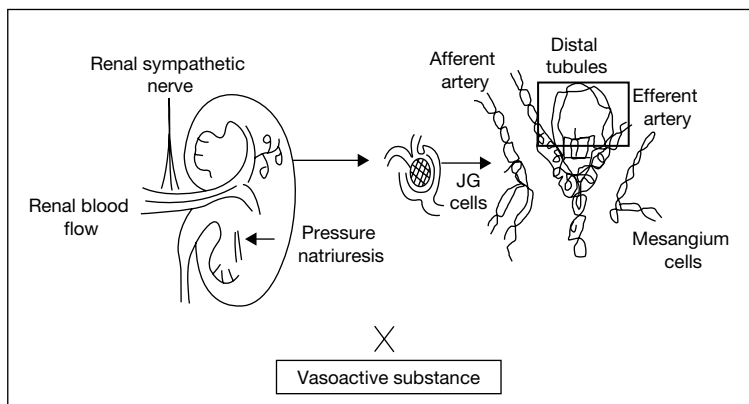
Fig. 1. Three major mechanisms that are involved, namely, renal blood flow, sympathetic nerve system, and pressure-natriuresis control.

Ang I is converted to the active, 8-amino-acid Ang II. Ang II is one the most potent renal vasoconstrictors, as well as a potent regulator of circulatory volume.

To delineate the role of kidney in the control mechanism of blood pressure, we describe three major mechanisms that are involved, namely, renal blood flow (RBF), sympathetic nerve system, and pressure-natriuresis control (illustrated in figure 1). Blood pressure regulation in the kidney involves the interplay of these factors.

RBF receives 25% of cardiac output and the normal kidney adjusts its vascular resistance so that the RBF is kept nearly constant over a wide range of perfusion pressures. This ability is maintained in hypertensive animals, although the RBF autoregulation is adjusted to higher perfusion pressure levels. In hypertension, as well as in congestive heart failure, the RBF is kept constant by an autoregulatory mechanism in spite of a reduction in cardiac output, thus maintaining adequate levels of glomerular filtration rate (GFR). There are two components to the autoregulation of RBF, the myogenic response of the afferent arteriole and the tubuloglomerular feedback by the juxtaglomerular apparatus (JGA).

Given the powerful influence of changes in renal hemodynamics (i.e., blood pressure, GFR and RBF) on urinary sodium excretion, it is evident that the influence of changes in renal sympathetic nerve activity (RSNA) on urinary excretion of sodium remains constant. By using electrical stimulation of the efferent renal sympathetic nerves at threshold frequencies that result in a decreases in RBF, a reversible decrease in urinary sodium excretion occurred

in the absence of changes in GFR, RBF and blood pressure, indicating that low-frequency renal sympathetic nerve stimulation increased overall renal tubular sodium reabsorption via a direct action on the renal tubule, independent of changes in renal hemodynamics. In a series of studies, it was found that the effects of RSNA on renin secretion from the JGA were graded with respect to the intensity of the RSNA that interacted with other mechanisms of renin secretion, i.e., the renal arterial baroreceptors through the effects of RBF and the renal tubular macular densa receptors through the amounts of urinary sodium excretion [3].

Pressure natriuresis refers to the effect of increased arterial pressure that leads to an increase renal sodium excretion, an effect that becomes especially powerful with long-term changes in blood pressure. The mechanisms of pressure natriuresis continue to operate until blood pressure returns to the initial set point, which is determined by multiple factors that influence renal excretory ability. When the RAS is fully functional, the long-term relation between arterial pressure and sodium excretion is extremely steep, so that minimal changes in blood pressure are needed to maintain sodium balance over a wide range of sodium intakes. Conversely, changes in activity of the RAS have a major influence on renal-pressure natriuresis, and the inability to adjust the activity of this system appropriately makes pressure natriuresis less effective [4].

As noted above, all mechanisms closely relate with the RAS. With these under consideration, we would like to view the JGA as the center of regulation of blood pressure in the kidney and/or the human body (illustrated in figure 2). Renin is secreted from the JGA via the macula densa. As physical stimulants, both pressure and flow mediate renin synthesis and secretion [5]. As chemical factors, inorganic and organic compounds stimulate renin synthesis and secretion. For example, Cl ion (inorganic stimulants) is shown to be a mediator of renin secretion. Moreover, as a biophysical stimulant, the role of renal sympathetic nervous stimulation might be important for regulation of renin secretion.

Kurokawa [6] noted in his review that Cl ions are essential for regulation of JGA and/or TGF. He introduced the studies by Holstein-Rathlou [7] who, using a Cl ion-sensitive microelectrode, revealed the presence of fairly regular oscillations at about 20 cycles/s in the distal tubular fluid Cl ion just beyond the macula densa, and of the proximal intratubular pressure, a reflection of single nephron GFR.

Chloride ions play an important role in the regulation of JGA as does the relationship between RSNA and JGA. The quantitative relationships are (1) substantial stimulation of JGA and antinatriuresis can occur with levels of RSNA that do not affect GFR and renal vascular resistance and (2) levels of RSNA that decrease RBF and GFR will stimulate JGA and produce antinatriuresis.
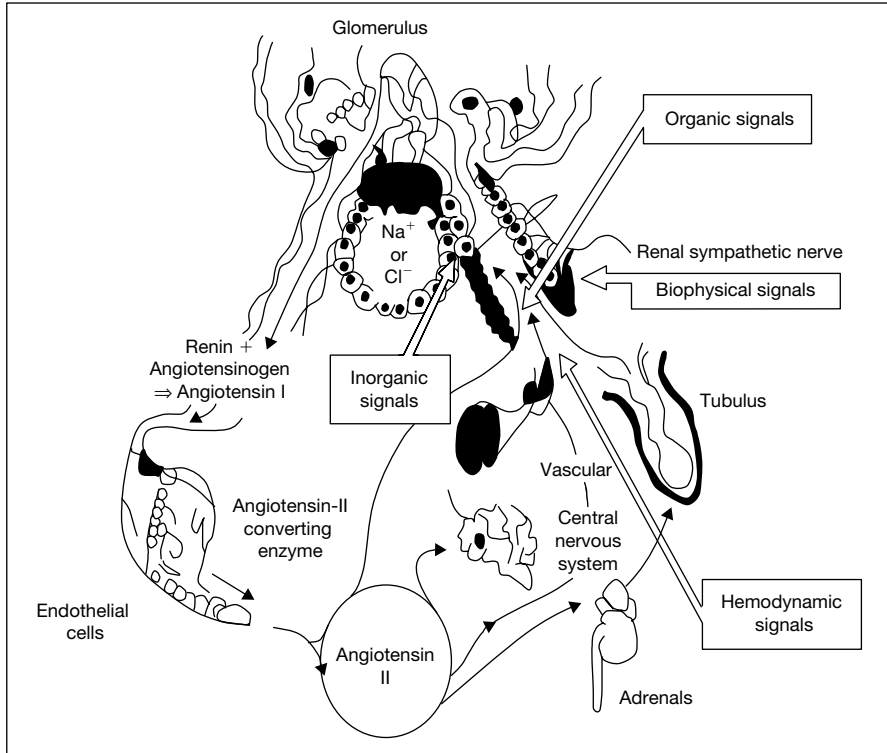
Fig. 2. View of the juxtaglomerular apparatus as the center of regulation of blood pressure in the kidney and/or the human body.

These regulator mechanisms of JGA prompted the examination of pathophysiological conditions in which it had been long suspected that increased RSNA and RBF played an important role in antinatriuresis and/or influenced the function of the JGA. These regulatory mechanisms are normally autoregulated under the control of neuro- and hormonal factors, such as Ang II, norepinephrine, vasopressin, etc. Among these factors, the RAS is the most important system for renal regulatory mechanisms of blood pressure. A growing body of evidence suggests that Ang II is involved in regulation of RBF, RSNA, pressure natriuresis and intraglomerular pressure feedback system, etc. These effects involve conversion of angiotensinogen (substrate) to Ang I by renin (enzyme) and subsequent conversion of Ang I to Ang II by ACE. Hypertension and congestive heart failure are important examples where this system plays a role. In focusing on these processes, our group has been investigating the complex pathophysiological processes. In this article, we have reviewed mainly the work

from our group; however, we acknowledge and recognize that many investigators in this field have made important contributions to understanding the mechanism of blood pressure regulation by the kidney.

Studies in Hypertension

Although there are many factors involved in the etiology of hypertension [8–15], the important role of the kidney in regulation of volume and vascular resistance makes it a prime suspect as a mediator of hypertension. Neurohumoral and possible other factors limit urinary sodium excretion, thereby expanding extracellular fluid volume or requiring higher renal perfusion pressure to permit adequate sodium excretion to prevent extracellular fluid volume expansion. Early studies of Bianchi et al. [16] and more definite well-controlled experimental studies of Rettig et al. [17] showed that 'blood pressure goes with the kidney'. Transplantation of the kidney from a genetically hypertension-prone donor rat, even when it had been kept normotensive from weaning by antihypertensive medications, caused progressive increase of blood pressure in a recipient animal, which was immunologically manipulated to prevent a rejection reaction. Also, human renal transplant studies showed that there is a genetic component associated with the renal factors that mediate hypertension. Thus, previously normotensive renal transplant recipients without a family history of hypertension, who receive a kidney from a donor with a family history of hypertension, develop hypertension more frequently and require more medication for blood pressure control compared to those patients who receive a kidney from a donor without a family history of hypertension [18]. To investigate more precise mechanisms of hypertension and the effects of antihypertensive medications on the regulatory factors such as RSNA, RBF, and pressure natriuresis, animal models of hypertension have been used.

Spontaneously Hypertensive Rats (SHR)

Various pathophysiological aspects of hypertension have been investigated using the SHR model.

The pressure-natriuresis mechanism is known to be impaired in SHR, and some studies have suggested an inadequate adaptation of the RAS to salt loading; however, no decisive evidence has been presented until recently. Takenaka et al. [19] compared the pressure-natriuresis response curves of SHR and Wistar-Kyoto (WKY) rats. The pressure-natriuresis relationship curve in SHR was shifted toward higher pressure in comparison to WKY rats. The inhibition of intrarenal RAS by MK-422 (ACE inhibitor) in SHR resulted in the excretion of more sodium at a given pressure, whereas no significant changes were observed

in WKY rats which showed significant changes in blood pressure, indicating that intrarenal RAS might be important for pressure-natriuresis mechanisms in SHR. Moreover, in SHR, administration of a kinin antagonist did not affect the recovered pressure-natriuresis relationship during intrarenal RAS inhibition with an ACE inhibitor. Similarly, administration of an angiotensin antagonist produced an increased sodium excretion accompanied by an increase in renal plasma flow. Conversely, administration of Ang I to WKY rats produced anti-natriuretic effects without any significant changes in renal hemodynamics. Following this work, Ikenaga et al. [20] clarified the role of nitric oxide (NO) in pressure natriureis in SHR. NO is well known as an important modulator of blood pressure and renal function [21–23]. They demonstrated that inhibition of NO synthesis using L-N^G-monomethyl-L-arginine (L-NMMA) markedly low-ered the slope of the pressure-natriuresis curve of WKY, while L-arginine administration improved that of SHR. These effects on the pressure-natriuresis response are considered to be mediated by NO, because they were effectively reversed by the concomitant infusion of L-NMMA and L-arginine. In all cases, there were no changes in the GFR, indicating that there was no filtered sodium load on the glomeruli. It has been suggested that suppression of tubular sodium reabsorption due to interstitial hydrostatic pressure elevation is essential to the mechanism of pressure-natriuresis response, and that papillary hemodynamics play a critical role in the regulation of the interstitial hydrostatic pressure. These findings prompt us to propose the hypothesis that NO participates in the pressure-natriuresis response through regulation of intrarenal blood flow distribution. Moreover, the deficiency in NO system might be one of the responsible factors for the impaired pressure natriuresis in SHR. Based on their studies, it was proposed that deficiency in NO and activation of the RAS system produced impaired pressure natriuresis in living animals as well as humans. From these studies, it is clear that NO plays an important role in regulation of blood pressure in the kidney. Kumagai et al. [24] examined the role of NO in relation to RBF and the sympathetic nervous system using conscious rabbits. In renal innervated rabbits, L-arginine increased RBF and decreased RSNA. In contrast, no changes occurred in any variable during D-arginine infusion. L-NMMA attenuated the RBF and RSNA responses to L-arginine. In renal denervated rab-bits, L-NMMA also attenuated the RBF response to L-arginine and abolished these responses but not in those of renal innervated rabbits. These findings indi-cate that exogenous L-arginine elicits a reduction in RSNA and that the reduction in RSNA could contribute to the increase in RBF as well as other mechanisms such as a direct vasodilator action of NO on vascular smooth muscle tone. In parallel with these studies, Jimbo et al. [25] examined a possible role of NO in modulating sympathetic nerve activity through its action on baroreceptor reflex arc. L-Arginine infusion decreased blood pressure, aortic, cervical, and renal

nerve activity without significant changes in heart rate. *L*-NMMA infusion increased blood pressure and aortic nerve activity and decreased heart rate, while it tended to increase cervical and renal nerve activity which was not statistically significant. From these results, it may be inferred that NO modulates efferent sympathetic nerve activity, not by altering the afferent or efferent limbs of the baroreceptor reflex arc, but by interacting with the sympathetic pathways in the central nervous system. Moreover, considering Ikenaga's study, it is suggested that the renal circulation is especially sensitive to NO formation.

We also examined the effects of antihypertensive drugs on baroreceptor reflexes in SHR. Evidence from other studies suggests that an arterial baroreceptor reflex mechanism modifies regional blood flow and that the effectiveness of the baroreceptor reflex mechanisms would be very limited if the resetting process is not reversible. Restoration of baroreceptor reflex function (i.e. normalization of reflex sensitivity and reversibility of baroreceptor resetting) is important in preserving internal organ function since it may alleviate the risk of decreasing regional blood flow. Kumagai et al. [26, 27] reported two remarkable findings. First, that a possible critical phase sensitive to intervention with antihypertensive treatment exists during the development of hypertension. Secondly, as expected, the effects of four different class of antihypertensive agents, namely, a diuretic, an ACE inhibitor, a β-blocker, and a calcium antagonist on baroreceptor reflex, calculated by using the relation between RSNA and mean blood pressure, were similar when these drugs were used early in the treatment of hypertension. In this experiment, attenuation of the development of hypertension is responsible for the restoration of impaired baroreceptor reflex control of RSNA and heart rate. In contrast to these findings, the late start of treatment with calcium antagonist or ACE inhibitor, but not a diuretic agent or β-blocker, moderately improved the RSNA gain, whereas only the calcium antagonist slightly improved the heart rate gain. In addition, none of the four agents with a late start of treatment improved the range of reflex sympathetic excitation. These studies clearly demonstrated that in SHR modulation of baroreceptor reflex depends on blood pressure control, if cardiovascular remodeling and vessel distensibility were not fully developed.

In parallel with the findings of Kumagai et al. [26, 27], Ichikawa et al. [28, 29] found that the responses of the afferent part of the baroreceptor to antihypertensive treatment were also impaired in SHR. In untreated SHR, the correlation curve of arterial pressure and aortic nerve activity was shifted to the right, that is, to a higher pressure level, and the maximum gain was depressed compared with untreated WKY rats. An equivalent decrease in arterial pressure with the four different antihypertensive drugs produced a leftward shift of the arterial pressure-aortic nerve activity correlation curve to a similar extent in SHR. From these findings it can be inferred that antihypertensive treatment

with the four different classes of agents equally enhances the arterial baroreceptor function through blood pressure reduction but not through specific depressor mechanisms at the early stage of hypertension. Ichikawa et al. [30] also examined the effects of long-term treatment with the four different classes of antihypertensive drugs on aortic baroreceptor activity in SHR with chronic hypertension. They found that (1) the four drugs induced baroreceptor resetting to a lower pressure level and that (2) baroreceptor sensitivity is augmented more by the calcium antagonist or the ACE inhibitor than by the diuretic agent or the β-blocker. These findings might be explained as follows: chronic hypertension induces changes in the aortic medial layers (such as smooth muscle hypertrophy and increased collagen content) that affect baroreceptor sensitivity through changes in vessel distensibility and/or mechanical coupling of the baroreceptors to the vessel. Calcium blockers and ACE inhibitors have been shown to prevent these medial changes to a greater extent than diuretics and β-blockers, probably by acting directly on vascular smooth muscle. These beneficial effects on the aortic media may contribute to the preserved baroreceptor sensitivity.

Dahl Salt-Sensitive Rats

In Dahl salt-sensitive (DS) rats, elevation of blood pressure has been shown to result from salt loading and renal transplantation from DS rats to Dahl salt-resistant (DR) rats is able to elevate the recipient's blood pressure. In DS rats, the pressure-natriuresis relationship is blunted compared to that of DR rats. These findings implicated an intrinsic defect in the kidney of DS rats.

Takenaka et al. [31] examined the role of prostaglandins on pressure natriuresis in DS rats. When DS rats are untreated, the pressure-natriuresis curve is blunted and secretion of prostaglandin E_2 is decreased in comparison to the DR rats. Treatment with indomethacin blunted the pressure-natriuresis curve in the DR rats, while no significant changes were observed in the DS rats. This study suggested that a decrease in renal prostaglandins plays some role in blunting of pressure natriuresis in DS rats.

Influence of Sex on Hypertension

Cardiovascular events due to hypertension differ between men and women. Moreover, the prevalence of hypertension is twice higher in postmenopausal women than in premenopausal women.

Increased sodium reabsorption by the kidney has been suggested to be a factor in this. Tominaga et al. [32] reported that decreases in sex hormones and increases in sodium sensitivity are important factors in the genesis of postmenopausal hypertension. Otsuka and Sasaki [33–35] investigated the effect of ovariectomy on pressure natriuresis in DS rats. The impaired pressure-natriuresis response of DS rats was further blunted by ovariectomy and that of DR rats was

not. The ovariectomized DS rats developed hypertension by salt loading earlier than sham-operated DS rats. This study indicated that ovariectomy enhances genetic salt sensitivity by blunting the pressure-natriuresis response, which precedes the development of overt hypertension in female DS rats.

Renovascular Hypertension

Since an animal model of renal hypertension was first produced by Goldblatt, renal hypertensive animal models have been used for investigation mainly focused on pathophysiological role of the RAS [36]. Nakamoto et al. [37] examined the effects of long-term oral administration of either *L*-arginine or the NO synthesis inhibitor, N-nitro-*L*-arginine on systemic and renal hemodynamics in dogs with chronic two-kidney, one-clip renovascular hypertension. Their study demonstrated that chronic inhibition of NO synthesis exacerbated renovascular hypertension in dogs. Furthermore, suppression of NO was associated with blunted activation of the circulating RAS during the evolution of renovascular hypertension. The ischemic kidney showed a greater depression of RBF and GFR in the presence of NO inhibition. This was associated with a significant reduction in RBF but not in GFR of the contralateral untouched kidney. In contrast, oral administration of *L*-arginine did not modify the magnitude of the hypertension produced by renal artery constriction, but it did have a beneficial effect on the residual function of the ischemic kidney. These findings led to the conclusion that NO provides a basic vasodilator tone that limits vasoconstrictor activity of the RAS during the evolution of renovascular hypertension. Again, the findings indicate that the balance between NO production and the activation of the RAS is critical for regulation and evolution of hypertension.

In clinical practice, there is still controversial whether calcium antagonists or ACE inhibitors are superior to protect end-organ damage due to hypertension. A number of studies examining the effects of these drugs on systemic and renal hemodynamics have been presented. However, very few studies comparing the effects of these two classes of hypertensive drugs on RSNA in hypertensive animals have been conducted. Kumagai et al. [38, 39] examined the different effects of an ACE inhibitor and a calcium antagonist on RBF and RSNA using two-kidney, one-clip renal hypertension in rabbits. First, the baroreflex control of RSNA and heart rate (HR) before and after reduction of blood pressure (BP) was similar in magnitude with an ACE inhibitor and a calcium antagonist. The maximum slopes of the curves relating BP to RSNA and HR in renovascular hypertension were significantly smaller than those in normotensive animals. In renovascular hypertensive animals, the maximum slope of BP-RSNA response curve was increased with ACE inhibitor compared with vehicle. In contrast, the maximum slope of BP-HR response curve was increased with the calcium antagonist compared with vehicle. These data indicate that in renovascular

hypertension, the baroreflex control of RSNA and HR are differently regulated with different classes of antihypertensive drugs. Further study revealed that these two drugs induced different RBF and RSNA responses. RBF increased consistently in response to BP reduction with an ACE inhibitor. The increment was associated with a decrease in plasma concentration of Ang II. In contrast, RBF decreased significantly after BP reduction with a calcium antagonist. The calcium antagonist increased the plasma concentration of Ang II and induced a smaller increase in RSNA than that induced with the ACE inhibitor. This study suggested that the more complex regulatory mechanisms of RBF and RSNA under the conditions of elevated BP due to endogenous Ang II.

Deoxycorticosterone Acetate (DOCA) Salt Hypertension

This model is known as a low-renin hypertension model [40–42]. In spite of many investigations [43], no precise role for ACE inhibitors and Ang II blockers has been implicated in this model. Using conscious DOCA salt dogs Naitoh et al. [44] demonstrated that the ACE inhibitors (captopril and imidaprilat) produced significant reductions in blood pressure and significant increases in RBF, GFR, and urinary excretion of sodium, while an AT1 receptor antagonist (losartan) caused significant increases only in urinary excretion of sodium without significant changes in blood pressure, RBF, and GFR. These investigators performed simultaneous infusion of a bradykinin receptor antagonist and found that it inhibited the ACE inhibitor induced reduction in blood pressure and increases in RBF. The results of their studies showed that in low-renin hypertension, inhibition of Ang II production in the kidney participates in the natriuretic action of ACE inhibitors. However, hypotensive and other renal effects are mainly due to the action of bradykinin. These results suggest that in the kidneys, the effects of ACE inhibitors and Ang II antagonists might be different.

Neurogenic Hypertension

Besides the hypertensive animal models such as SHR, Dahl rats, renovascular hypertension, etc., that have been studied extensively, another model namely neurogenic hypertension has been less investigated. Matsukawa et al. [45] attempted to elucidate the interaction between the SNS and the RAS in neurogenic hypertension produced by sinoaortic-denervated and norepinephrine-infused conscious, unrestrained rabbits. They found that in sympathetic-activated animals postsynaptic interaction between norepinephrine and Ang II is important in regulation of blood pressure.

Ryuzaki et al. [46, 47], using sinoaortic-denervated rabbits, provided convincing evidence for association between neurogenic hypertension and the kidney. They demonstrated that renal nerve stimulation contributed to neurogenic

hypertension through a combination of elevation of plasma vasopressin as a result of sinoaortic denervation and renal afferent nerve stimulation.

Glucocorticoid-Induced Hypertension

Both clinical [48] and experimental [49–54] studies clearly demonstrated that glucocorticoid excess produces elevation of blood pressure; however, precise renal as well as cardiac hemodynamics had not been clarified until Nakamoto's study [55, 56]. He found that administration of a low dose of glucocorticoid did not produce hypertension, while large doses induced elevation of blood pressure with reduction of cardiac output and markedly increased the total peripheral resistance. Moreover, he demonstrated that depressor system such as prostaglandins and bradykinins played an important role in regulation of blood pressure in this model [57]. From these studies it is suggested that renal mechanisms are at least in part involved in pathogenesis and regulation of blood pressure elevation in glucocorticoid excess hypertension.

Studies in Heart Failure

Recent clinical and experimental studies have demonstrated that the blockade of the RAS produced an improvement of symptoms and survival rate of patients with congestive heart failure. We examined the role of vasopressin in congestive heart failure induced by rapid right ventricular pacing in dogs. In the dogs with impaired cardiac function, effective RBF and GFR were decreased mainly due to reduction of cardiac output. In these dogs, plasma renin activity, norepinephrine and vasopressin were all elevated. Murakami et al. [58] provided interesting data by studying the dogs with impaired cardiac function. They compared the acute effects of an ACE inhibitor and an angiotensin type 1 receptor antagonist on cardiac output and RBF. Interestingly, these two types of drugs showed distinct effects; captopril increased both cardiac output and RBF, however, losartan increased RBF but failed to alter cardiac output. Furthermore, Matsumoto et al. [59] found a synergistic action with an ACE inhibitor and a neuroendopeptidase inhibitor which together produced an improvement of cardiac output and RBF in dogs with congestive heart failure. The findings of these studies indicated that in congestive heart failure regulation of cardiac output and RBF is mutually dependent. Naitoh et al. [60] clearly showed that when the heart is failing, vasopressin plays an important role with respect to hemodynamics as well as renal circulation. Combined administration of vasopressin-1 and -2 antagonists produced a marked improvement in cardiac output ($+30\%$) and renal plasma flow ($+50\%$). Moreover, in dogs with impaired renal function and reduced GFR (-15% compared to the normal), vasopressin antagonist improved

the GFR by 35%. In addition, Okada et al. [61–64] have provided evidence for the crucial role of vasopressin in hypertensive animals.

Studies in Obesity

The relationship between obesity and hypertension is now widely recognized. Experimental studies have shown that weight gain raises blood pressure and clinical studies showed that weight loss is effective in lowering blood pressure in most hypertensive patients. In obesity, a close relationship has been proposed to exist between impaired natriuresis and increased RSNA and hypertension; however, there have been few studies directly addressing this relationship. Suzuki et al. [65, 66], using a genetically obese rat, Wistar fatty rat, found that in spite of no apparent impairment of baroreceptor reflex, RSNA was increased. In addition, without salt loading, blood pressure was not elevated even though pressure natriuresis was dysregulated. Taken together, obesity-induced hypertension might be intimately related to salt loading which stimulates RSNA and produces volume in impaired pressure natriuresis.

Conclusions

The syndrome of hypertension is intimately related to kidney function, and there is good evidence that each can manifest effects in the other. Our current studies are likely to provide clues for understanding the pathophysiology of hypertension and heart failure relating to regulatory mechanisms of the kidneys.

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Salt, Blood Pressure, and Kidney

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It is widely known that excessive salt intake plays a crucial role in the development of hypertension in humans as well as animals. Several epidemiological studies have demonstrated that prevalence of hypertension is greater in people on a higher salt diet. Indeed, the INTERSALT study showed the positive correlation of the incidence of hypertension to the amount of salt intake among people in 34 different countries [1]. However, the response of blood pressure (BP) to salt excess differs among individuals with essential hypertension. In our previous studies [2, 3], according to the BP response to salt loading, hypertensive patients could be divided into two groups – salt-sensitive and non-salt-sensitive ones (fig. 1). Not only some essential hypertensives but also the other young borderline hypertensives exhibited the increased salt sensitivity of BP [4]. Moreover, there is a significant correlation between the elevation of BP with salt loading and the reduction of BP with the subsequent treatment of diuretics, suggesting that the individual response to salt loading and restriction might be attributable to salt retention and depletion, respectively. Thus, it is a plausible hypothesis that susceptibility of BP to salt intake depends upon the ability of the kidney to excrete sodium (Na) in the urine.

Salt Sensitivity of Blood Pressure and Renal Sodium Excretion

Despite the same amount of salt intake, in our previous study, urinary Na excretion during the high salt diet was significantly decreased in salt-sensitive patients as compared with non-salt-sensitive patients (fig. 1); Na retention was significantly greater in salt-sensitive than non-salt-sensitive patients [2]. Especially, the reduction in urinary Na excretion apparently occurred during the
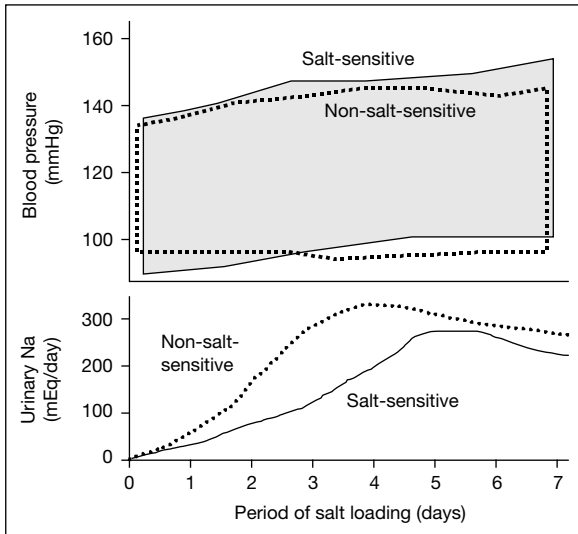
Fig. 1. Salt-induced blood pressure (BP) rise associated with decreased urinary sodium (Na) excretion in salt-sensitive hypertension. According to the BP response to salt loading, hypertensive patients could be divided into two groups – salt-sensitive and non-salt-sensitive ones. Despite the same amount of salt intake, urinary Na excretion during the high salt diet was significantly decreased in salt-sensitive patients as compared with non-salt-sensitive patients.

early period of high salt diet in salt-sensitive hypertensives. Generally, when salt loading after the low salt diet starts to be given, urinary Na excretion gradually increases, leading to the elevation of BP by Na retention. In turn, Na excretion reaches and further exceeds the Na intake (so-called 'escape') by pressure natriuresis, and finally returns to the equal level to the net Na intake. The delayed escape of natriuresis caused the greater Na retention in salt-sensitive patients than in non-salt-sensitive ones, resulting in the greater elevation of BP. Supporting it, the salt loading-induced rise in BP was accompanied with the greater increment of cardiac output (CO) in salt-sensitive patients [2]. The increase in BP was positively correlated with retained Na and increase in CO. Therefore, the impaired renal function for Na excretion plays an important role in salt-induced hypertension in humans.

Guyton's Renal Function Curve

Guyton [5] proposed that all types of hypertension basically have the impaired renal function for Na excretion and indicated the abnormality of the
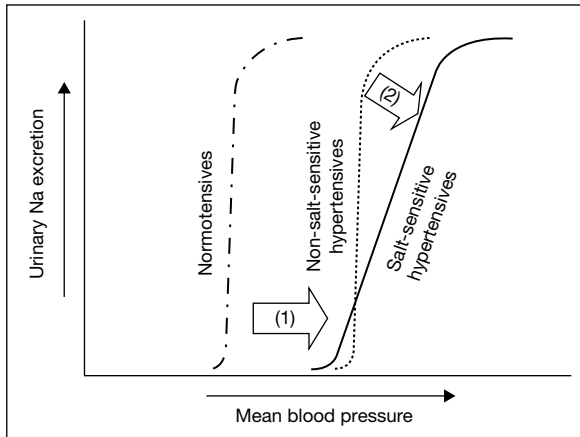
Fig. 2. Renal function curve in non-salt-sensitive and salt-sensitive hypertension. (1) In patients with essential hypertension, the renal function curve is shifted to the right suggesting that increased renal perfusion pressure might compensate the decreased renal ability to excrete sodium (Na). On the other hand, (2) the renal function curve of salt-sensitive patients is not only shifted to the right but its slope is also decreased, whereas the slope of the curve of non-salt-sensitive patients is still steep. That is, the slope of the curve indicates salt-sensitivity of blood pressure.

renal function curve that depicts the relationship between urinary output of Na and mean BP levels in several hypertensive animals. Urinary output of Na equilibrates the net Na intake at the point where the renal function curve and the net Na intake curve cross. The equilibrium point predicts the long-term level to which BP with changes in salt intake is achieved. If renal function curve is normal, salt loading never increases BP: Na retention transiently occurs, and it increases BP, in turn, resulting in natriuresis. Therefore, BP returns to the level before salt loading [6]. Thus, the slope of renal function curve is apparently steep in normotensive subjects, in whom the BP increase is merely a little despite salt loading. However, in patients with essential hypertension, the renal function curve is shifted to the right, suggesting that high BP is associated with the increased renal perfusion pressure, which might compensate the decreased renal ability to excrete Na in the urine, in order to maintain the normal body Na content (fig. 2). Indeed, in both salt-sensitive and non-salt-sensitive patients with hypertension, the renal function curve is shifted to the right. However, the renal function curve of salt-sensitive patients is not only shifted to the right but its slope is also decreased, whereas the slope of the curve in non-salt-sensitive patients is still steep; the slope of the curve indicates the salt sensitivity of BP [2, 7].
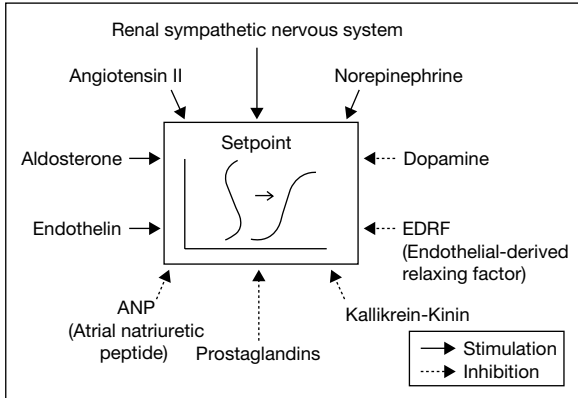
Fig. 3. Factors influencing the renal function curve. Extrarenal factors such as hormones and the sympathetic nervous system affect the renal function curve. Not only the excess of norepinephrine, angiotensin II, aldosterone and endothelin, but also the deficiency of atrial natriuretic peptide, prostaglandins, dopamine, endogenous digitalis-like substance(s), endothelium-derived relaxing factor and adrenomedullin could shift the curve to the right and/or decrease its slope.

Possible Mechanism(s) for the Impaired Renal Function of Sodium Excretion in Salt-Sensitive Hypertensives

There is growing evidence suggesting many intra- and extrarenal factors influence the renal function curve (fig. 3). The slope of the renal function curve is also altered by these factors. According to the hypothesis of renal origin, several investigators have proposed an 'abnormal tubulo-glomerular (T-G) feedback mechanism', and 'nephron heterogeneity' which is based upon imbalance of the nephron function and the renin secretion in the individual nephron [8]. Furthermore, Keller et al. [9] recently reported that the kidney of patients with essential hypertension had fewer glomeruli than that of normotensive subjects, and the remaining glomeruli in hypertensives were apparently larger, suggesting hyperfiltration.

However, some investigators have demonstrated that extrarenal factors such as hormones and the sympathetic nervous system are involved in the development and maintenance of hypertension in humans and animals. Not only the excess of norepinephrine (NE), angiotensin II, aldosterone and endothelin, but also the deficiency of atrial natriuretic peptide, prostaglandins, dopamine, endogenous digitalis-like substance(s), endothelium-derived relaxing factor (nitric oxide), adrenomedullin, etc., could shift the curve to the right and/or decrease its slope. Thus, the mechanisms of salt-induced hypertension in
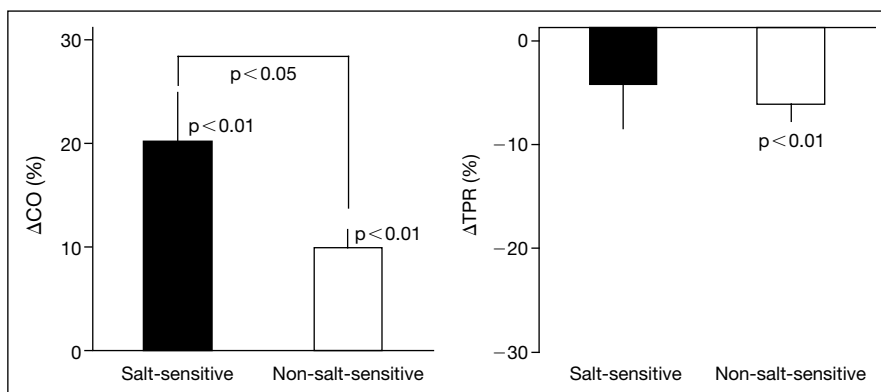
Fig. 4. Systemic hemodynamic changes with salt loading in salt-sensitive and non-salt-sensitive hypertension. Salt loading caused the apparent increase in cardiac output (CO) and the inappropriate decrease in total peripheral resistance (TPR) in salt-sensitive hypertensive patients. ΔCO: changes in CO with salt loading; ΔTPR: changes in TPR with salt loading.

salt-sensitive patients with essential hypertension should be complicated. Several different mechanisms might be involved in salt-induced BP rise in individuals with salt-sensitive essential hypertension.

Involvement of the Increased Renal Sympathetic Activity in Salt-Sensitive Patients

In salt-sensitive patients, we previously reported that plasma NE was indeed decreased during the early period of salt loading, but subsequently returned toward the level of the low Na diet. Interestingly, it was accompanied with the consistent BP rise and the peculiar systemic hemodynamic changes: the apparent increase in CO and the inappropriate decrease in total peripheral resistance with the 7-day salt loading (fig. 4), which might be attributable to not only volume retention but also the increased sympathetic activity [2]. Moreover, we found the abnormal regional hemodynamic changes with salt loading in salt-sensitive hypertensives: renal vascular resistance was significantly increased but forearm vascular resistance was decreased, followed by the marked increase in forearm blood flow but the absence of the increased renal blood flow with salt loading (fig. 5) [10]. These hemodynamic changes resemble those in the 'fight-or-flight reaction', which is induced by the increased hypothalamic NE discharge. Since renal sympathetic nerve activity
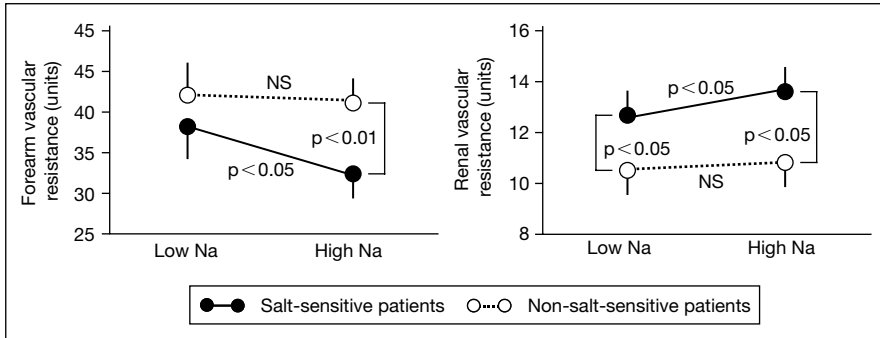
Fig. 5. Regional hemodynamic changes with salt loading in salt-sensitive and non-salt-sensitive hypertension. Renal vascular resistance was significantly increased but forearm vascular resistance was decreased with salt loading in salt-sensitive patients with essential hypertension. These hemodynamic changes resemble those in the 'fight-or-flight reaction', which is induced by the increased hypothalamic noradrenergic discharge. Low Na = low sodium intake; high Na = high sodium intake.

is well known to decrease urinary Na excretion, the increase of renal sympathetic activity might play a crucial role in the development of salt-sensitive hypertension.

Using salt-sensitive animal models, we found the importance of the renal sympathetic nerve activity in salt-sensitive hypertension. By directly monitoring renal nerve discharge, basal renal sympathetic tone was significantly increased in young salt-sensitive spontaneously hypertensive rats (SHR) as compared to normotensive Wistar-Kyoto rats (WKY) [11]. It is well known that either BP rise or elevated stimulation of aortic depressor nerve decreases renal nerve discharge, through the central vasomotor centers. Salt loading enhanced the inhibitory response of renal nerve activity to the electrical stimulation of aortic depressor nerve in the normotensive WKY, whereas the response to aortic depressor nerve stimulation was markedly suppressed by salt loading in SHR. It suggests that salt loading increases renal sympathetic nerve activity in salt-sensitive SHR, which is mediated by the central nervous system. Resultantly, salt loading decreased baroreceptor reflex sensitivity in SHR but not in WKY.

Moreover, using the NE turnover method, we demonstrated the increased renal sympathetic nerve activity in young salt-sensitive SHR. Tissue NE turnover rate is estimated from the decline of tissue NE content after the administration α-methyl-*p*-tyrosine, the inhibitor of tyrosine hydroxylase, the rate-limiting enzyme of NE synthesis [12]. Salt loading increased renal NE turnover in salt-sensitive SHR selectively, but it did not affect the turnover in
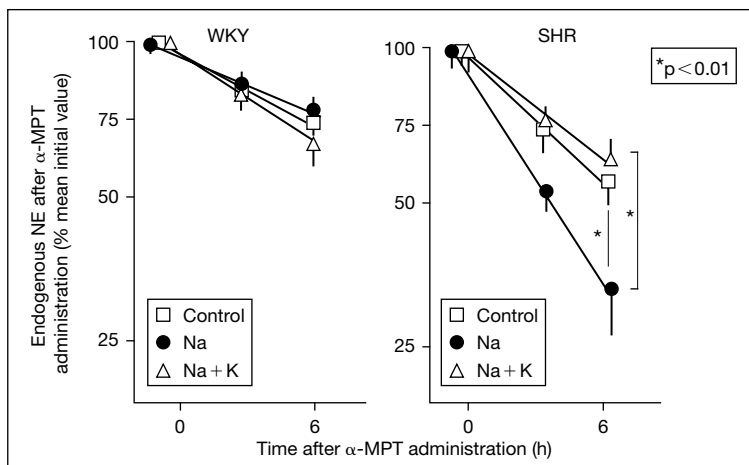
Fig. 6. Renal norepinephrine (NE) turnover in sodium (Na) and/or potassium (K) loaded spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Renal NE was measured after α-methyl-*p*-tyrosine (α-MPT) administration. Salt loading increased renal NE turnover in SHR, but not in WKY. K supplementation ameliorated NE turnover in salt-loaded SHR. Control, control (0.66% salt) diet-fed rats; Na, high (8.0%) salt diet-fed rats; Na+K, high salt and high (8.0%) potassium chloride diet-fed rats.

WKY (fig. 6) [13]. Moreover, the response of NE turnover in the kidney and the hypothalamus to cold exposure was markedly enhanced by salt loading in SHR but not in WKY (fig. 7), suggesting the selective increase in renal nerve activity is intimately related to the abnormal central noradrenerigic mechanism. It is consistent with the result of the abnormal response of renal nerve activity to aortic nerve stimulation in salt-sensitive SHR [11]. According to the abnormal central noradrenergic mechanisms, we demonstrated that air-jet stress decreased urinary Na excretion without changes in renal blood flow and glomerular filtration rate in deoxycorticosterone acetate (DOCA)-salt rats, a model of salt-sensitive hypertension, but not in control rats. But, renal denervation abolished the inhibitory response of urinary Na excretion to air stress in DOCA-salt rats [14]. Accordingly, the electrical stimulation of renal nerve has been demonstrated to induce Na retention, by the direct inhibition of Na reabsorption in the proximal tubules, the decreased renal blood flow, and the increased renin secretion, in a dose-dependent manner. Thus, the abnormal centro-renal sympathetic nervous system may play a key role in the development of salt-sensitive hypertension. Supporting this hypothesis, renal denervation could inhibit the development of salt-sensitive hypertension in salt-sensitive SHR [11], DOCA-salt rats [14] and salt-loaded obese dogs [15], through natriuresis.
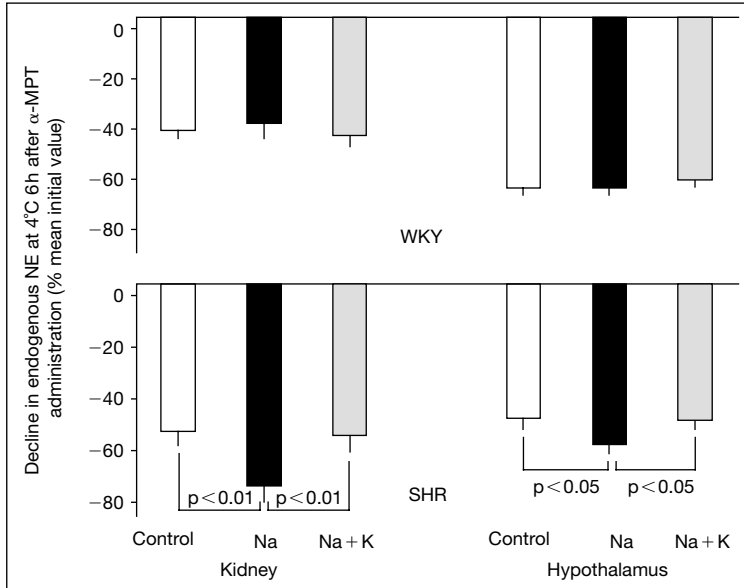
Fig. 7. Decline in endogenous norepinephrine (NE) in kidney and hypothalamus to cold exposure in sodium (Na) and/or potassium (K) loaded spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Tissue NE was measured cold exposure (4°C) and 6 h after α-methyl-*p*-tyrosine (α-MPT) administration. Salt loading enhanced them markedly and K supplementation normalized. Control = control diet-fed rats; Na = high Na diet-fed rats; Na+K = high Na and high K chloride diet-fed rats.

According to the selective increase in renal nerve activity, Esler et al. [16] reported that the renal NE spillover rate was selectively increased in patients with essential hypertension, especially obese salt-sensitive hypertensives. Moreover, Hollenberg et al. [17] demonstrated that mental stress could decrease renal blood flow markedly in patients with essential hypertension. Normotensives with a positive family history of hypertension had an abnormal response of renal blood flow to metal stress, associated with Na retention, but normotensives with a negative family history of hypertension did not, thus suggesting salt sensitivity of BP might be a genetic predisposition, although it is still controversial.

Since the increased renal sympathetic nerve activity plays a central role in salt-sensitive hypertension, sympatholytic agents might not only prevent hypertension but also be efficacious therapy for hypertensive patients. However, the long-term treatment of a sympatholytic agent, α-methyldopa, occasionally returns toward the level before the treatment, because of BP reduction-induced
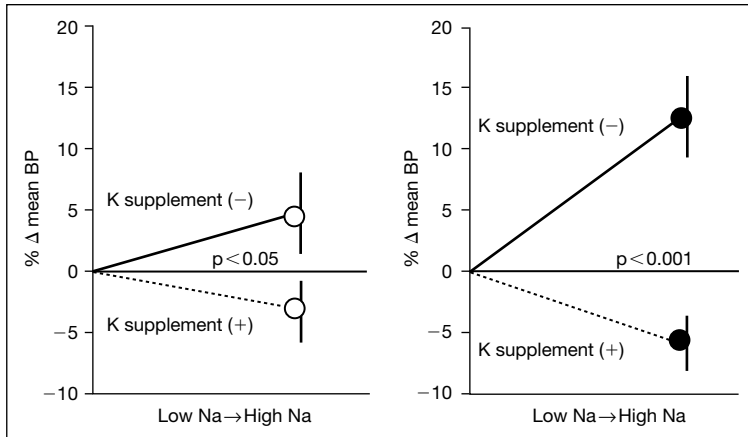
Fig. 8. Antihypertensive effect of potassium (K) supplementation on salt-induced blood pressure rise in salt-sensitive (right) and non-salt-sensitive hypertensive (left) patients. The depressor effect of K supplement was greater in salt-sensitive patients than non-salt-sensitive ones. % Δ mean BP, percent change in mean blood pressure; Low Na = low sodium intake; high Na = high sodium intake.

Na retention: pseudo-tolerance. In fact, the additional treatment of diuretics decreases BP rapidly, associated with natriuresis [18]. The systemic sympatho-inhibition induces Na retention through systemic vasodilation-induced BP reduction, which overcomes the Na excretion enhanced by the inhibition of renal sympathetic nerve activity. If a renal-specific sympatholytic agent could be developed, the agent as well as diuretic would be one of the first choices for the therapy of salt-sensitive hypertension. However, at present, no such renal-specific sympatholytic agents are available.

Potassium Supplementation in Salt-Sensitive Hypertension

In contrast to the pressor action of salt, potassium (K) has been known to have an antihypertensive effect. We demonstrated that K supplementation inhibited a salt-induced BP rise in patients [19] and rats [20] with salt-sensitive hypertension. The patients who had taken the potassium chloride (KCl) supplement (96 mEq/day) showed an apparently lower BP rise with changes in salt intake from 25 to 250 mEq/day than patients who had not taken the KCl supplement (fig. 8) [19]. Moreover, KCl supplementation also suppressed the salt-induced rise in BP in young salt-sensitive patients with borderline hypertension [4]. In animal studies, concomitantly, KCl supplementation inhibited

the development of salt-sensitive hypertension in DOCA-salt hypertensive rats [20] and salt-loaded salt-sensitive SHR [13, 21].

The antihypertensive effect of K was accompanied with an increase in urinary Na excretion and the resultant decrease in extracellular fluid volume [19, 20], suggesting that the antihypertensive effect of K might be mainly attributable to natriuresis. Moreover, K supplementation reduced the salt-induced elevation of renal vascular resistance in salt-sensitive SHR [21]. Thus, K may not only normalize the salt-induced regional hemodynamic changes, but also the abnormal renal function curve in salt-sensitive hypertension. Moreover, it led us to the hypothesis that the normalization of the impaired renal function with K supplementation might be intimately related to the sympatho-inhibition. Supporting it, K supplementation during the high salt diet decreased plasma NE to a greater extent in patients with essential hypertension as compared to those without K supplementation [19]. Consistently, salt-induced enhancement of NE turnover was suppressed by K supplementation in DOCA-salt rats [22] and salt-sensitive SHR (fig. 6) [13]. Moreover, K supplementation could attenuate salt-induced augmentation of renal and hypothalamic NE turnover response to cold exposure (fig. 7), suggesting that the inhibitory effect of K on salt-induced BP rise in salt-sensitive hypertension is mediated by the normalization of the abnormal central noradrenergic mechanisms. Accordingly, the inhibitory response of urinary Na excretion to air jet was abolished by K loading in DOCA-salt rats [14], as observed in DOCA-salt rats with renal denervation. Thus, K supplementation inhibited salt-induced BP elevation, possibly through natriuresis, which is intimately related to centro-renal sympatho-inhibition.

Renal Damage in Salt-Sensitive Hypertension

A high prevalence in cardiovascular diseases has been demonstrated in patients with salt-sensitive hypertension [23]. Salt-sensitive hypertensive patients have several risk factors for cardiovascular events (table 1). In particular, salt-sensitive patients are prone to suffer from the end-stage renal diseases. Abnormal renal hemodynamics is intimately related to not only Na retention but also the poor prognosis of hypertensive renal damage. Salt loading decreased intraglomerular pressure in non-salt-sensitive patients, whereas it increased intraglomerular pressure in salt-sensitive patients [24]. The increased Na reabsorption in the proximal tubulus decreases distal chloride (Cl) flow in the macula densa, induces vasodilation of the afferent arterioles, and results in the increased intraglomerular pressure by T-G feedback mechanism. According to the higher intraglomerular pressure, patients with salt-sensitive hypertension had the high prevalence of microalbuminuria [25]. Some investigators

Table 1. Clinical characteristics in salt-sensitive hypertension

Increase in insulin resistance
Low serum HDL cholesterol and high serum LDL cholesterol
Elevated intraglomerular pressure
High incidence of microalbuminuria
High incidence of non-dippers
Abnormal vascular endothelial function
High incidence of cardiovascular events

HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Table 2. Renal microcirculation and susceptibility to its damage in salt-sensitive and non-salt-sensitive hypertension model animals

	Dahl S rats	SHR
Systolic blood pressure	↑	↑
Intraglomerular pressure	↑	→
Glomerulosclerosis	Prone	Resistant

Dahl S rats = Dahl salt-sensitive rats; SHR = spontaneously hypertensive rats.

demonstrated the increased intraglomerular pressure and resultantly increased susceptibility to glomerulosclerosis in a hereditary salt-sensitive model, Dahl salt-sensitive (S) rats, but were not in a non-salt-sensitive model, SHR (table 2). Therefore, in salt-sensitive hypertension, salt-induced changes in renal microcirculation, such as afferent arteriolar vasodilation and/or efferent arteriolar vasoconstriction, increase intraglomerular pressure, resulting in the development of glomerulosclerosis as well as microalbuminuria.

Recently, it has been proposed that oxidized low-density lipoprotein (ox-LDL) plays an important role in the progression of atherosclerosis. Because vascular endothelial dysfunction triggers the development of atherosclerosis, the role of ox-LDL receptor in endothelial cells has been focused. A novel endothelial ox-LDL receptor, lectin-like ox-LDL receptor-1 (LOX-1) [26], has been considered to mediate ox-LDL-induced endothelial dysfunction, possibly through the upregulation of monocyte chemoattractant protein-1 and vascular cell adhesion molecule-1 expression. Since LOX-1 expression is upregulated by the mechanical stress, shear and stretch [27, 28], we can speculate that the

increased intraglomerular pressure accelerates the development of renal glomerulosclerosis possibly through LOX-1 overexpression. In fact, aortic LOX-1 expression was enhanced in both salt-loaded Dahl S rats and non-salt-sensitive SHR through BP rise. But, renal LOX-1 expression was increased in salt-loaded Dahl S rats alone [29]: the extent of its expression was consistent with that of progression of renal damage. Using both in situ hybridization and immunohistochemical methods, LOX-1 expression appeared in glomeruli but not in tubuli. Thus, LOX-1 may play a key role in the development of renal damage in salt-sensitive hypertension, through the overexpression of LOX-1 induced by the increased intraglomerular pressure.

Insulin Resistance and Salt-Sensitive Hypertension

Recently, several investigators proposed the intimate relationship between metabolic syndrome and salt sensitivity of BP: salt-sensitive hypertension exhibited insulin resistance [30, 31]. In patients with essential hypertension the salt-induced rise in BP was positively correlated to steady-state plasma glucose (SSPG) during high salt diet [30]. Moreover, salt loading per se elevates the plasma glucose response to glucose ingestion [32]. Therefore, we measured insulin resistance in Dahl S and salt-resistant (R) rats with salt loading precisely [31]. As a result, in salt-loaded Dahl S rats, both glucose infusion rate during hyperinsulinemic euglycemic clamp and insulin-stimulated 2-deoxyglucose uptake into isolated skeletal muscle were significantly decreased, suggesting that salt loading caused insulin resistance. In contrast, these parameters were not affected by salt loading in Dahl R rats. Insulin resistance in salt-sensitive hypertension may contribute to the susceptible cardiovascular diseases.

Salt loading has recently been demonstrated to enhance oxidative stress in salt-sensitive hypertension [33–35]. Increased insulin resistance in salt-loaded Dahl S rats may also be related to oxidative stress because a membrane-permeable superoxide dismutase mimetic, Tempol, ameliorated insulin resistance in Dahl S rats [32]. The administration of buthionine sulfoxide (BSO), glutathione synthase inhibitor, which inhibits reactive oxygen species (ROS) elimination through glutathione depletion, could decrease insulin sensitivity in rats and cultured adipocytes, associated with the impaired translocation of GLUT-4 into plasma membrane. Thus, ROS overproduction with salt loading may be involved in the development of insulin resistance in salt-sensitive hypertension.

K has been reported to have an antioxidant action in cultured cells [36] and rats [37]. We also confirmed that K decreased plasma 8-iso-prostaglandin $F_{2\alpha}$ and urinary 8-hydroxyl-2′-deoxyguanosine (8-OHdG), parameters of oxidative
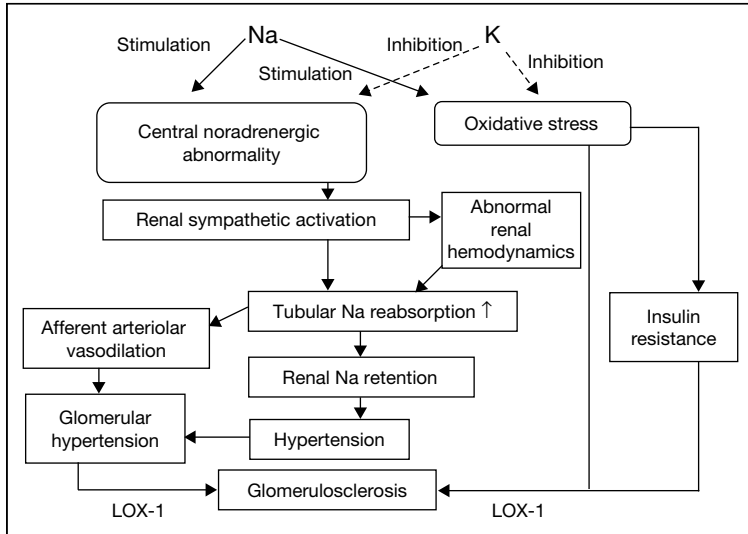
Fig. 9. Mechanisms that salt loading induces hypertension and renal damage and that potassium supplementation ameliorates them.

stress, in DOCA-salt rats. When K was supplemented in salt-loaded Dahl S rats, both glucose infusion rate during hyperinsulinemic euglycemic clamp and insulin-stimulated glucose uptake into skeletal muscle were ameliorated. Thus, K antagonizes against salt-induced insulin resistance, possibly through its antioxidant action. It is consistent with Tobian's finding that K exerts a cardio-vascular-protective action, independent of a BP-lowering effect [38]. In addition, we demonstrated that K supplement decreased renal LOX-1 expression in DOCA-salt rats, associated with the significant reduction in urinary protein. Because LOX-1 is upregulated by oxidative stress [39], this effect may also be due to its antioxidant effect as well as its depressor action. Therefore, K exhibits an organ-protective effect, possibly via not only antihypertensive but also antioxidant actions, both of which antagonize against salt-induced cardiovascular damages.

These findings are compatible with the results from a clinical mega-study using thiazide diuretics. In a subanalysis of the SHEP (Systolic Hypertension in the Elderly Program) study [40], chlorthalidone was effective in decreasing cerebrovascular events in elderly hypertensive patients. However, hypokalemic patients had no beneficial effect from diuretics, despite the similar BP reduction. Therefore, K depletion may offset the beneficial effect of BP lowering induced by diuretics, suggesting that K exerts organ-protective effects by the other mechanisms than its depressor action.

Conclusion

Salt loading caused a volume expansion-induced rise in BP through the impaired renal function for Na excretion, which may be attributable to renal-specific sympathetic activation (fig. 9). Increased sympathetic nerve activity in the kidney may cause Na retention, through the direct action on tubular Na reabsorption and via renal hemodynamic changes, and a decreased renal blood flow with unchanged glomerular filtration rate. Increased Na reabsorption in the proximal tubulus decreases Cl flow at the macula densa, resulting in the afferent arterial vasodilation by T-G feedback. Abnormal intraglomerular hemodynamics is associated with the increased intraglomerular pressure, which stimulates LOX-1 expression in glomeruli, resulting in the progression of end-stage renal diseases. In addition, Na does not only cause hypertension by the increased central noradrenergic-renal sympathetic activity, but also induces cardiovascular damages including glomerulosclerosis via insulin resistance and LOX-1 upregulation. In contrast, K ameliorates a salt-induced rise in BP and salt-induced cardiovascular damages, by inhibiting the sympathetic activation and the reduced ROS production, respectively.

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Involvement of Renal Sympathetic Nerve in Pathogenesis of Hypertension

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Network of the Sympathetic Nervous System and Baroreflex

Figure 1 demonstrates the network of the sympathetic nervous system (SNS) [1–8]. The rostral ventrolateral medulla (RVLM, shaded area) contains neurons that stimulate the SNS and determine blood pressure (BP). If electrophysiological activity of the RVLM bulbospinal neurons is increased, it activates peripheral sympathetic nerves to the heart, the kidney (renal sympathetic nerve activity, RSNA), and arterioles, thus elevating BP. Figure 1 also illustrates the arterial baroreflex that makes BP stable. The baroreflex is a negative feedback system. When BP is elevated, activities of arterial baroreceptors at carotid sinus and vagal afferent nerves (IXth and Xth cranial nerves) are increased, and neuronal activity of the caudal VLM (CVLM) is increased via the nucleus tractus solitarius (NTS). Since synaptic transmission from CVLM neurons to the RVLM is mediated by an inhibitory amino acid (γ-amino butyric acid, GABA) [1, 2], the neuronal activity of the RVLM is suppressed, and efferent sympathetic nerves (such as RSNA) and BP are decreased to the original level.

Conversely, if BP is reduced by standing or antihypertensive drugs, the activities of vagal afferents, the NTS, and CVLM neurons are reduced. Then, the activity of RVLM neurons is increased, and efferent sympathetic nerve activities should be activated. Thus, BP is increased to the original level.
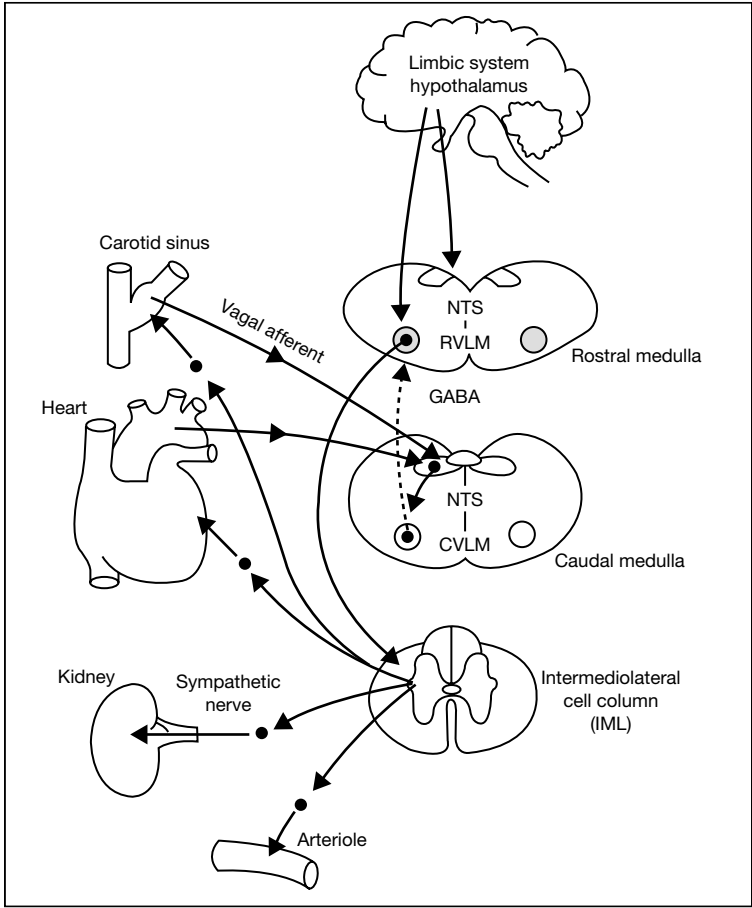
Fig. 1. The SNS and arterial baroreceptor reflex. RVLM (shaded area) = rostral ventrolateral medulla; CVLM = caudal ventrolateral medulla; GABA = γ-amino butyric acid; NTS = nucleus tractus solitarius. Activated RVLM neuron increases peripheral sympathetic nerves to the heart, kidney, and arterioles, thus inducing hypertension.

The arterial baroreflex mentioned above is the so-called high-pressure baroreflex. The cardiopulmonary baroreflex (low-pressure baroreflex) is also important to maintain sodium and water balance. When circulating plasma volume increases, that is, sodium and water balance is positive, this signal stimulates left atria receptors. This information decreases RSNA via vagal afferent-mediated projections to the RVLM. The decreased RSNA increases sodium excretion. Conversely, when circulating plasma volume is decreased, RSNA increases to
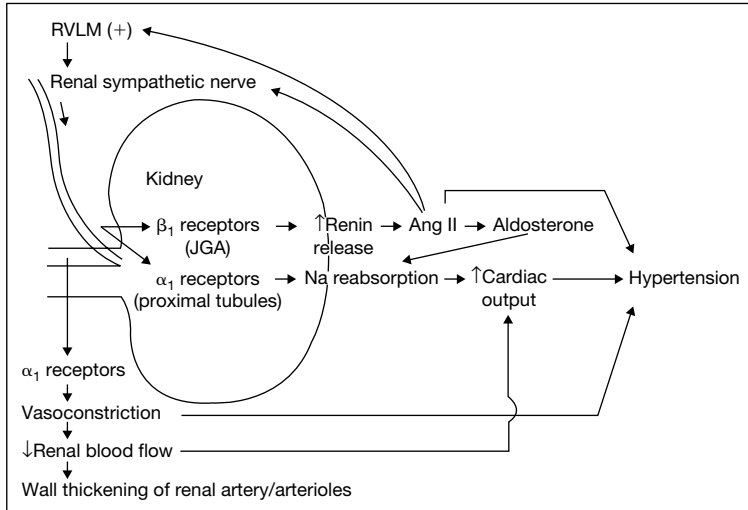
Fig. 2. Functional roles of renal sympathetic nerve in the initiation of hypertension. JGA = juxtaglomerular apparatus; RVLM = rostral ventrolateral medulla. Increased renal sympathetic nerve activity (RSNA) produces renin release (then genesis of angiotensin II, Ang II), sodium reabsorption, and reduction in renal blood flow, thus inducing hypertension.

enhance sodium reabsorption. Therefore, circulating plasma volume is tuned minutely and directly by RSNA.

Roles of Renal Sympathetic Nerve in Kidney Function

Figure 2 illustrates functional roles of RSNA in initiation of hypertension. When RSNA is elevated, renin is released via β_1 receptors, and sodium reabsorption occurs via α_1 receptors [6]. α_1 receptors mediate vasoconstriction of renal vessels and reduce renal blood flow (RBF). The enzyme renin changes angiotensinogen to angiotensin I (Ang I). Angiotensin-converting enzyme cleaves Ang I to produce angiotensin II (Ang II). Ang II induces vasoconstriction, sodium reabsorption at the proximal tubules, aldosterone production, and activation of the SNS. Ang II has an important presynaptic action on renal sympathetic nerve terminals of renal tubules to facilitate the release of norepinephrine and sodium reabsorption [7]. Involvement of the SNS in Ang II-induced hypertension is supported by observation that sympathectomy prevented the development of the Ang II hypertension [9]. Ang II also increases RSNA by acting

on the RVLM neurons in the central nervous system [3–8] (see Central Neuronal Mechanisms by Which Ang II Activates the Peripheral SNS). Using power spectral analysis, Townend et al. [10] reported that Ang II infusion enhanced the sympathetic component of heart rate variability in young normotensive subjects. Therefore, the SNS (RSNA) and Ang II interact to make a mutual cycle [3–8, 11, 12]. Ang II further promotes proliferation of vascular smooth muscles and cardiac hypertrophy. Aldosterone induces sodium reabsorption at the distal tubules. All of these factors are responsible for the initiation and development of hypertension through increases in cardiac output and/or total peripheral resistance (fig. 2).

DiBona and Kopp [13] demonstrated frequency-dependent effects of external stimulation of renal sympathetic nerves in rats. When the nerve stimulation is weak, renin release occurs. If the nerve stimulation is stronger, sodium reabsorption at the proximal tubules is found. When much stronger stimulation is applied to renal nerves, RBF decreases.

Autoradiographic localization of innervation at juxtaglomerular granular cells implies a direct physiological role of RSNA in renin release. Electrical stimulation of renal nerves as low as $0.5\,Hz$ increases renin release. Since this very low level of stimulation does not change sodium reabsorption or RBF, this low stimulus is a direct effect of RSNA on the juxtaglomerular cells. Other factors that activate renin release include decreased renal perfusion pressure, decreased perfusion pressure at the carotid baroreceptors, head-up tilt, and volume depletion. In these conditions, RSNA stimulates β_1 receptors to release renin, which is abolished by renal denervation.

Hollenberg et al. [14] demonstrated different RBF responses in normotensive and hypertensive subjects to psychological stimuli, such as nonverbal IQ test, which activate RSNA (fig. 3). Changes in BP and heart rate induced by the stimuli were identical in essential hypertensive patients and normotensive subjects with and without a family history of hypertension. However, they found a substantial reduction in RBF with increases in plasma renin activity and aldosterone concentration in hypertensive patients. In contrast, RBF was increased in normotensive subjects without a family history. This is a good example showing that externally activated RSNA reduces RBF in hypertensive patients. Also, these results suggest that difference in RSNA response is produced by the central nervous system.

On the other hand, we examined whether spontaneous RSNA actually regulates BP and RBF. Sakata of our laboratory [15] has for the first time established an experimental system to simultaneously record BP, HR, RSNA to the left kidney, and RBF of the left renal artery in conscious rats. RSNA is recorded with stainless wire electrodes, and RBF is recorded with pulsed Doppler flow probe. We record BP, HR, RSNA, and RBF to an A/D converter at 2,000 Hz.
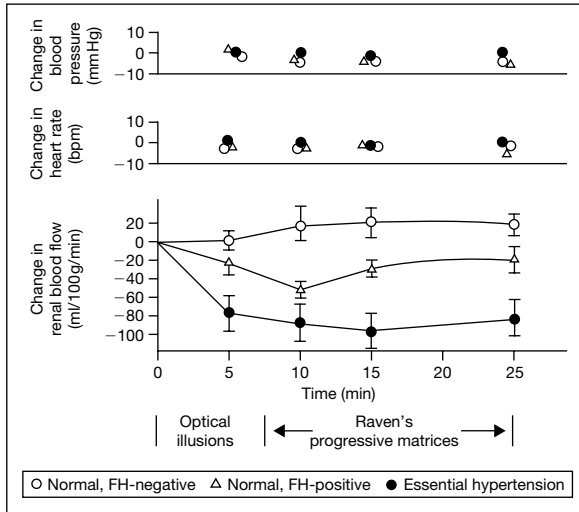
Fig. 3. Renal blood flow was decreased by increased renal sympathetic nerve activity in response to two types of psychological stimuli in essential hypertensive patients. FH = family history of hypertension [cited from 14].

We clearly demonstrated that an increase in RSNA preceded elevation of BP and decrease in RBF, when the recording was low-pass filtered <0.1 Hz [15]. These data suggest that spontaneous RSNA actually constricts the renal artery and reduces RBF, thus increasing BP. Also, since in the presence of normal arterial baroreflex, an increase in BP should precede a decrease in RSNA, our results suggest a cardiovascular regulatory system independent of the arterial baroreflex.

Role of the Renal Sympathetic Nerve in the Maintenance of Sodium Balance

The physiological consequences of maintaining normal balance of circulating plasma volume contain important neural elements [16]. The spontaneous level of RSNA attenuates the natriuretic and diuretic response, since bilateral renal denervation induces significant sodium excretion. In response to increased or decreased sodium intake, RSNA decreases or increases, respectively, thus increasing or decreasing urinary sodium excretion. The rapid change in urinary sodium excretion is abolished by bilateral renal denervation. When the rapid and minute response of RSNA is impaired, regulation of urinary

sodium excretion becomes to depend on slow long-term controllers, such as humoral hormones, RBF, and glomerular filtration rate. Thus, RSNA is critical to the control of the rate at which appropriate sodium balance is achieved during changes in sodium intake. The baroreflex also participates in the maintenance of sodium balance [17, 18]. When sodium intake is increased, RSNA should decrease and sodium excretion increases. However, if baroreflex is impaired, the increase in sodium intake does not suppress RSNA and induces sodium retention, thus initiating hypertension.

Complex interactions are found between sodium intake, RSNA, renin-Ang II-aldosterone system, arginine vasopressin, and environmental stress in the control of BP and circulating plasma volume [3–8, 11–13, 15–19]. Arginine vasopressin, which promotes water reabsorption, suppresses RSNA [11, 12]. As mentioned earlier, Ang II and RSNA behave as a vicious cycle. For example, angiotensin-converting enzyme inhibitor attenuated the antinatriuretic response to electrical stimulation of renal sympathetic nerves [13]. However, in the state of sodium depletion, RSNA and Ang II are stimulated together to increase sodium reabsorption and maintain sodium balance.

The clinical relevance of the interaction between dietary NaCl intake, RBF, renal vascular resistance, and RSNA has been demonstrated in normotensive and borderline hypertensive subjects [13, 20]. Responses of these parameters to changing from supine to upright posture were determined during terms of low and high NaCl diet. This change in posture produces reflex activation of RSNA. In normotensive subjects, the decreases in BP and increases in renal vascular resistance (due to increased RSNA) induced by upright posture were similar during low and high dietary NaCl. In contrast, in borderline hypertensive subjects, the results were different. On low dietary NaCl intake, BP did not decrease, and the increase in renal vascular resistance by upright posture did not differ from that in normotensive subjects. On the other hand, on high dietary NaCl, BP increased, and the increase in renal vascular resistance induced by upright posture was significantly larger than that observed in normotensive subjects. Thus, the high dietary NaCl augmented RSNA response and renal vasoconstriction to upright posture in borderline hypertensive subjects.

Environmental or psychoemotional stress increases RSNA, whereas excess sodium intake should suppress RSNA through low-pressure baroreflex. However, in some genetically hypertensive rats, sodium intake does not suppress the stress-induced increase in RSNA, thus initiating hypertension. Environmental stress is translated into an increase in peripheral RSNA via the limbic-hypothalamic-bulbar autonomic centers [13]. The development of hypertension in genetically hypertensive rats is accelerated by high dietary NaCl alone or by environmental stress alone [21]. The combination of genetic predisposition, high dietary NaCl intake, and stress results in more severe hypertension than any factor alone.
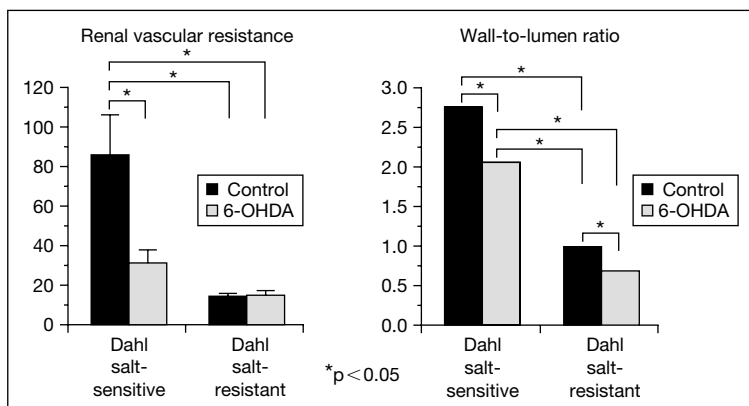
Fig. 4. Higher renal vascular resistance (black column, left panel) and wall-to-lumen ratio of renal vessels (black column, right panel) in Dahl salt-sensitive hypertensive rats than those in Dahl salt-resistant rats were reduced by chemical destruction of renal sympathetic nerves by 6-hydroxydopamine (6-OHDA, shaded column) [cited from 22].

In humans, psychoemotional stress acts at various sites in the central nervous system to override the normal regulatory system and baroreflex, resulting in augmented RSNA. The net results in the kidney would oppose the sodium excretion, promote renal sodium retention, and induce hypertension.

In hypertension and congestive heart failure, such minute interactions are impaired, and enhanced RSNA decreases sodium excretion [3–8, 12, 13]. Thus, excess sodium intake cannot suppress RSNA or Ang II, thus inducing hypertension and edema. Increased RSNA was actually documented in rats and patients despite sodium retention in congestive heart failure, nephritic syndrome, and hypertension [13].

Renal Structural Changes by Enhanced RSNA

So far, we have discussed roles of RSNA in renal function. On the other hand, Wu et al. [22] demonstrated that RSNA also affects structural changes in renal vessel walls. In Dahl salt-sensitive hypertensive rats, renal vascular resistance (control, black column in fig. 4, left) and wall-to-lumen ratio of renal vessels (wall thickness, black column in fig. 4, right) were higher than those in Dahl salt-resistant rats. After peripheral sympathetic nerves were chemically destructed with 6-hydroxydopamine, not only BP and renal vascular resistance (shaded column in fig. 4, left) significantly decreased, but also the

wall-to-lumen ratio (shaded column in fig. 4, right) was reduced. These data strongly suggest that RSNA induces trophic effects and structural changes in renal vessels.

Potentiated SNS in Hypertension

Overactivity of the SNS has been implicated in the initiation and development of various types of hypertension including essential hypertension, since the potentiated SNS increases cardiac output and total peripheral resistance [3–8]. Esler et al. [23] have demonstrated that renal norepinephrine spillover, a precise index of RSNA, was increased in patients with essential hypertension. The basal level of muscle sympathetic nerve activity was elevated in borderline hypertensive subjects as compared with normotensive subjects [24], and mental stress increased the nerve activity [25]. Julius et al. [26] showed by their longitudinal observation that sympathetic nerve activity was supposed to be elevated in hyperkinetic borderline hypertension. The overactivity the SNS is also responsible for the antinatriuretic state in renal diseases, such as nephrotic syndrome.

Spontaneously hypertensive rats (SHR) have been used as a model of human essential hypertension. Since sympathetic nerve activities to various organs are basically the same, RSNA is representative for the systemic SNS. RSNA recorded in the conscious state was increased in SHR as compared in normotensive Wistar-Kyoto (WKY) rats [27, 28]. Complete denervation of bilateral renal nerves reduces the magnitude of hypertension in various hypertensive models, such as SHR, two-kidney, one-clip Goldblatt rats, aortic coarctation dogs, obesity hypertensive dogs, and deoxycorticosterone acetate-salt hypertensive rats [13].

Ang II Receptor Blocker Reduces RSNA

Kumagai et al. [19] examined the relationship between RSNA and Ang II in the regulation of RBF in conscious renovascular hypertensive rabbits, when BP was equally reduced by intravenous infusion with ACE inhibitor (captopril) and calcium channel blocker (nicardipine). During captopril infusion, RBF consistently increased as reduction in BP. In contrast, RBF initially increased when mean arterial pressure was reduced to 80 mm Hg with nicardipine, but then it decreased when mean arterial pressure was reduced to 70 mm Hg. Since plasma concentration of Ang II was increased with nicardipine infusion, vasoconstriction due to baroreflex-stimulated RSNA and Ang II may have overcome
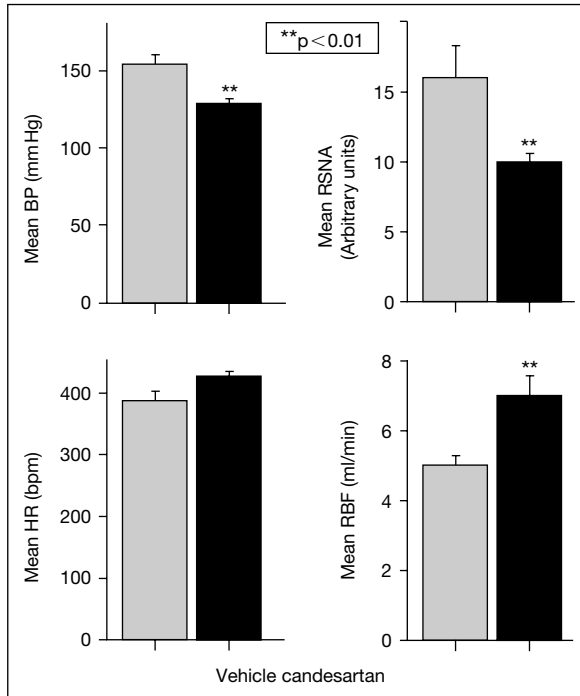
Fig. 5. Two-week oral treatment with candesartan, Ang II receptor blocker (black column), significantly (p < 0.01 vs. vehicle treatment) suppressed renal sympathetic nerve activity (RSNA) despite a decrease in blood pressure (BP) and an increase in renal blood flow (RBF) in conscious spontaneously hypertensive rats [cited from 15].

vasodilatation, thus reducing RBF. Our data imply that interaction between RSNA and Ang II, as well as underlying BP level, plays a substantial role in determining RBF.

When we reduce BP with a calcium channel blocker, RSNA is significantly elevated [19]. In contrast, when we infused candesartan (Ang II receptor blocker, ARB) intravenously for 10 min in conscious SHRs, RSNA did not increased despite BP reduction.

Furthermore, we found that in conscious SHR given candesartan orally for 2 weeks, mean value of RSNA was smaller than in SHR given vehicle despite a significant reduction in BP and increase in RBF (fig. 5) [15]. Also, we reported that long-term oral treatment with candesartan improved impaired baroreflex in SHR [29]. We speculate that the improvement in baroreflex can explain partly the decrease in RSNA in SHR given candesartan. The baroreflex function in SHR was also improved by angiotensin-converting enzyme inhibitor [30].

Grassi et al. [31] showed that in obese hypertensive patients, 3-month treatment with candesartan reduced muscle sympathetic nerve activity. These data in rats and humans [15, 31] strongly suggest that candesartan decreases systemic sympathetic nerve activity. Overactivity of the SNS is generally accepted as a risk factor for cardiovascular events, such as myocardial infarction [32]. Although calcium channel blockers are frequently used in hypertensive patients, caution must be exercised to their reflex sympathoactivation [33]. In this regard, ARB is promising for decreasing the cardiovascular events, since this drug reduced the SNS.

High Linearity and Low Nonlinearity of Neural Regulation in Hypertension

In normal condition, various regulatory factors, such as the renin-angiotensin system, the SNS, arterial baroreflex, nitric oxide, and depressor hormones, make complex interaction to maintain normal homeostasis. In other words, nonlinearity (complexity) of neural regulation of the cardiovascular system is high and linearity is low in the normal condition. In contrast, in congestive heart failure, patients with impaired baroreflex, epilepsy, and elderly patients, the nonlinearity is reduced [34]. Actually, Huikuri et al. [35] reported that patients with a reduced nonlinear component of heart rate regulation after acute myocardial infarction showed a poor prognosis. Therefore, we examined whether nonlinear correlations between RSNA-BP and RSNA-RBF were reduced in SHR [15]. By recording BP, RSNA, heart rate, and ipsilateral RBF in the conscious state, we compared linear (coherence of transfer function) and nonlinear (mutual information method [36]) correlations between WKY and SHR. We found coherence peaks of the transfer function from RSNA to BP and from RSNA to RBF at 0.05 and 0.80 Hz, i.e., below respiratory- and cardiac-related fluctuations. We speculate that such fluctuations (oscillations) are generated in the central nervous system (for example, the RVLM neurons), as Malliani et al. [37] advocated, but not in the baroreflex.

We demonstrated that the coherence (linearity) was significantly higher and the nonlinearity was lower in SHR than in WKY. Moreover, 2-week oral treatment with candesartan reduced the linearity and increased the nonlinearity in SHR [15]. As mentioned earlier, candesartan, which blocks the renin-angiotensin system, reduced RSNA despite BP reduction.

Since candesartan reduced the linearity and increased the nonlinearity, the higher linearity and lower nonlinearity in SHR can be explained by strong dependence on only one or two predominant neurohumoral systems (the SNS and the renin-angiotensin system), and/or by a decrease in the number of

neurohumoral systems (NO and baroreflex) [15]. By contrast, the higher non-linearity (complexity) in normotensive WKY may imply that regulation of BP and RBF depends on various neurohumoral systems and not solely on the SNS or the renin-angiotensin system. From these data, we consider that the nonlinearity of cardiovascular regulation is determined by the central nervous system (the RVLM, the hypothalamus), but not by peripheral vasculature [38].

Central Neuronal Mechanisms by Which Ang II Activates the Peripheral SNS

When activity of the RVLM neurons is increased, peripheral sympathetic nerves to the heart, kidney and arterioles are activated, and BP elevates [1–5, 7, 18]. To determine mechanisms of increased SNS and interaction between Ang II and the SNS in the central nervous system, we performed a whole-cell patch-clamp technique on RVLM neurons [39, 40]. First, to examine neuronal mechanisms underlying the initiation of hypertension, we compared electrophysiological characteristics of RVLM neurons of neonatal WKY rats and SHR [40]. Using the whole-cell patch-clamp technique, Matsuura et al. [40] examined the properties of RVLM neurons in brainstem-spinal cord preparations in which the sympathetic neuronal network is preserved. The baseline membrane potential of irregularly firing neurons was shallower (-50.1 ± 0.6 vs. -52.0 ± 0.6 mV) and the firing rate was faster (3.0 ± 0.2 vs. 2.0 ± 0.2 Hz) in SHR (n = 56) than in WKY (n = 38). These results imply that activities of RVLM neurons are higher in SHR than in WKY.

Then, superfusion with Ang II (6 µmol/l) induced significant depolarization and an increase in firing rate in RVLM neurons of SHR ($+4.8 \pm 0.6$ mV) but not of WKY [40]. In contrast, candesartan (0.12 µmol/l) induced significant membrane hyperpolarization (-4.1 ± 0.6 mV) and a decrease in the firing rate in RVLM neurons of SHR but not of WKY. Thus, candesartan can reduce the higher activity of RVLM neurons.

Therefore, basal electrophysiological properties of RVLM neurons and their response to Ang II differ between neonatal WKY rats and SHR before BP differs. The higher activity of the RVLM neurons increases RSNA and BP in SHR. The significant hyperpolarization and decreased firing rate caused by candesartan suggest that locally generated Ang II (probably by astrocyte and glia) actually binds to Ang II type 1 receptors on RVLM neurons, thus tonically inducing the higher activity of RVLM neurons in hypertensive rats. These differences in RVLM neurons suggest a mechanism possibly leading to future BP elevation. In support of our data, DiBona and Jones [41] demonstrated that candesartan microinjected into the RVLM area reduced BP and RSNA in rats fed

a low sodium diet, in which the renin-Ang II system was stimulated. These results of the central nervous system [40, 41] account for mechanisms of decreased RSNA by oral treatment with candesartan [15]. Inhibitory effect of candesartan on the RVLM neurons may override the sympathoexcitation by BP reduction due to oral candesartan.

Conclusions

Renal sympathetic nerve participates in pathogenesis of hypertension through renin release (Ang II production), sodium reabsorption, and RBF reduction. Ang II induces vasoconstriction, aldosterone production, and stimulation of the SNS. Therefore, the SNS and Ang II behave as a vicious cycle. Since the elevated SNS and reduced nonlinearity of cardiovascular regulation are risk factors for cardiovascular events, ARB, such as candesartan, is a beneficial drug to prevent the events.

Acknowledgements

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Blood Pressure Regulation and Renal Microcirculation

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The kidney handles urea metabolites and excretes acids, and regulates systemic blood pressure (BP) not only by modulating sodium balance but also by actively producing vasoactive substances, including angiotensin II (AngII), prostaglandin (PG) and nitric oxide (NO). Although it is readily expected that tubular Na transport affects systemic BP, elevated renal microvascular tone requires higher renal perfusion pressure to establish a new homeostatic level that would efficiently excrete sodium. This formulation, the pressure-natriuresis theory, was originally proposed by Guyton et al. [1].

Several investigators have attempted to characterize the intrarenal hemodynamics in humans quantitatively. Traditionally, such approaches have included Gomez's formulas [2] and, more recently, the application of the renal function curve (pressure-natriuresis relationship) [1, 3]. Recently, Kimura et al. [4] applied renal function curves to characterize the renal hemodynamics in hypertensive patients. They categorized hypertension into two subsets of patients, sodium-sensitive and non-sodium-sensitive, and found that the pressure-natriuresis curve exhibited a parallel rightward shift in non-sodium-sensitive hypertension (with rightward shift of autoregulatory range) whereas the slope of the curve was blunted in sodium-sensitive patients (without changes in autoregulatory range). Such clear definition of sodium sensitivity allows characterization and categorization of hypertension according to the sodium sensitivity (table 1). They further evaluated the effect of nicardipine on the intrarenal hemodynamics in hypertensive patients; nicardipine reduced predominantly elevated preglomerular resistance from 9,300 to 7,400 $dyn/s/cm^{-5}$, whereas no changes were noted in postglomerular resistance. Studies using renal function curves in humans are in full accordance with experimental observations using several videomicroscopic techniques [5–9]. In this review,

Table 1. Classification of hypertension [adopted from Kimura et al.: J Hypertens 1997;15:1055]

Changes in pressure-natriuresis curve	Salt-insensitive parallel shift of curve	Salt-sensitive blunted slope of curve	
Sites of responsibility	*Vasculature:* Main renal artery Interlobular artery Afferent arteriole	*Glomerulus:* Basement membrane	*Tubulus:* Proximal Henle's loop Distal collecting duct
Mechanisms	Increases in preglomerular resistance	Decrease in coefficient	Increased tubular Na reabsorption
Human disease	Renovascular hypertension Essential hypertension	Glomerulonephritis Hypertension in blacks	Primary aldosteronism
Animal models	Two-kidney, one-clip hypertension Spontaneously hypertensive rats	Dahl salt-sensitive rats	DOCA hypertension Zucker obese rats

we characterized renal microcirculation with special references to renal autoregulatory mechanisms. Furthermore, the microvascular responsiveness to several types of disorders that affect renal function has also been evaluated.

In vitro and in vivo Observations of the Renal Microcirculation

Assessment of the renal microcirculation in humans requires several assumptions for calculation of renal microvascular hemodynamics. Furthermore, the extensive manipulation such as the use of pharmacological tools cannot necessarily be conducted in humans. Nevertheless, direct assessment of the renal microvascular response is required to determine the renal microvascular responses to certain disease conditions or vasoactive substances. In this regard, recent advances in renal physiology enable us to observe renal microvessels indirectly [10, 11] or directly (table 2).

Edwards [12] initially developed the dissected renal microvessels to observe directly the renal microvascular responsiveness. More recently, Ito and Carretero [13] succeeded in isolating the renal afferent (AA) and efferent arteriole (EA), with an attached glomerulus and a thick ascending limb of Henle's loop. This preparation possesses both microvascular and tubular components and thus enables us to assess the net effects on the renal microvasculature. Casellas and Carmines et al. [6] developed an in vitro technique that allows

Table 2. Methods for assessing the renal microcirculation

Methods	Group (first author)	Characteristics
Indirect		
Pressure-natriuresis curves	Gomez [2]	
	Kimura [4]	
Laser Doppler	Pallone [11]	
	Cowley [50]	
Micropuncture	Thurau [10]	
	Bell [105]	
Direct		
Isolated microvessels	Edwards [12], Yuan [5]	Without glomeruli
	Ito [13]	With glomeruli
Juxtamedullary nephrons	Navar [6]	Blood-perfused
Hydronephrotic kidneys	Steinhausen [19]	In vivo
	Loutzenhiser [7, 14]	In vitro
Needle-type CCD camera	Yamamoto [16]	Intravital

direct visualization of the juxtamedullary nephron circulation. The kidney is isolated from the rat, and the papilla is folded back to expose the renal microvasculature of juxtamedullary nephrons. The perfusate consists of reconstituted blood (hematocrit, 33%), and renal microvessels can be observed at the juxtamedullary portion, with preserved perivascular structures adjoining the glomerulus. Using in vivo hydronephrotic rat kidney, Steinhausen et al. [9] introduced a novel technique which allows us to visualize the renal microcirculation in situ. In their experiments, vasoactive agents are applied topically to the surface of the kidney, thus eliminating systemic effects in an intact and in vivo setting. Recently, Loutzenhiser et al. [14, 15] developed a model of the isolated perfused hydronephrotic kidney that facilitates direct observation of the renal microvasculature under in vitro conditions. Hydronephrosis is induced in donor rats by unilateral ureteral ligation. After 8–10 weeks, the hydronephrotic kidneys are excised and studied using a modification of the isolated perfused technique. The perfused kidney is placed on the stage of an inverted microscope modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom surface. Finally, Yamamoto et al. [16] developed a novel technique utilizing an intravital needle-type CCD videomicroscopy. Due to miniaturization of the probe with a tapered end, the physical pressure on the area of investigation and the manipulation of the surrounding tissue were minimized. This novel instrument may allow direct observation of the renal microvasculature, without impairment of the vasculature and perivascular tissues that may modulate the vasomotor tone of the adjacent vascular beds.

Voltage-Dependent Calcium Channels in Renal Microcirculation

A number of investigators attempted to characterize the renal microvascular beds. We used the isolated perfused hydronephrotic kidney model to characterize the distribution of voltage-dependent Ca channel (VDCC) and their activity in the renal microcirculation [17]. Because membrane depolarization induced by high K level elicits preferential activation of VDCCs, this pharmacologic tool may clarify their role in mediating renal vascular tone. A medium high in KCl (30 mEq/l) causes marked constriction of the AA, but only a modest decrement in EA diameter. Consequently, nifedipine reversed the KCl-induced AA constriction. Carmines et al. [18] conducted an elegant study by directly assessing the intracellular Ca concentration ($[Ca]_i$) of isolated rabbit glomerulus with attached AA and EA. They demonstrated that high K-induced depolarization elevated the $[Ca]_i$ of the AA from 150 to 196 nM, whereas $[Ca]_i$ of the EA was reduced from 188 to 148 nM, probably because of a decrease in electrochemical gradient driving force of Ca leak into cells. They also demonstrated that nifedipine completely prevented the high K-induced rise in $[Ca]_i$. In addition to the indirect method of VDCC activation by membrane depolarization, Steinhausen et al. [19] activated VDCCs directly by an L-type Ca channel agonist, Bay K-8644. They demonstrated that Bay K-8644 caused a preferential constriction and vasomotion of the AA, an observation qualitatively similar to that subtending KCl-induced activation of VDCCs. In summary, it is concluded that L-type VDCCs predominate at the AA, and are sparse or functionally silent at the EA. Such functional heterogeneity of the renal microvasculature greatly influences the actions of Ca antagonists on renal hemodynamics.

In contrast to a predominant activity of L-type VDCCs on the AA, recent developments in the structural modification of the Ca antagonist provide novel information on the role of other VDCCs such as N-type and T-type Ca channels. Cilnidipine inhibits N-type as well as T-type Ca channels [20], and dilates EAs [21]. Furthermore, efonidipine causes prominent EA dilation nearly identical in magnitude with the AA dilation [22, 23]. Because the traditional Ca antagonists act on L-type VDCCs, their effects on the EA are most likely attributable to some of their additional properties rather than to class effects. In this regard, Ozawa et al. [24] have recently demonstrated that the EA dilation by some Ca antagonists is mediated by the blocking action on T-type VDCCs. Indeed, mibefradil, a selective T-type VDCC antagonist, potently reverses the AngII-induced EA constriction. Similarly, nilvadipine and aranidipine, both of which possess T-type as well as L-type VDCC-blocking activity [25, 26], share the same properties with efonidipine with regard to the EA action. Thus, the results

obtained from novel Ca antagonists clearly indicate the functional presence of T-type VDCCs in both AAs and EAs.

Cortical Autoregulation

The kidney maintains a relatively constant renal blood flow, glomerular filtration rate (GFR) in the face of the alterations in renal and systemic arterial pressure. This phenomenon (i.e., renal autoregulation) constitutes an important determinant of the glomerular protection, to which the vascular tone of the AA contributes exclusively. The pressure-induced constriction of the AA requires the integrity of two homeostatic mechanisms, i.e., myogenic response of the AA and tubuloglomerular feedback (TGF) mechanism [27]. Various disorders, including diabetes mellitus (DM) and chronic renal failure, seem to impair these two mechanisms.

In DM, the kidney manifests elevated GFR (glomerular hyperfiltration) and glomerular capillary pressure (GCP) that subsequently leads to the development of renal injury. In an animal model of DM, such as streptozotocin-induced DM rats, AA resistance is decreased [28–30], and renal autoregulatory response to elevated renal arterial pressure is impaired. Analogous findings have been reported in obese non-insulin-dependent DM rats [31]. Several vasoactive substances, including NO [32, 33] and vasodilatory PGs [34, 35], are proposed as a factor responsible for the glomerular hyperfiltration. There have been divergent results on the role of NO in DM kidneys. A specific neuronal NO synthetase (nNOS) inhibitor, s-methyl-L-thiocitrulline revealed that nNOS activity was activated [36], leading to augmented NO production and the subsequent glomerular hyperfiltration in DM kidneys. Carmines et al. [37–39] demonstrated enhanced activity of ATP-sensitive K channels and derangement in L-type VDCCs in the AA of DM kidneys. Sharma et al. [40, 41] reported that transforming growth factor-β, which is up-regulated in DM kidneys, inhibits pressure-induced AA constriction.

Another major issue that has not been clarified so far is the mechanism for glomerular hypertension in chronic renal disease. Traditionally, the depressed function of injured nephrons is compensated by the hyperfunction of the remaining intact glomeruli [42]. Although this theory, i.e., intact nephron hypothesis or glomerular hypertension theory, has long been accepted, the intrarenal process to glomerular hypertension has not been determined. Cortical glomerular dysautoregulation observed in remnant kidneys could be involved [43]. It has been reported that insulin-like growth factor, which dilates AAs from normal rats, is increased after subtotal nephrectomy, although this substance is reported to restore autoregulatory response of AAs from

subtotally nephrectomized rats [44]. Alternatively, it is possible that transforming growth factor-β participates in the diminished AA tone in chronic renal injury [40, 45]. Clearly, the determination of this issue requires further investigations.

Medullary Autoregulation

Renal medulla plays a pivotal role in BP regulation. Diminished papillary blood flow is observed in various hypertensive models, including the spontaneously hypertensive rat (SHR), Dahl salt-sensitive (DS) rat and deoxycorticosterone acetate-salt hypertension [46–48]. In juxtamedullary nephrons, AA and EA blood flow is autoregulated above 100 mm Hg [6, 27]. However, autoregulatory capacity of papillary blood flow depends on volume status and species [6]. While papillary blood flow is well autoregulated even in volume-expanded dogs, it is autoregulated above 75–100 mm Hg in euvolemic but not volume-expanded rats [6, 49, 50]. This papillary dysautoregulation may relate either functionally to NO-induced descending vasa recta (DVR) dilation [51] or anatomically to shunt vessel which directly connects AA to DVR bypassing glomerulus, thus only partially autoregulating the blood flow due to the lack of TGF [50]. In response to elevations of BP, increments in papillary blood flow may account for increases in renal interstitial pressure, decreases in proximal tubular reabsorption and resultant natriuresis at lower BP or during volume-expansion. Lymph channels are sparse in outer medulla, and absent in inner medulla [11]. Small increase in papillary capillary pressure due to dysautoregulation could accumulate significant amount of transdate in medulla, elevating interstitial pressure that transmits to cortex easily because the kidney is encapsulated. Proximal tubules are major site of actions for interstitial pressure because this segment is the most susceptible to changes in pressure [52]. Pressure-induced decreases in AngII elicit rapid redistribution of apical proximal tubule Na/H exchanger out of microvilli to endosomal pools, contributing to decrease in proximal tubular reabsorption [53]. Indeed, pressure-induced decrease in proximal tubular reabsorption is an important response. Since single nephron GFR, plasma flow, peritubular capillary oncotic and hydrostatic pressures are maintained constant in spite of elevation of BP, reduced proximal tubular reabsorption is needed to increase NaCl delivery to macula densa (MD), providing a signal for TGF that BP has been elevated. At higher BP or in euvolemia, pressure natriuresis occurs in distal nephron [54, 55]. Amiloride and thiazide prevented pressure natriuresis above 100 mm Hg in euvolemic animals. Pressure-induced increases in NO should suppress NaCl reabsorption in distal nephron including collecting duct [56]. NO could derive from

preglomerular vascular endothelium and MD. Anatomically, the interlobular artery runs next to the collecting duct. MD cells are characterized by the lack of Tamm-Horsfall glycoprotein, the presence of cyclo-oxygenase-2 (COX-2) and nNOS [57–59]. When TGF is active, the elevation of $[Ca]_i$ in MD stimulates NO synthesis, reducing reabsorption of collecting duct (feed-forward theory).

Arginine vasopressin (AVP) increases water reabsorption acting on aquaporin channels in collecting ducts through V2 receptors, diluting medullary tonicity [11, 60]. Constrictor actions of AVP on medullary circulation facilitate direct antidiuretic effects of this peptide [11]. Excess water in medullary interstitium should be removed by ascending vasa recta. AVP binded to V1 receptors on AAs and EAs of juxtamedullary nephrons, and constricted these arterioles at lower doses than those of superficial nephrons [6, 61]. DVR possessed V1 receptors and AVP constricted DVR [62]. AVP-induced decreases in DVR blood flow allow ascending vasa recta to take more water out of medullary interstitium. Conversely, any increases in DVR blood flow give isotonic fluid to medullary interstitium, washing out medullary hypertonicity. Of importance, pressure-induced natriuresis was intact in animals with diabetes inspidus [63]. While chronic administration of AVP into medullary interstitium failed to alter papillary blood flow and BP, the infusion of V1 agonist decreased papillary blood flow and increased BP [50]. Stimulation of V2 receptors with DDAVP relaxed DVR preconstricted by V1 agonist [62].

AngII regulates papillary circulation. AngII constricted both AAs and EAs of juxtamedullary nephrons [6]. Pericytes of DVR possess AT1 receptors [64]. AngII opens Cl channels and depolarizes DVR pericytes, constricting DVR by activating L-type VDCCs [65, 66]. In general, AngII flattens the slope of the pressure-natriuresis relationship, making the animal salt-sensitive [67]. AngII blockade sharpens the pressure-natriuresis curve in physiological conditions [67, 68]. Furthermore, intrarenal administration of AngII decreases papillary plasma flow and sodium excretion without changes in total GFR and renal plasma plow [69]. AngII seems to be involved in reduced papillary blood flow in hypertension models. Medullary administration of captopril lowered BP in SHR [70]. Chronic medullary infusion of candesartan increases papillary blood flow and decreases BP in SHR, but not Wistar-Kyoto rat (WKY) [71]. Hypertension in obesity manifests the resetting of pressure natriuresis, possibly due to histological changes in renal medulla that may activate the renin-angiotensin system [72].

NO manifests a great influence on medullary circulation. NO synthesis inhibition constricts AAs and EAs of blood-perfused juxtamedullary nephrons, suggesting that flow-induced NO has tonic vasodilatory effects on arterioles [6]. NO also counteracts AngII-induced constriction of DVR, juxtamedullary AAs

and EAs [73, 74]. NOS is rich in vasa recta and medullary collecting duct, and blood in DVR shows lower hematocrit than arteriole [11]. NO activity is higher in medulla than cortex [73]. Intravenous administration of nitro-*L*-arginine flattened the pressure-natriuresis curve of WKY but not SHR [75]. Systemic NO synthesis inhibition also increased renal AngII content [68]. Local inhibition of medullary NO synthesis decreases papillary blood flow, blunts pressure natriuresis, and elevates BP [50]. Conversely, administration of *L*-arginine into renal interstitium increases papillary blood flow and ameliorates pressure natriuresis in the DS rat and SHR [46, 47]. Furthermore, the obese Zucker rat shows diminished pressure natriuresis compared to the lean Zucker rat [76]. The medullary level of NO in the obese Zucker rat does not increase in response to elevation of BP. Troglitazone partially corrects blunted pressure natriuresis in the obese Zucker rat, suggesting that insulin resistance is involved [76].

PGs also regulate papillary blood flow. COX inhibition decreases papillary blood flow, increasing the risk of papillary necrosis [11]. COX inhibitor may increase BP [77]. Medulla is rich in PGs, and PGE_2 is higher in medullary interstitium than cortex [73]. Interstitial cell (COX-1 and 2), loop of Henle (COX-2) and collecting duct (COX-1) are the sites of PGE_2 production [78]. COX inhibitor constricted juxtamedullary AAs but not EAs, suggesting tonic vasodilatory effects of PGE_2 [12, 73]. PGE_2 counteracted DVR constriction by AngII [65]. PGE_2 reduces NaCl reabsorption in loop of Henle and collecting duct. Elevation of interstitial pressure increases PGE_2 excretion and facilitates pressure natriuresis [51]. Inhibition of COX blunted the slope of the pressure-natriuresis curve [67]. Compared to the Dahl salt-resistant (DR) rat, the DS rat manifested both decrease in PGE_2 excretion and blunted pressure natriuresis [79]. NaCl reabsorption in loop of Henle was increased in the DS rat [80]. The difference in pressure natriuresis between DS and DR rats disappeared with intravenous infusion of indomethacin, which flattened the pressure-natriuresis curve of the DR rat but not DS rat.

Myogenic Response

Pressure-induced (myogenic) constriction is seen in various vascular beds, including renal, cerebral and skeletal muscle arteries [81]. Increasing pressure depolarizes vascular myocytes, underlying myogenic constriction [81, 82]. In the kidney, myogenic constriction is seen in preglomerular (AA, interlobular and arcuate arteries), but not postglomerular vessels (EA). Since vascular resistance is inversely related to its diameter, AA plays the most important role in autoregulatory adjustments of renal vascular resistance. Furthermore,

pressure-induced constriction is observed in renal vasculature without endothelium, so vascular myocytes work as both sensor and effecter to pressure [81].

There is a consensus that Ca entry through L-type VDCCs underlies AA myogenic constriction [6]. However, ionic mechanisms mediating membrane depolarization during AA myogenic constriction are not well defined. The blocker to 20-hydroxyeicosatetraenoic acid (20-HETE) synthesis, 17-octadecynoic acid, abolished renal autoregulation, and 20-HETE closes Ca-activated K channels [83]. However, AA myogenic constriction was preserved in the presence of either tetraethylammonium (Ca-activated K channel blocker), barium (rectifying K channel inhibitor), glibenclamide (ATP-sensitive K channel blocker) or 4-aminopyridine (voltage-gated K channel inhibitor) [84–87]. In addition, Cl channels play small role in AA myogenic constriction, because of the lack of effects of IAA-94 [88]. Mechanosensitive cation channels conduct Na and Ca, and 'translate' mechanical signals to electrical ones [89]. These channels may underlie myogenic response, because both gadolinium and streptomycin, inhibitors of mechanosensitive cation channels, arrested AA myogenic constriction [89]. Interestingly, recent studies have demonstrated that transient receptor potential (TRP) channels, which conduct Na and Ca, regulated myogenic tone [90], and were expressed on AA myocytes [91]. However, genes encoding mechanosensitive channels have not been cloned yet. AA myogenic responsiveness is modulated by autacoids. A subconstrictor dose of AngII or endothelin potentiated AA myogenic constriction [15, 86, 92]. While inhibition of PG synthesis did not alter AA myogenic constriction [93], dibutyl cyclic AMP (cAMP), that mimics PG actions, inhibited AA myogenic constriction [unpubl. observations]. Inhibition of endogenous NO synthesis potentiated AA myogenic constriction [93], and 8-bromo-cyclic GMP (cGMP), which imitates NO effects, attenuated AA myogenic constriction [94]. AA myogenic responsiveness is altered in various pathological conditions, and contributes to pathogenesis of diseases. Elevations of renal arterial pressure from 110 to 151 mm Hg did not constrict AAs in renovascular (2K1C) hypertension [95]. In SHR, threshold of renal arterial pressure to induce AA myogenic constriction was shifted to higher pressure (120 mm Hg) than WKY (100 mm Hg), consistent with that autoregulation range is shifted to higher pressure in SHR [96]. However, maximal AA myogenic responses are similar between WKY and SHR, compatible with the findings that GCP of SHR is comparable to WKY. Furthermore, in the DS rat, threshold pressure to induce AA constriction was also shifted to higher pressure (120 mm Hg) than the DR rat (100 mm Hg). The DS rat manifested decrements in maximal AA myogenic constriction compared to the DR rat, consistent with that autoregulation of GFR is deranged in the DS rat [79, 97]. In the DS rat, 30 mM KCl elicited smaller AA constriction than the DR rat, suggesting that a given membrane depolarization induces smaller

arteriolar constriction due to alterations in Ca pump in the DS rat. In addition, obese Zucker rats showed higher threshold pressure (120 mm Hg) to induce AA myogenic constriction than lean Zucker rats (100 mm Hg). Impaired myogenic AA responsiveness at 180 mm Hg was seen in the obese Zucker rat [31]. These autoregulatory abnormalities were restored by troglitazone, an insulin resistance improver, in the obese Zucker rat.

Interlobular artery (ILA) also exhibits myogenic constriction. Since intravascular pressure in the middle of ILA was at least partly autoregulated [98], ILA considerably participates in autoregulation of cortical nephrons. Similarly to AA, L-type VDCC inhibition blocked myogenic constriction of all segments in ILA [99]. However, pressure elevations constrict large ILA (close to main renal artery) via differing mechanisms from small ILA (close to glomerulus). Myogenic constriction of small ILA was inhibited by gadolinium. Thus, mechanosensitive cation channels on small ILA are opened by pressure, thereby eliciting membrane depolarization and allowing Ca entry through L-type VDCCs [99]. In contrast, myogenic constriction of large ILA involves pressure-induced stimulation of phospholipase C, similarly to arcuate artery [81]. Like cerebral artery [82], myogenic constriction of large ILA was blocked by IAA-94, a Cl channel blocker [unpubl. observations], suggesting that Cl channels may underlie pressure-induced depolarization of large ILA. Altered myogenic responsiveness of ILA also contributes to pathogenesis of diseases. In contrast to AA, small ILA of SHR exhibited stronger myogenic responsiveness than WKY [100]. This enhanced myogenic constriction of ILA prevents the transmission of systemic BP to AA and then glomerulus. Blunted transmission of systemic pressure results in both the changes in threshold pressure for AA to induce myogenic constriction toward higher pressure and rightward shift of the entire autoregulatory curve (descending autoregulation). In the DS rat, ILA exhibited diminished myogenic responsiveness, similarly to AA [97]. This is consistent with the findings that GCP is higher in the DS rat than DR rat, thereby maintaining whole kidney GFR similar to the DR rat, in spite of a smaller number of glomeruli in the DS rat [101].

Tubuloglomerular Feedback

TGF is kidney-specific mechanism which strongly regulates GCP. MD initiates TGF, and the effecter of TGF is the AA. TGF accounts for about half of autoregulatory adjustments of renal vascular resistance [27]. TGF may exert a stronger influence on juxtamedullary nephron [102], compensating the lack in contribution of ILA to autoregulation. Since TGF constricts terminal part of AA, it increases upstream pressure (ascending autoregulation), potentiating

myogenic constriction, and influencing autoregulatory capacity of adjacent nephrons [103, 104]. An increase in either NaCl concentration or tubular flow at the level of MD augments the reabsorption through Na-2Cl-K co-transporter, and subsequently elicits Ca mobilization in MD cells, allowing the release of chemical mediator [6, 105]. There are at least three candidates for mediator of TGF: adenosine, ATP and 20-HETE. In A1 receptor knockout mice, TGF was completely abolished [106]. Elevation of renal arterial pressure increased renal interstitial ATP concentration, and the saturation of purinergic receptors with ATP inhibited TGF responses [6, 107]. MD cells release ATP through maxianion channels [108]. ATP can be cleaved extracellularly to adenosine by ectonucleotidases [106]. ATP and/or adenosine reach extraglomerular mesangial (Groomagtigh) cells first and then transmit TGF signals to AA [109]. The mediator depolarizes Groomagtigh cells by opening Cl and/or non-selective cation channels [110]. This membrane depolarization could transmit via gap junctions (at most 3–4 cells) to adjacent mesangial cells and then to AA [103]. In general, AAs are more sensitive to electrophysiological stimulus than EAs [6, 17]. ATP and/or adenosine directly constrict AAs by activating L-type VDCCs [6]. The mediator may also prepare AA ryanodine receptor to induce tubular flow oscillation [111, 112]. However, ionic mechanisms mediating AA membrane depolarization by TGF are open question to be addressed. Because peritubular capillary infusion of IAA-94 inhibited AngII-induced decrease in GCP but not TGF-induced ones [113], Cl channels are unlikely to be involved. Rectifying K channels, which are opened by mild hyperkalemia and inhibited by barium, may buffer TGF-induced AA depolarization [85, 87]. The TGF response was attenuated during acute hyperkalemia [114], and barium enhanced the TGF response [115]. Alternatively, an inhibitor of 20-HETE production, 17-octadecynoic acid, blocked TGF response [116]. Hydroxylase of cytochrome P-450 produces 20-HETE in the thick ascending loop of Henle. It depolarizes the arcuate artery by closing Ca-activated K channels, activating L-type VDCCs [83]. In any case, Ca influx through L-type VDCCs mediates TGF-induced AA constriction [6].

Various autacoids modulate TGF. First, AngII is a strong positive modulator of TGF. About half of AngII-induced AA constriction comes from enhancement of TGF [117]. AngII-induced increase in thromboxane participates in TGF augmentation [118, 119]. Sites of actions of AngII in enhancing TGF are not well defined. AngII stimulates both luminal Na-2Cl-K co-transporter and basolateral Na/H exchange at MD [120]. AngII also depolarizes mesangial cells by gating Cl channels, possibly facilitating the transduction of TGF signal [121]. Direct effects of AngII on AA in enhancing TGF are also proposed [122]. Mechanisms mediating AngII-induced glomerular microcirculatory actions merit comments. AngII elicits membrane depolarization in AAs, but not EAs [123].

In AAs, AngII binds to AT1 receptors, and stimulates phospholipase C possibly via Gq proteins, elicits inositol trisphosphate-induced Ca release (IICR) which increases open probability of Ca-activated Cl channels, thereby depolarizing membrane and inducing Ca influx through L-type VDCCs [124, 125]. In addition, AngII closes rectifying K channels in juxtaglomerular cell, allowing membrane depolarization [126]. In EAs, AngII causes IICR and Ca influx [125]. The diacylglycerol pathway activates Ca influx through TRP channels, voltage-independent receptor-operated Ca channels, in EAs upon AT1 receptor stimulation [91]. AngII-induced IICR also gates T-type VDCCs in EAs [127, 128]. AngII also constricts mesangial cells, lowering ultrafiltration coefficient [129]. AngII plays some role in the pathogenesis of hypertension. SHR shows a parallel shift of the pressure-natriuresis relationship toward higher pressure (table 1), and this abnormality is attributable to increased preglomerular resistance [130]. Of interest, enhanced TGF may account for an increased renal vascular resistance of SHR [131]. Intrarenal angiotensin-converting enzyme inhibition shifted the pressure-natriuresis curve to lower pressure in SHR [132], and AT1 receptor blockade corrects the enhanced TGF in this strain [133].

Secondly, NO attenuates AA constriction by TGF. NO is produced in the juxtaglomerular apparatus by endothelial NOS (arteriole) and nNOS (MD). Inhibition of nNOS enhances TGF-induced AA constriction [57], and nitroprusside attenuates pressure-induced constriction of AA close to glomerulus [94]. NO attenuates AngII-induced enhancement of TGF [134]. NO antagonizes constrictor actions of AngII or norepinephrine on AAs and EAs [73, 135]. NO stimulates guanylate cyclase with utilization of cGMP as second messenger, and appears to work exclusively through protein kinase G in AAs [136]. cGMP activates protein kinase G, which increases open probability of Ca-activated K channels, hyperpolarizing membrane and then inactivates L-type VDCCs [87]. Protein kinase G may also gate ATP-sensitive K channels. cGMP facilitates Ca reuptake into microsome, decreasing $[Ca]_i$ [135]. Because AA constriction by high KCl is relatively resistant to NO [136], normal K equilibrium and electrochemical potentials are required for vasodilatory effects of NO. Thus, NO would activate protein kinase G via cGMP, thereby opening K channels on AAs and inhibit TGF-induced AA constriction.

Thirdly, PGE_2 inhibits TGF. PGE_2 is produced in MD (COX-2) and mesangial cells (COX-1). Effects of PGE_2 on AA constriction by TGF interact with NO [57]. COX inhibition may decrease GFR [77]. RT-PCR study indicates that EP3 and EP4, but not EP1 or EP2 receptors are expressed in AAs [137]. Vasodilatory actions of PGE_2 on AAs are mediated by EP4 receptors, and PGE_2 dilates AAs at least in part through adenylate cyclase-cAMP pathway. Indeed, forskolin and dopamine attenuate TGF [108, 138]. cAMP facilitates

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Role of Renal Eicosanoids in the Control of Intraglomerular and Systemic Blood Pressure during Development of Hypertension

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Studies suggest that glomerular hemodynamics is critically involved not only in the pathogenesis of hypertension, but also in the mode of progression of renal dysfunction [1]. The juxtaglomerular apparatus (JGA), consisting of the glomerular afferent and efferent arterioles as well as the specialized tubular epithelial cells called macula densa, plays a critical role in the regulation of glomerular hemodynamics and renin release [2]. Kimura and Brenner [3] proposed three major renal mechanisms leading to the development of hypertension: an increased pre-glomerular vascular resistance, a decreased whole kidney ultrafiltration and an increased tubular sodium reabsorption. They suggest that preglomerular vasoconstriction causes salt-resistant hypertension, whereas a reduced renal mass and alterations of renal sodium handling result in the development of salt-sensitive forms of hypertension. They also suggest that salt sensitivity is associated with an increased intraglomerular pressure, and hence a higher risk of developing glomerulosclerosis and chronic renal failure. This article reviews the mechanism by which the JGA regulates glomerular capillary pressure (P_{GC}), as well as its alterations observed in various conditions associates with systemic hypertension. We paid a particular attention to the role of renal eicosanoids (arachidonic acid metabolites, fig. 1), which play important roles in the control of renal vascular resistance and renal sodium handling [4], in the control of P_{GC} during development of hypertension. The reader is referred to recent reviews concerning the contribution of other factors to the control of P_{GC} during development of hypertension [5] or detail vascular actions of eicosanoids on renal circulation [4, 6].
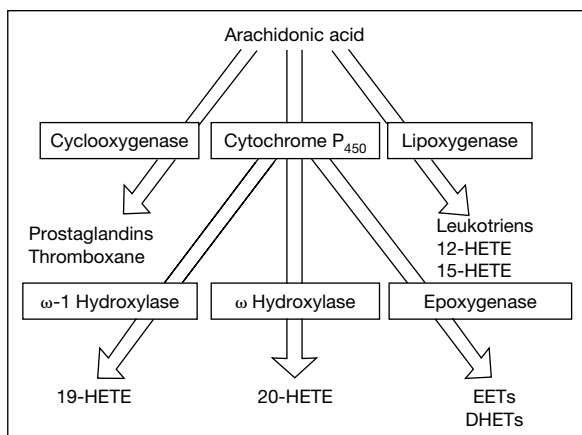
Fig. 1. Three major pathways of eicosanoids formation are described: (1) cyclooxygenase, (2) lipoxygenase and (3) cytochrome P_{450} monooxygenase. Cytochrome P_{450}-dependent metabolites were described as structural formula. The 20- and 19-hydroxyeicosatetraenoic acid (HETE) are formed by ω and (ω-1)-hydroxylation. Epoxidation results in the formation of four epoxyeicosatrienoic acids (EETs), which can be enzymatically hydrolyzed by epoxide hydrolase to the corresponding dihydroxyeicosatrienoic acid (DHETs).

P_{GC} and Clinical Pictures

There is substantial evidence that P_{GC} greatly influences the rate of the progression of renal dysfunction. The P_{GC} is found to be normal in non-salt-sensitive (salt-resistant) essential hypertension, whereas it is elevated in such cases as diabetes mellitus, various renal diseases and salt-sensitive (essential) hypertension.

Micropuncture studies in the spontaneously hypertensive rats (SHR), a model of human essential hypertension, and its normotensive control Wistar-Kyoto rats (WKY) have revealed that despite a significant difference in systemic blood pressure and renal vascular resistance, P_{GC} is the same in both strains [7]. This demonstrates that in SHR, renal vascular resistance is high due to a strong constriction of the preglomerular vessels. Calculation of P_{GC} according to Gomez's equation suggests that in human essential hypertension P_{GC} is also within the normal range with increased preglomerular resistance, whereas postglomerular efferent arteriolar resistance remains essentially unchanged [8]. Among various preglomerular vascular segments, the afferent arteriole is likely to be the major contributor to the elevated vascular resistance. Such functional alterations are reflected in the histology of human kidneys. Because of the strong constriction of afferent arterioles (and distal segment of interlobular

arteries), glomeruli are protected from systemic hypertension, thereby maintaining a relatively normal architecture. In contrast, afferent arterioles and interlobular arteries show hypertrophy and sclerotic changes due to a long-lasting pressure overload. The increased afferent arteriolar resistance with normal P_{GC} is also reflected in clinical pictures. Namely, hypertension should precede renal dysfunction and proteinuria. There would be less proteinuria and the progression of renal dysfunction would be slow because of the normal P_{GC}. However, progressive luminal narrowing of afferent arterioles and a subsequent fall in glomerular blood flow would induce glomerular ischemia, ultimately leading to the renal failure. Alternatively, long-lasting pressure overload can damage the autoregulatory vasoconstrictor behavior of afferent arterioles (see below), which permit the direct transmission of elevated systemic pressure to glomeruli and facilitate glomerular hypertension leading to a glomerular structural injury and a progressive loss of renal function. Although elevated preglomerular vascular resistance protects the glomerulus from systemic hypertension, this compensation impairs the ability of the kidney to excrete salt and water, and contributes to the inappropriate retention of extracellular fluid and the development of salt-sensitive hypertension. Thus, elevated preglomerular vascular resistance also contributes to the development of salt-sensitive hypertension [9, 10].

The strong constriction of afferent arterioles may be important in the pathogenesis of hypertension. Norrelund et al. [11] measured afferent arteriolar diameter in one kidney removed at the age of 7 week from F_2 generation of SHR and WKY, and kept these uninephrectomized rats until the age of 23 weeks. They found that rats which had smaller afferent arteriolar diameter at 7 weeks developed hypertension, whereas those with larger diameter remained normotensive. In addition, these authors have shown that when the young rats were given antihypertensive drugs, treatments associated with increased afferent arteriolar diameter resulted in lower systemic blood pressure in adulthood even after the cessation of the therapy [12]. These results suggest that exaggerated afferent arteriolar constriction may be essential for future development of hypertension.

Substantial differences in renal hemodynamic adaptation to a high salt intake are evident between salt-sensitive and salt-resistant hypertensive patients [13]. During a low salt diet both salt-sensitive and salt-resistant patients have similar mean arterial pressure, glomerular filtration rate (GFR), effective renal blood flow (RBF) and filtration fraction (FF). On the other hand, during a high salt intake effective RBF increases in salt-resistant but decreases in salt-sensitive patients without change in GFR in either group; FF and P_{GC} decrease in salt-resistant but increase in salt-sensitive patients. Salt-sensitive hypertension is characterized by an inability of the kidney to excrete unnecessary amounts of sodium loaded into the body. This is likely due to either a decreased ultrafiltration coefficient or increased tubular reabsorption. When dietary sodium intake

is increased under such abnormalities, body fluid volume, and hence systemic blood pressure increase, leading to an elevated P_{GC} and therefore an increase in the GFR. With such increased GFR, more sodium is loaded to the tubules to maintain sodium balance. Thus, in salt-sensitive hypertension, glomerular hypertension is a common feature regardless of the cause of hypertension, such as diabetic nephropathy, primary aldosteronism, chronic glomerulonephritis and essential hypertension in black populations [14]. Regardless of initial insults, however, glomerular hypertension causes endothelial, mesangial and podocyte injuries, which ultimately results in glomerulosclerosis [15]. This decreases the number of functioning nephrons and further elevates P_{GC}, thereby resulting in a vicious cycle. Thus, clinical pictures of glomerular hypertension would be the presence of more proteinuria from an early stage, faster decline in renal function, and glomerulosclerosis but not arteriosclerosis seen in essential hypertension. Glomerular hemodynamics is important in the pathophysiology of hypertension, particularly it greatly influences the mode of progression of renal dysfunction. It is therefore important to understand first the basic mechanisms that regulate glomerular hemodynamics, second alterations and possible mechanisms that lead to progression of renal dysfunction.

Mechanisms that Control the Glomerular Hemodynamics

Four mechanisms operate at the JGA to control the P_{GC}. Namely, sympathetic nervous system, myogenic response, macula densa-mediated tubuloglomerular feedback (TGF) and various local hormones. Among these, two intrinsic mechanisms, the myogenic response and TGF, operate mainly the afferent arteriole. In order to study each of these mechanisms in detail, we have developed preparations of isolated microperfused afferent arteriole alone, or together with the macula densa. We have found that increasing luminal pressure of the afferent arteriole caused constriction at the proximal segment (myogenic response) [16], while increasing NaCl concentration at the macula densa caused constriction at the terminal segment of the afferent arteriole (TGF) [17]. Indeed, when the Cl^- concentration near the macula densa and the proximal tubular pressure (an index of single nephron GFR) were measured simultaneously in the same nephron, it was found that they oscillate in a synchronous fashion at a rate of 2 times per minute, and that any small changes in Cl^- concentration are immediately followed by changes in pressure in an opposite direction [18]. This is most likely due to a fine tuning of afferent arteriolar resistance at the distal end. Thus, the myogenic response and the TGF exist in series along the afferent arteriole. The myogenic response is the first to respond to changes in systemic pressure, and any changes that cannot be prevented by the myogenic response are now well

compensated by the TGF in a single cycle. This perhaps is the reason why the kidney exhibits very efficient autoregulation over a wide range of systemic blood pressure.

On the other hand, efferent arteriole does not exhibit myogenic response, and there are uncertainties about the role of macula densa in the regulation of efferent arteriolar resistance, although Ren et al. [19] recently provided some data suggesting it. Perhaps important mechanisms are autocoids, among which angiotensin II (Ang II) is well known and studied extensively. When we studied the effect of Ang II in isolated afferent and efferent arterioles, we found stronger constriction in the efferent than in the afferent arteriole [20]. The higher sensitivity of efferent arteriole to Ang II may be due, at least in part, to the lack of modulation by nitric oxide (NO), since L-NAME (an inhibitor of NO synthase; NOS), enhanced Ang II action only in the afferent arteriole. Thus, the P_{GC} is controlled very precisely by well-balanced constriction and dilation of the afferent and efferent arterioles.

Mechanisms for Altered Glomerular Hemodynamics in Hypertension

Salt-Resistant Hypertension

Consistent with micropuncture studies in SHR, the estimated P_{GC} is within the normal range in human non-salt-sensitive hypertension [7]. It has been reported that during the developmental phase of hypertension in SHR, both the myogenic response and the TGF are exaggerated, contributing to the elevated afferent arteriolar resistance [21–23]. The mechanism of exaggerated myogenic response is not clear at present. However, cytochrome P-450 (CYP-450)-dependent metabolites of arachidonic acids may be involved. Imig et al. [24] have demonstrated that 20-HETE (CYP-450 ω-hydroxylase metabolite) participates in the myogenic response-induced afferent arteriolar vasoconstriction, whereas EETs (CYP-450 epoxygenase metabolite) attenuate it. Interestingly, renal production of 20-HETE or EETs is elevated or reduced, respectively, during the development of hypertension in SHR [25]. Thus, it is possible that altered CYP-450-dependent metabolism of arachidonic acids may play a role in the exaggerated myogenic response in SHR during development of hypertension.

On the other hand, Ang II, neuronal NOS and oxidative stress at the macula densa have been implicated in the exaggerated TGF response in SHR [22, 23, 26]. In addition to these factors, Brannstrom and Arendshorst [27] have demonstrated the involvement of thromboxane A_2 (TxA_2), which is increased in SHR kidney [28, 29], in the enhanced TGF activity in young (7-week-old) SHR. They found that synthesis inhibition or receptor blockade of TxA_2 attenuates

the enhanced TGF activity close to that observed in normal rats. Thus, increased endogenous TxA_2 is likely to contribute to the enhanced TGF activity in young SHR, which may lead to a rightward shift in the pressure-natriuresis curve, promoting the development of hypertension. It may also be possible that TxA_2 mediates Ang II-induced enhancement of TGF activity, since Ang II stimulates the renal production of TxA_2 [30, 31].

In addition to such alterations in intrinsic mechanisms, renal vascular response to Ang II is known to be exaggerated during the development of hypertension in SHR [32, 33]. Such exaggerated responses to Ang II may be responsible, at least in part, for the elevated preglomerular vascular resistance in young SHR [34, 35]. We have demonstrated that vasoconstrictor action of Ang II is exaggerated in SHR afferent arterioles before development of hypertension (4- to 5-week-old) compared with age-matched WKY [36]. It is unlikely that increased AT_1 receptor levels can be responsible for the exaggerated afferent arteriolar Ang II responsiveness because upregulation of renal vascular AT_1 receptors was not observed in SHR [37]. Instead, exaggerated Ang II reactivity in SHR has been attributed to an impaired buffering capacity of prostaglandins (PGs) associated with a decreased cAMP production [38, 39]. Arendshorst et al. [38, 39] have found that cyclooxygenase inhibition with indomethacin abolished the strain differences in Ang II action between SHR and WKY, and suggested an involvement of impaired buffering effect of vasodilator PGs. On the other hand, we have previously demonstrated an impaired function of the AT_2 receptor in SHR afferent arterioles before the development of hypertension [36]. Since activation of AT_2 receptor in afferent arterioles causes endothelium-dependent vasodilation by stimulating the release of EETs and modulates the vasoconstrictor actions of Ang II mediated by AT_1 receptor [40], impaired function of AT_2 receptor would account, at least in part, for the difference in Ang II action between SHR and WKY afferent arterioles [36]. In addition, we have also suggested a possibility that in the afferent arterioles activation of AT_1 receptor stimulates the production of 20-HETE, which in turn mediates the vasoconstrictor action of Ang II on this vascular segment [41]. Thus, increased renal production of 20-HETE (during development of hypertension) may also responsible for the exaggerated vasoconstrictor action of Ang II in SHR afferent arterioles. Alternatively, since 20-HETE causes direct vasoconstriction in afferent arterioles [42], increased renal production of 20-HETE itself may participate in the elevated vascular resistance of afferent arterioles in essential hypertension.

Possible involvement of isoprostanes, which are generated by peroxidation of arachidonic acids by oxygen radicals [43], is also suggested. It has been reported that plasma levels of isoprostanes are increased in SHR kidney [44] and that isoprostanes decrease RBF and GFR by preferentially constricting the preglomerular vasculature [45]. Taken together, isoprostanes may contribute to

the pathogenesis of elevated preglomerular vascular resistance in SHR, however, precise vascular actions of isoprostanes on the glomerular hemodynamics in the hypertensive states are to be defined.

Salt-Sensitive Hypertension

The glomerular hypertension seen in diabetes and renal diseases can be due to either decreased afferent arteriolar resistance or increased efferent arteriolar resistance, or both. In diabetes, the pathogenesis of glomerular hemodynamic abnormalities that cause glomerular hypertension is multifactorial. Elevated efferent arteriolar resistance may be due to an increased intrarenal renin-angiotensin system or decreased vasodilator substances such as NO or PGs. On the other hand, impaired calcium/potassium channels in vascular smooth muscle cells, several humoral factors (such as atrial natriuretic peptide, NO or insulin) and attenuated intrinsic mechanisms (myogenic response and TGF) are thought to be involved in the decreased afferent arteriolar resistance [46]. Among them, Hayashi et al. [47] demonstrated an involvement of vasodilator PGs in the pathogenesis of attenuated myogenic responses. Using the isolated perfused hydronephrotic kidney technique, they found that inhibition of PG synthesis normalized the (attenuated) myogenic response of diabetic afferent arterioles. The myogenic response is also attenuated in Dahl salt-sensitive hypertensive rats and Fawn-hooded rats, permitting transmission of systemic blood pressure to the glomerulus and thereby causing glomerular hypertension.

Several hormonal and autocrine systems (including the renin-angiotensin and PG systems) have been involved in the pathophysiology of salt sensitivity. Suppression of the renin-angiotensin system is one important mechanism for both the immediate and long-term increase in sodium excretion following the increased sodium intake. Hall et al. [48] have demonstrated in the dog that when the activity of the renin-angiotensin system cannot be modulated, blood pressure becomes salt-dependent. In accordance with this finding, several studies have reported that plasma renin activity is inappropriately suppressed in patients whose blood pressure increases on a high sodium diet, and that the blunted renin response correlates with a diminished salt-induced renal vasodilation. In patients with impaired renal function, the rate of sodium excretion per nephron increases, as evidenced by an exaggerated fractional sodium excretion [49, 50]. In addition, the fractional sodium excretion was found to correlate positively with the rate of urinary excretion of PGE_2, a major cyclooxygenase metabolite of arachidonic acids in the kidney, in patients with chronic glomerulonephritis. This finding suggests that the renal PG system plays an important role in the control of excretory functions of residual nephrons [49, 50]. This notion is supported by the study of Kennedy et al. [51] demonstrating a development of salt-sensitive hypertension in mice with targeted disruption of the EP_2 receptor, which mediate vasodilator

actions of PGE_2. Thus, it is thought that PGE_2 facilitates the ability of the kidney to increase sodium excretion, thereby protecting systemic blood pressure from a high-salt diet, and that impaired function of PG system (especially PGE_2) contributes to the development of salt-sensitive hypertension. However, it is not completely understood how PG system is involved in the salt sensitivity of blood pressure. It has been reported that by dilating descending vasa recta (which increases medullary blood flow), PGE_2 may enhance the sodium excretion, thereby protecting systemic blood pressure from a high-salt diet [52].

It may be also possible that sodium depletion increases the renal level of PGs, which in turn affects glomerular hemodynamics and tubular functions, resulting in reduced systemic blood pressure. It is interesting to note that in salt-sensitive hypertension in humans and animals, RBF does decrease upon salt loading, which may contribute to salt-retention and development of hypertension [13, 14]. Such decreases in RBF are associated with increases in P_{GC}, which are attributed to increased efferent arteriolar resistance. In order to define the role of PGs in the control of glomerular microcirculation, we developed in vitro preparations in which isolated efferent arterioles are perfused either from their distal end (retrograde perfusion) or from the end of afferent arterioles through the glomerulus (orthograde perfusion) [53]. Since the efferent arteriolar perfusate passes through the glomerulus only in orthograde perfusion, vasoactive substances released by the glomerulus could modulate vascular reactivities in the downstream efferent arteriole. We found both Ang II and norepinephrine (NE) caused much weaker constriction of the efferent arteriole in orthograde than in retrograde perfusion, while inhibition of PG synthesis with indomethacin augmented the vasoconstriction only in orthograde perfusion (fig. 2). These results suggest that the glomerulus may control its own capillary pressure (and hence the rate of ultrafiltration) by releasing PGs and thereby adjusting the resistance of the downstream efferent arterioles. Thus, it is speculated that sodium depletion may increase glomerular synthesis of PGs, which in turn dilate the efferent arterioles. Thus, in the setting of salt-sensitive hypertension, Ang II- or NE-induced efferent arteriolar constriction may become stronger because of an impaired buffering effect of PG together with an inappropriate suppression of renal Ang II and an activated sympathetic nervous system [13].

Although specific PG involved in the control of efferent arteriolar resistance is not clear from our results, we speculate that it may be PGI_2 because PGI_2 but not PGE_2 exerts dilator effect on the efferent arteriole [4]. On the other hand, Campese [13] proposed another possibility based on the findings that both PGE_2 and PGI_2 antagonize the vasoconstrictor actions of NE and Ang II on preglomerular afferent arterioles, whereas only PGI_2 but not PGE_2 was effective on the postglomerular efferent arterioles [4]. An increase in intracellular calcium concentration ($[Ca^{2+}]_i$) stimulates PGE_2 but not PGI_2 release, while changes in
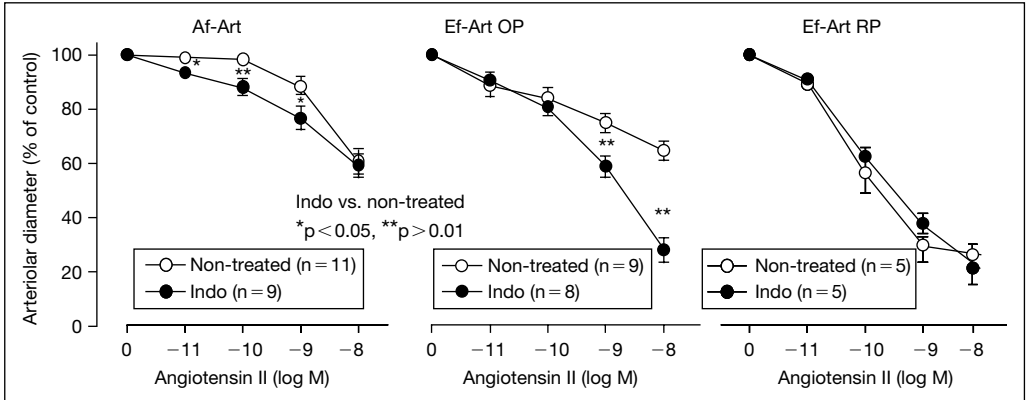
Fig. 2. Effect of indomethacin (Indo, a cyclooxygenase inhibitor, $50 \, \mu M$) pretreatment on angiotensin II-induced constriction in afferent (Af-Art) or efferent arterioles with orthograde (Ef-Art OP) or retrograde perfusion (Ef-Art RP). Note that pretreatment with indomethacin significantly augmented angiotensin II-induced vasoconstriction in efferent arterioles with orthograde but not retrograde perfusion.

extracellular calcium ion in vivo may selectively alter renal PGI_2 synthesis [54]. This raises the possibility that a high dietary NaCl intake may increase $[Ca^{2+}]_i$ in renal glomerular arterioles which in turn may cause a greater increase in PGE_2 versus PGI_2. Thus, he proposed the possibility that such imbalance in the production of these two PGs would lead to a relative decrease of preglomerular compared with postglomerular vascular resistance and to an increase in FF and P_{GC}. These hypotheses remain to be clarified.

Badr et al. [55] have reported that inhibition of leukotriene receptors decreases P_{GC} in rats. It is well known that lipoxygenase metabolites are primarily produced in response to inflammation and injury, and that they have effects on renal hemodynamics in inflammatory diseases and glomerulonephritis [4]. Thus, in addition to PG system, lipoxygenase metabolites may also be involved in the pathogenesis of glomerular hypertension in salt-sensitive hypertension observed in such renal diseases. However, further studies examining the mechanism for leukotriene-induced glomerular hypertension under disease states are clearly required.

Conclusion

In conclusion, the glomerular hemodynamics seems to be very important not only for the pathogenesis of hypertension but also the progression of renal

dysfunction in various renal diseases. In non-salt-sensitive essential hypertension, P_{GC} is normal with a significant constriction of preglomerular arterioles, whereas in various renal diseases P_{GC} is elevated. There is substantial evidence that glomerular hypertension plays an important role in the pathogenesis glomerulosclerosis, thereby contributing to the progressive nature of the decline in renal function in various renal diseases. Since transmission of systemic blood pressure to the glomerulus is facilitated, strict control of systemic blood pressure would be critical in order to prevent the progression of renal dysfunction. In addition, measure to improve autoregulation (low protein diet) and/or dilate efferent arteriole (inhibition of the renin-angiotensin system) would be important. Thus, understanding the mechanism that regulates glomerular hemodynamics as well as its alterations under various pathological conditions would be important not only for the basic or clinical research but also for a good management of patients with hypertension and/or renal diseases. In addition, as mentioned in this review, since alterations of renal PG system plays an important role in the pathophysiology of various forms of hypertension, attention should be paid when non-steroidal anti-inflammatory drugs (NSAIDs) are used in (salt-sensitive) hypertensive patients.

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Novel Aspects of the Renal Renin-Angiotensin System: Angiotensin-(1–7), ACE2 and Blood Pressure Regulation

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Continual discoveries over the past several years have broadened understanding of the functional characteristics of the renin-angiotensin system (RAS) through identification of novel biochemical components with diverse functional actions. Particularly within the kidney these emerging members of the RAS are best characterized at this point in time. As depicted in figure 1, the conventional representation of the RAS cascade begins with the formation of angiotensin I from the protein precursor angiotensinogen by renin and the subsequent conversion to Ang II by ACE. The angiotensin subtype 1 (AT_1) receptor specifically binds Ang II and mediates the majority of the actions of the peptide within the kidney including vasoconstriction, sodium retention, cellular injury and hypertrophy [1, 2]. However, identification of additional subtype angiotensin receptors (AT_2, $AT_{(1–7)}$) and elucidation of processing enzymes forming Ang-(1–7) has demonstrated that this arm of the RAS serves to balance or mitigate the cellular actions of the Ang II-AT_1 axis within the kidney [3–6], specifically and the whole organism in general. In this chapter, we review the current knowledge for a role of Ang-(1-7) in the regulation of renal function.

ACE and ACE2

The discovery of ACE was a pivotal achievement in the elucidation of the role of the RAS in the regulation of blood pressure, as well as providing a
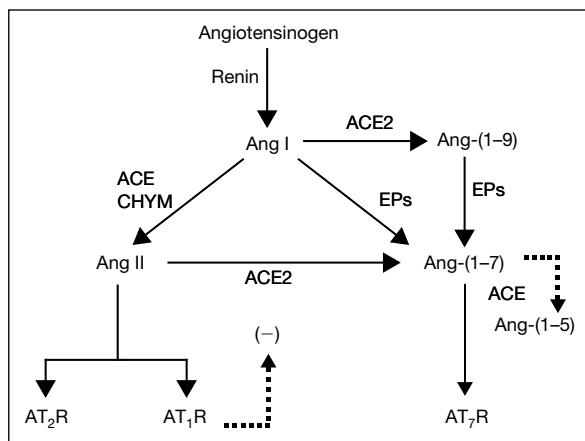
Fig. 1. Processing pathways for the formation and metabolism of angiotensin-(1–7) in the kidney. Once formed from angiotensinogen by renin, angiotensin (Ang) I can be processed to Ang II or Ang-(1–7) by the pathways illustrated. Several neutral endopeptidases (NEP) involved in the formation of Ang-(1–7) from Ang I or Ang-(1–9) in the kidney include neprilysin, prolyl endopeptidase and thimet oligopeptidase [44]. The critical positions of converting enzyme (ACE) and its recently identified homolog ACE2 with respect to reciprocal formation and metabolism of Ang II and Ang-(1–7) emphasize the potential contribution of each enzyme to the overall regulation of the renin-angiotensin system.

unique therapeutic target in the treatment of hypertension and renal disease. Indeed, ACE inhibition attenuates the decline in renal function in both experimental and clinical forms of diabetes and hypertension-related renal disease. The role of ACE in the regulation of blood pressure expanded with our demonstration that ACE readily hydrolyses Ang-(1–7) to Ang-(1–5), thus regulating the vasodepressor and antiproliferative effects of Ang-(1–7) (fig. 1) [7, 8]. In keeping with these findings, we also showed that ACE inhibition increased the half-life of Ang-(1–7) approximately 6-fold, as well as augmenting endogenous levels of the peptide [9–11]. Blockade with the selective Ang-(1–7) antagonist *D*-[Ala[7]]-Ang-(1–7) or a monoclonal antibody against the peptide reversed the blood-pressure-lowering actions of the ACE inhibitor lisinopril [12, 13]. Thus, increased levels of Ang-(1–7) may account for up to 30% of the antihypertensive actions of ACE inhibition. Interestingly, Ang-(1–7) and the hematopoietic fragment acetyl-Ser-Asp-Lys-Pro are the only two endogenous substrates known to be exclusively cleaved by the N-terminal catalytic domain of human ACE [8, 14]. Although selective inhibitors against both catalytic domains of somatic ACE have recently been developed, the functional significance of the two domains is not presently known [15, 16].
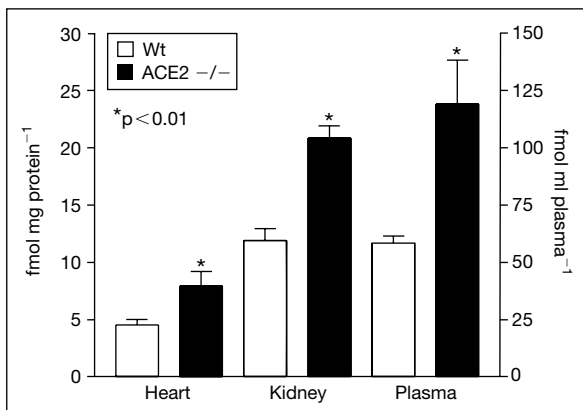
Fig. 2. Increased circulating and tissue angiotensin II concentrations in ACE2 knock-out (ACE2−/−) mice. Data adapted from Crackower et al. [24].

In the past 3 years, a new homolog of ACE termed ACE2 was identified by two separate groups, independently [17, 18]. In contrast to ACE, ACE2 is not inhibited by ACE inhibitors such as captopril or lisinopril, nor does it share the same catalytic properties. In this regard, ACE2 contains a single catalytic site that corresponds to the C-terminal domain of somatic ACE. ACE2 exhibits carboxypeptidase activity cleaving a single amino acid residue at the carboxyl terminus. Although ACE2 was originally reported to cleave Ang I to Ang-(1–9), kinetic studies suggest that the conversion of Ang II to Ang-(1–7) is much preferred [19]. Indeed, metabolism studies in both human and mouse heart revealed that Ang I was converted to Ang-(1–9) by carboxypeptidase A, whereas ACE2 was predominant in the conversion of Ang II to Ang-(1–7) [20, 21]. In addition, ACE2 exhibits the highest efficiency (kcat/km) among Ang-(1–7)-forming enzymes and a 500-fold greater kcat/km for Ang II as compared to Ang I. Similar to ACE, ACE2 exists in both soluble and membrane-associated forms with high expression in the kidney, heart, brain and testes [22, 23]. Multiple forms of ACE2 are present in various tissues and may arise from either post-translational glycosylation or alternative splicing of the ACE2 mRNA. The physiological relevance of these forms awaits further investigation.

Our studies in the ACE2 knockout model demonstrated higher circulating and tissue levels of Ang II suggesting that reductions in ACE2 expression may lead to higher endogenous levels of Ang II and contribute to the cardiac and renal pathologies associated with this model [24] (fig. 2). Several hypertensive models including the SHR and Sabra salt-sensitive rat exhibit both reduced mRNA levels and protein expression of ACE2 [24]. Cooper and colleagues [25]

reported that the renal expression of ACE2 was reduced in streptozotocin-induced type I diabetes and reversed by chronic ACE inhibitor therapy with ramipril. Moreover, ACE2 maps to a QTL region on the X chromosome that is highly associated with the hypertensive phenotype [24]. In this regard, our recent data demonstrated that estrogen depletion and a high salt diet reduced the expression of ACE2, but increased ACE within the kidneys of female salt-sensitive mRen.2 Lewis congenic rats [26]. This imbalance of ACE/ACE2 may contribute to the profound effects of increased sodium intake on the development of hypertension and renal injury in this model. Furthermore, Brosnihan et al. [27] reported greater immunocytochemical staining for Ang-(1–7) and ACE2 in the kidney of pregnant rats. These novel findings suggest that the enhanced expression of the Ang-(1–7)/ACE2 pathway contributes to the maintenance of blood pressure in the presence of an activated RAS during pregnancy. Although relatively little is known about the direct regulation of renal ACE2 and its role in the development of hypertension, our preliminary data suggest that Ang II may exert a negative influence on the expression of the enzyme through the AT1 receptor [18, 28]. Overall, these results would support the view that ACE2 may play a vital role to regulate the expression of both Ang II and Ang-(1–7) in the kidney (see functional aspects of Ang-(1–7) below).

Renal Actions of Angiotensin-(1–7)

Ang-(1–7) is present in the circulation and in many tissues including the kidney at concentrations that are comparable to Ang II [29]. Indeed, the levels of the peptide in plasma, renal tissue and urine are altered during physiologic or pathophysiologic conditions including those associated with changes in sodium intake and blood volume. Similar to the distribution of ACE2, we found that Ang-(1–7) is present in proximal renal tubules of both the mouse (see fig. 3) and the rat kidneys, as well as the distal convoluted tubules and collecting ducts [30, 31]. Ongoing studies clearly support the concept of a complete RAS within the proximal tubule including the expression of renin, ACE, angiotensinogen and the AT_1 receptor [32–39]. The localization of ACE2 to the tubular region now provides compelling evidence for the direct conversion of Ang II to Ang-(1–7) in this locale. Although the attenuation of the AT_1-mediated actions is clearly important, the product resulting from the ACE2-dependent metabolism of Ang II exhibits significant functional effects within the kidney as well.

It is important to emphasize that the processing pathways for Ang-(1–7) in the circulation and kidney are distinct. The endopeptidase neprilysin is the major activity forming Ang-(1–7) from Ang I or Ang-(1–9) in the circulation [11, 40–42]. Although plasma levels of neprilysin are low to non-detectable, the
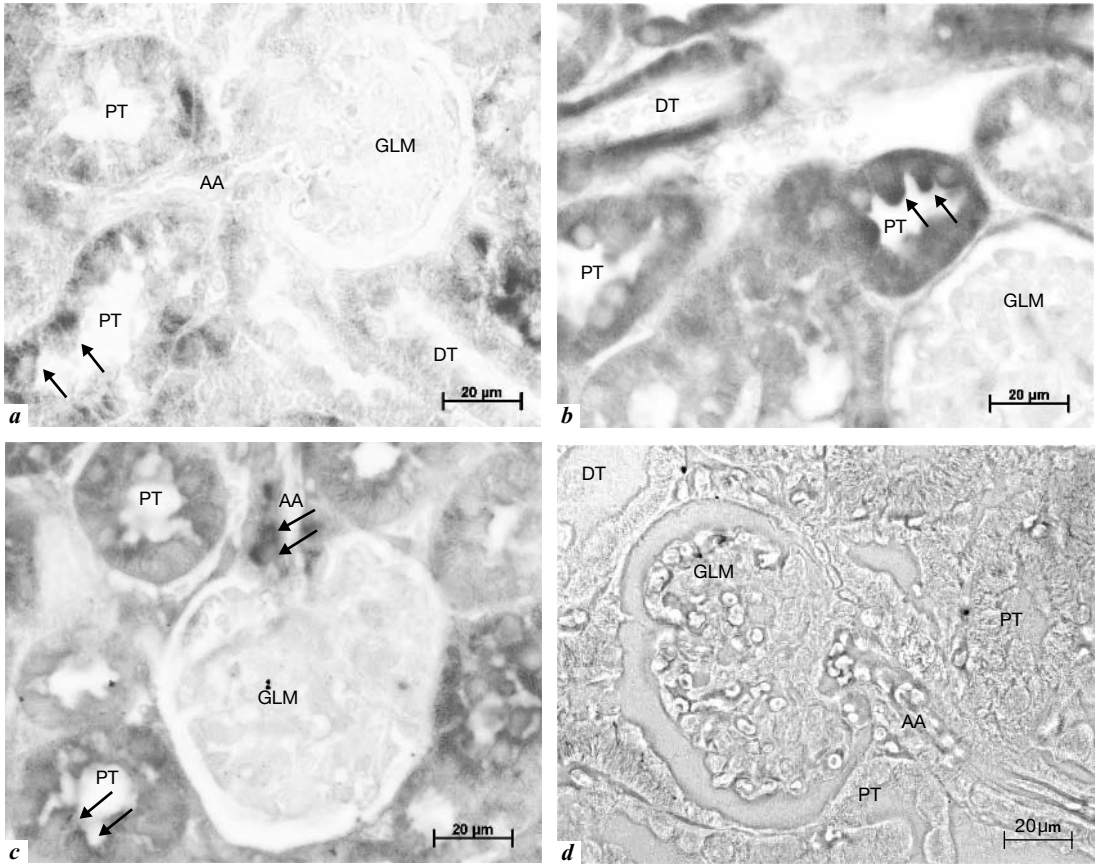
Fig. 3. Immunocytochemical localization of angiotensin-(1–7), ACE2 and mas protein in the mouse kidney. Photomicrographs (×100) of mouse kidney sections reveal immunostaining for Ang-(1–7) (top left panel), ACE2 (top right panel) and the mas receptor (bottom left panel). The bottom right panel is control tissue (×100) in the absence of the primary antibody. The arrows indicate pronounced staining on the apical aspect of the proximal tubules. PT = Proximal tubule; DT = distal tubule; GLM = glomerulus; AA = afferent arteriole.

enzyme is appropriately localized to the ectocellular surface of endothelial and smooth muscle cells to contribute to the formation of Ang-(1–7). In the kidney, Ang-(1–7) is the primary product formed in preparations of isolated proximal tubules and exists in urine at significantly higher levels than Ang II [43]. Neprilysin may contribute to both the formation as well as the degradation of the peptide [43]. Neprilysin cleaves Ang I to Ang-(1–7), but continues to metabolize Ang-(1–7) at the Tyr^5-Ile^6 bond to form Ang-(1–4) and Ang-(5–7) [43–45]. Indeed, neprilysin inhibitors augment the urinary levels of Ang-(1–7) in both

human and rat [30, 46]. We have recently shown that the combined ACE/ neprilysin inhibitor omapatrilat augmented renal and urinary levels of Ang-(1–7), but produced a blunted Ang-(1–7) response in plasma in comparison to ACE inhibition alone [42]. Interestingly, chronic treatment with omapatrilat was also associated with increased expression of ACE2 suggesting a novel mechanism to enhance the conversion of Ang II to Ang-(1–7) within the kidney.

The presence of Ang II and Ang-(1–7) in kidney supports the concept of important and divergent actions for the two peptides. For Ang II, the actions include potent, but differential, vasoconstriction of diverse segments of the renal microvasculature, retention of sodium and water by activation of various transporters in the proximal epithelium, as well as a stimulus for inflammation and oxidative stress. However, the majority of actions of Ang-(1–7) are in opposition to those of Ang II. Infusion of Ang-(1–7) into the renal artery leads to diuresis and natriuresis accompanied by modest increases in glomerular filtration rate (GFR) [47–49]. Ang-(1–7) induces vasodilation of pre-constricted afferent arterioles by local release of nitric oxide [50]. Ang-(1–7) and its metabolite Ang-(3–7) are potent inhibitors of Na^+,K^+-ATPase activity in isolated convoluted proximal tubules and the renal cortex [51, 52]. In renal tubular epithelial cells, Ang-(1–7) inhibits transcellular flux of sodium which was associated with activation of phospholipase A_2 (PlA_2) and suggests that the peptide also influences the Na^+/H^+ exchanger [53]. Interestingly, the inhibition of sodium transport by Ang I is markedly potentiated by the ACE inhibitor captopril suggesting either a shift in processing pathways from Ang II to Ang-(1–7) or the marked reduction in the metabolism of the peptide in the proximal tubule epithelial cells. The chronic and pronounced diuresis following omapatrilat treatment was associated with large increases in urinary excretion of Ang-(1–7) and enhanced immunocytochemical staining of the peptide as well as ACE2 in the kidney [31, 42]. This diuretic effect is consistent with the localization of the Ang-(1–7) in multiple areas of the renal tubules (fig. 3) [42]. Moreover, that the peptide may negatively influence aquaporin 1 expression in renal epithelial cells emphasizes the potential role of the peptide in the handling of water within the kidney [54]. Indeed, aquaporin 1 protein expression was markedly reduced in the kidneys from *tissue* ACE-depleted (*tis*ACE−/−) mice that are polyuric and hypotensive [55]. Developed by Bernstein and colleagues [56], tissue ACE activity is essentially absent in this model, however, circulating levels of the enzyme are still evident. Our characterization of the renal expression of angiotensins revealed markedly depleted levels of both Ang II and Ang I, but a sustained amount of Ang-(1–7) (fig. 4, left panel) [57]. As expressed as a percentage of the total angiotensin content, Ang-(1–7) represents the predominant peptide (>65%) in the kidneys of the *tis*ACE(−/−) mice, while Ang II and Ang I falls to <25 and 10%, respectively (fig. 4, right panel). The large increase
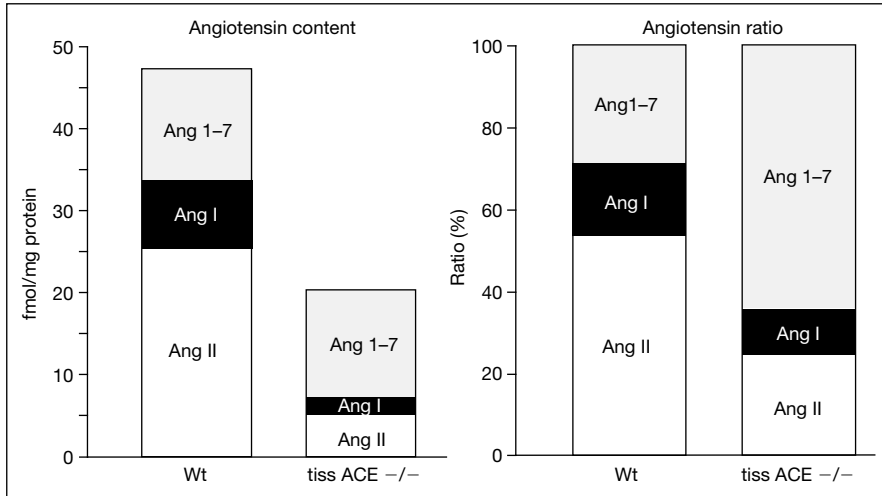
Fig. 4. Renal concentrations (left panel) and peptide ratios (right panel) of angiotensins in the kidneys of wild-type (Wt) and tissue ACE null (*tis*ACE−/−) mice. Data from Modrall et al. [57].

in the ratio of Ang-(1–7) to Ang II may well contribute to the reduced blood pressure, as well as the inability to concentrate urine in this strain.

The renal actions of Ang-(1–7) and Ang II are also comparable in several situations. Similar to Ang II, Ang-(1–7) exhibited biphasic effects on the transport of bicarbonate in perfused straight proximal tubules of normotensive rats [58]. Moreover, blockade with an AT_1 antagonist abolished both the inhibitory and stimulatory effects of the peptide. The intraluminal introduction of Ang-(1–7) stimulates transport in the loop of Henle, but does not affect reabsorption in either the proximal or distal tubule [59]. In this case, the absence of effects may reside from the high luminal concentrations of Ang-(1–7) in the proximal or distal segments of the tubule. A similar situation occurs with Ang II where application of an AT_1 antagonist or ACE inhibitor attenuates basal reabsorption in the proximal tubule [60]. It is possible that the natriuretic effects of high doses of Ang II ($>10^{-9}$ M) may arise from the ACE2-dependent conversion of Ang II to Ang-(1–7) at the epithelial surface of the proximal tubule. In water-loaded Wistar rats, low doses of Ang-(1–7) given subcutaneously promote an antidiuretic action [61, 62]. Ang-(1–7) stimulates ouabain-insensitive Na^+-ATPase activity in the ovine renal cortex, however, the peptide abolished the Ang II-dependent stimulation of this ATPase [63]. Burgelova et al. [64] recently showed that the intrarenal administration of Ang-(1–7) produced natriuresis and blocked the antinatriuretic actions of Ang II. Finally, similar to the

expected actions of Ang II, Ang-(1–7) also increased in oxidative stress markers such as TBARS, an indicator of lipid peroxidation. These effects were accompanied by a reduction in both superoxide dismutase and glutathione peroxidase activities [65]. Together these studies emphasize the complexity of the actions and interactions of Ang-(1–7) and Ang II. Thus, the overall state of activity of the RAS and site(s) of the nephron exposed to both Ang II and Ang-(1–7) clearly influence the ultimate physiological action observed.

Signaling Mechanisms and Receptors

The cellular signaling responsible for the actions of Ang-(1–7) in the kidney most likely involve mobilization of arachidonic acid and its subsequent processing through pathways yielding vasodilator and natriuretic products. The depressor response to Ang-(1–7) in the pithed rat was first shown to be dependent upon cyclooxygenase pathways [66]. Hilchey and Bell-Quilley [67] showed that infusion of Ang-(1–7) into the isolated, perfused kidney increased prostacyclin levels in both the urine and the venous outflow. The natriuretic and diuretic actions of Ang-(1–7) in the perfused kidney are also associated with increased levels of prostacyclin that are attenuated by the cyclooxygenase inhibitor indomethacin [67]. Ang-(1–7) infusion into SHR caused diuresis and natriuresis during the early days of infusion, concomitant with increases in urinary prostaglandins [68]. We showed in hypertensive rats treated with lisinopril and losartan that the COX inhibitor indomethacin caused similar increases in blood pressure to either an antibody to Ang-(1–7), a neprilysin inhibitor or the Ang-(1–) receptor antagonist [13]. These treatments were not additive suggesting that vasodilatory prostaglandins mediate the effects of Ang-(1–7) following ACE/AT$_1$ blockade. In proximal tubular epithelial cells, Ang-(1–7) stimulates PlA$_2$ activity that would lead to the release of arachidonic acid [53]. In addition, picomolar concentrations of Ang-(1–7) stimulate phosphatidylcholine incorporation in the renal cortex providing one potential source for the arachidonic acid substrate [63]. In isolated rat proximal tubules, the Ang-(1–7)-dependent inhibition of ouabain-sensitive rubidium (^{86}Rb) influx was attenuated by blockade of the cytochrome P450 (CPY450) system, but not COX inhibition [45, 69]. These data are consistent with the inhibitory effects of epoxygenase products on sodium transport, but contrast to stimulation of the vascular CYP450 system by Ang II whose products are likely to contribute to the potent vasoconstrictor effects of the peptide within the kidney [70, 71]. The differential actions of Ang II and Ang-(1–7) may explain the apparent contrasting actions of the CYP450 system on the renal vasculature and the tubular epithelium regarding the overall regulation of blood pressure.

As recently reviewed by Diz et al. [72], there are at least three potential mechanisms to account for the actions of Ang-(1–7). These include the activation of antihypertensive systems such as prostaglandins and nitric oxide release through a novel non-AT_1, non-AT_2 receptor $[AT_{(1-7)}]$ that is sensitive to the antagonist [D-Ala^7]-Ang-(1–7); Ang-(1–7)-dependent actions that are attenuated by either AT_1 or AT_2 receptor antagonists in addition to [D-Ala^7]-Ang-(1–7), and the homologous or heterologous down-regulation or desensitization of AT_1 receptors by Ang-(1–7). We discuss here the evidence for a novel receptor that mediates the actions of Ang-(1–7) in the kidney. Santos, Khosla, and colleagues [73] initially described the unique antagonist [D-Ala^7]-Ang-(1–7) that selectively attenuates several of the actions of Ang-(1–7), but not those of Ang II. The natriuretic and diuretic effects of Ang-(1–7) and actions on the afferent arteriole are blocked by [D-Ala^7]-Ang-(1–7), suggesting that the renal actions of Ang-(1–7) encompass both tubular and vascular binding sites that are non-AT_1/AT_2 receptors. Intrarenal administration of D-[Ala^7]-Ang-(1–7) to rats elicits a fall in GFR, urine volume and sodium excretion, demonstrating an intrinsic effect of renal Ang-(1–7) in normotensive animals [64, 74]. In the afferent arteriole, Ang-(1–7) exhibits potent vasodilatory effects that are blocked by D-[Ala^7]-Ang-(1–7) and nitric oxide synthase inhibitors, but not AT_1 or AT_2 antagonists [50]. Furthermore, the Ang-(1–7)-dependent inhibition of Na^+,K^+-ATPase activity (^{86}Rb uptake) in isolated proximal tubules from normotensive rats – consistent with natriuretic actions within the kidney – are blocked by D-[Ala^7]-Ang-(1–7), but not AT_1 or AT_2 antagonists [45].

The molecular evidence favoring *at least one unique receptor* that mediates the actions of Ang-(1–7) was recently revealed by Santos, Walter and colleagues [75]. These investigators reported both binding and functional data that the *mas* receptor gene codes for an Ang-(1–7) binding site. Studies in *mas* receptor-deficient mice indicated a loss of Ang-(1–7) binding in the kidney of these animals associated with the absence of vascular depressor responses to Ang-(1–7), as well as its antidiuretic effect in water-loaded mice [75]. Although Hanley and colleagues [76] originally reported the *mas* orphan protein was the Ang II receptor, the functional actions were due to the presence of residual AT_1 receptors on mas-transfected oocytes that mediated the Ang II-dependent influx of calcium [77]. Tallant et al. [78] recently found that antisense oligonucleotides or small interfering RNAs (siRNAs) to *mas* attenuated the responses to Ang-(1–7) and were associated with a reduction in the expression of the *mas* protein. Utilizing an affinity-purified antibody to the mas receptor protein, we demonstrate pronounced staining in the proximal tubules of the mouse kidney, primarily on the apical aspect of the tubule (see bottom panel of fig. 3). The positive staining for *mas* in the renal afferent arteriole is also consistent with the direct vasodilatory effects of the peptide in this vessel and the increase in GFR.

These immunocytochemical data reveal a similar distribution for Ang-(1–7), ACE2, and the *mas* receptor within the tubular epithelium of the kidney (fig. 3). Moreover, the discrete localization of these novel components provides evidence for an alternative RAS within the proximal tubule epithelium that may antagonize the actions of Ang II in this renal compartment.

Acknowledgements

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Role of Aldosterone Blockade in the Management of Hypertension and Cardiovascular Disease

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Aldosterone is a major regulator of extracellular fluid volume and the major determinant of potassium metabolism [1–5]. These effects are mediated by the binding of aldosterone to the mineralocorticoid receptor in target tissues, primarily the kidney. Volume is regulated through a direct effect on the collecting duct, where aldosterone causes an increase in sodium retention and an increase in potassium excretion. The reabsorption of sodium ions produces a fall in the transmembrane potential, thus enhancing the flow of positive ions, such as potassium, out of the cell into the lumen. The reabsorbed sodium ions are transported out of the tubular epithelium into the renal interstitial fluid and from there into the renal capillary circulation.

In recent years, there has been a paradigm shift with respect to our understanding of aldosterone's widespread effects on the heart, the vasculature, and the kidney [6–11]. The endocrine properties of aldosterone have assumed a broader perspective involving nonclassic actions in nonepithelial cells found in nonclassic target tissues, including the heart, the vasculature, and the kidney [6, 9–17].

There is increasing evidence that aldosterone can have an effect on vascular remodeling and collagen formation, and a nongenomic action to modify endothelial function. Among the most intriguing effects of aldosterone are its impact on fibrosis and activity associated with a cell surface receptor in certain target tissues, including endothelial cells [6, 7, 18–21]. These actions contribute substantively to the pathophysiology of congestive heart failure, including its progressive nature, as well as progressive renal dysfunction. This new information has spurred interest in the development of a selective antagonist to block aldosterone's effect,

not only because of its antihypertensive and diuretic effects, but also primarily because of its potential cardiovascular and renal protective effects.

In this review, I will briefly consider the expanding role of aldosterone and the broad spectrum of nongenomic effects. It is becoming increasingly evident that these effects, occurring independently of hemodynamic factors, contribute to enhanced cardiovascular risk manifested by congestive heart failure and progressive renal disease. I also will review the recent clinical and experimental trials with the selective aldosterone blocker (SAB) eplerenone (Inspra®). This agent is currently approved for the treatment of hypertension and is under review for the additional indication in patients with heart failure. Such trials have established eplerenone's role as an effective and safe antihypertensive agent, and also have enhanced our understanding of the role of aldosterone in the pathophysiology of cardiovascular and renal disease. Based on these studies, it is appropriate to view eplerenone as a promising agent for achieving a further reduction in cardiovascular and renal disease morbidity and mortality, and for enhancing patient well-being.

Traditional Concept

In its capacity as a mineralocorticoid hormone, aldosterone has receptor-ligand endocrine properties on epithelial cell sodium and potassium exchange in classic target tissues, such as the kidneys, colon, and salivary and sweat glands [1–3]. By virtue of its effects on the distal renal tubular cell, aldosterone is involved in the regulation of sodium and body water homeostasis, and consequently participates in the regulation of blood pressure [4]. As a consequence of activation of the renin-angiotensin-aldosterone system, the increase in circulating aldosterone enhances renal sodium retention and potassium excretion. Consequently, aldosterone potentiates the occurrence of stroke, coronary artery disease, myocardial infarction, and sudden cardiac death.

Nongenomic Effects of Aldosterone

In contrast to the classical effect of aldosterone on its nuclear receptor, its 'neoclassical' function involves a different second messenger system, that is, activation of the sodium/hydrogen antiporter. In contrast to genomic-mediated events, these nongenomic effects occur within seconds to minutes rather than minutes to hours [12]. An aldosterone-mediated effect on sodium/hydrogen antiporter activity has been demonstrated in renal cells [22], vascular smooth muscle cells [14, 17], endothelial cells [23], and leukocytes [24]. Further studies

are necessary to fully understand the physiologic or pathophysiologic relevance of these rapid effects of aldosterone.

Cardiovascular Effects of Aldosterone

Several lines of evidence have provided a theoretic framework supporting the role of aldosterone in mediating cardiovascular disease. Recent data indicate that aldosterone blocks the uptake of norepinephrine and that aldosterone antagonists improve the uptake of norepinephrine [25]. Furthermore, aldosterone enhances vascular collagen turnover and exacerbates heart rate variability [26] and depresses baroreceptor reflex function [27]. Experimental data with aldosterone blockade indicate that aldosterone also may play a role in promoting endothelial dysfunction [28, 29], coronary vascular inflammation and subsequent interstitial fibrosis [30, 31], and activation of matrix metalloproteinases and subsequent ventricular remodeling [32].

Are Cardiovascular Events Attributable to Aldosterone per se? Lessons from Primary Aldosteronism

Although studies clearly document an enhanced cardiovascular risk for patients with primary aldosteronism, it has been argued that these cardiovascular events are explained by the presence of accompanying hypertension. In order to dissociate the contribution of aldosterone from hypertension, several clinical studies have compared the incidence of cardiovascular events between patients with primary aldosteronism and those with essential hypertension. Left ventricular hypertrophy (LVH) is more common and severe in patients with primary aldosteronism than in patients with other types of hypertension. For example, Denolle et al. [33] studied the degree of LVH in age- and sex-matched patients with renovascular hypertension, primary aldosteronism, or pheochromocytoma. Among the three groups, patients with primary aldosteronism demonstrated greater left ventricular mass index than those with renovascular hypertension or pheochromocytoma. Another study [34] compared the level of LVH in matched patients with primary aldosteronism or essential hypertension. Despite similar blood pressure, age, and gender distributions, patients with primary aldosteronism had a more severe level of LVH than those with essential hypertension, and the level of left ventricular wall thickness was directly correlated to plasma aldosterone levels.

Role of Aldosterone in Mediating Progressive Renal Disease

In analogy with its effects on the cardiovascular system, aldosterone also exerts adverse effects on the kidney. I have recently reviewed the rapidly

emerging and extensive evidence for aldosterone as a mediator of progressive renal disease [9–11]. Subsequently, several experimental and clinical studies have expanded our understanding of aldosterone and the kidney. Although the role of angiotensin II in mediating progressive renal disease has been documented extensively, recent evidence also implicates aldosterone (*independent* of the renin-angiotensin system) as an important pathogenetic factor in progressive renal disease.

Although many studies have demonstrated a beneficial effect of angiotensin-converting enzyme (ACE) inhibition and angiotensin II receptor blockers (ARBs) in retarding progressive renal disease, these interventions do not differentiate between the relative contributions of renin-angiotensin versus aldosterone. To evaluate the possible contribution of aldosterone per se, Rocha et al. [8, 35] conducted a series of experiments in spontaneously hypertensive stroke-prone rats (SHRSP) that succeeded in dissociating the relative contributions of aldosterone and the renin-angiotensin system. In one study of saline-drinking (1% NaCl) SHRSP ingesting a stroke-prone rodent diet [35], a model known to induce severe hypertension and glomerular and vascular lesions characteristic of thrombotic microangiopathy, these investigators demonstrated that mineralocorticoid receptor blockade with spironolactone markedly attenuated urinary protein excretion. Histologic examinations revealed fewer nephrosclerotic and cerebrovascular lesions in the spironolactone group than in the placebo group (p < 0.01 and p < 0.001, respectively). Notably, systolic blood pressure did not differ between the two groups at any time during the study.

In a subsequent study, Rocha et al. [8] evaluated whether an aldosterone infusion would reverse the renal-protective effects of captopril therapy in SHRSP. Treatment with the ACE inhibitor captopril reduced endogenous aldosterone levels, prevented the development of proteinuria, and prevented the development of glomerular and renal vascular lesions. However, subsequent aldosterone infusion reversed the ability of captopril to confer this protection. The aldosterone-infused, captopril-treated rats demonstrated proteinuria, renal vascular lesions, and glomerular lesions despite ACE inhibitor treatment. Interestingly, systolic blood pressure in captopril-treated SHRSP receiving the aldosterone infusion was not significantly different than in SHRSP treated with captopril alone. Thus, the renal injury induced by aldosterone developed independently of blood pressure increases, suggesting a more direct tissue effect of aldosterone.

The precise mechanisms by which aldosterone promotes target organ dysfunction have not yet been established. As I have detailed in a recent review [36], several possible mechanisms may account for the ability of aldosterone to promote fibrosis and target organ dysfunction (table 1). These include plasminogen activator inhibitor (PAI-1) expression and consequent alterations of

Table 1. Potential mechanisms by which aldosterone produces fibrosis and collagen formation

Potentiation of the pressor responses of angiotensin II
Inhibition of norepinephrine uptake in vascular smooth muscle cells and myocardial cells
Participation in vascular smooth muscle cell hypertrophy
Modulation of the effect of angiotensin II on plasminogen activator inhibitor-1 (PAI-1) expression
Stimulation of transforming growth factor-β_1 (TGF-β_1) synthesis
Acting through cyclooxygenase-2 (COX-2)
Stimulation of osteopontin
Generation of reactive oxygen species (ROS)
Induction of endothelial dysfunction

vascular fibrinolysis [37, 38], stimulation of transforming growth factor-β_1 [39], and stimulation of reactive oxygen species [40].

Another possible mechanism relates to the potential proinflammatory effects of angiotensin II and aldosterone. By way of background, recent studies have documented vascular inflammatory damage in the hearts of aldosterone/salt, uninephrectomized rats [30]. Rocha et al. [41] demonstrated that aldosterone plays a major role in angiotensin II-induced vascular inflammation in the heart and implicated cyclooxygenase-2 (COX-2) and osteopontin as mediators of the vascular myocardial injury.

Therapeutic Role of Aldosterone Blockade

Heart Failure: RALES and EPHESUS Clinical Trials

Chronic heart failure is an increasing burden to healthcare. Pharmacologic treatment with ACE inhibitors and β-blockers improves survival and reduces hospitalizations in patients with low left ventricular ejection fraction [42, 43]. Despite these improvements in therapy, long-term mortality remains high in patients with heart failure [44], mandating a need for new treatment strategies to reduce the burden of mortality and morbidity in this condition.

Several important clinical trials support the cardioprotective effects of aldosterone blockade in patients with heart failure. The Randomized Aldactone Evaluation Study (RALES) [7] examined the effect of spironolactone on overall morbidity and mortality in patients with severe heart failure treated with standard therapy, including an ACE inhibitor, a loop diuretic, and digoxin, combined with either a nonhemodynamic dose of spironolactone (25 mg/day) or placebo.

Spironolactone-treated patients demonstrated a 30% reduction in the risk of death from all causes compared with the placebo group. This reduction in mortality was largely attributed to a reduction in death from progressive heart failure and sudden cardiac death. The RALES investigators attributed the beneficial actions of spironolactone to the drug's favorable effects on myocardial and vascular fibrosis and its ability to increase myocardial uptake of norepinephrine, in addition to its anticipated ability to prevent sodium retention and potassium loss.

Because it was unknown whether aldosterone blockade would confer cardiovascular benefit among patients with acute myocardial infarction complicated by left ventricular dysfunction, Pitt et al. [45] recently investigated the effects of eplerenone in this setting. Specifically, the Eplerenone Post-AMI Heart Failure Efficacy and Survival Study (EPHESUS) examined whether selective aldosterone blockade with eplerenone would be beneficial in reducing morbidity and mortality. During the EPHESUS study, eplerenone 25–50 mg/day reduced morbidity and mortality, and reduced hospitalization rates in patients with post-myocardial infarction heart failure who already were receiving optimal medical therapies, including ACE inhibitors and β-blockers. Eplerenone is the first agent to demonstrate incremental benefit in addition to ACE inhibitors and β-blockers in improving outcomes in patients with post-myocardial infarction heart failure. The EPHESUS investigators proposed that the beneficial effects of eplerenone were attributable to several nonrenal mechanisms. Eplerenone reduces coronary vascular inflammation and the risk of subsequent development of interstitial fibrosis in animal models of myocardial disease [30]. Eplerenone also reduces oxidative stress, improves endothelial dysfunction [29], and decreases activation of matrix metalloproteinases and improves ventricular remodeling [32]. In summary, the EPHESUS study supports the clinical paradigm of adding eplerenone to the treatment regimen for optimal management of heart failure.

Hypertension

Eplerenone is the first member of a novel antihypertensive class, the SABs, and has a unique mechanism of action that lacks the adverse endocrine effects observed with non-SABs. Multiple recent studies have demonstrated that eplerenone reduces blood pressure significantly in a wide range of patient populations [46–59] (table 2). The achieved reductions in blood pressure are comparable with many other commonly used antihypertensive agents, including ARBs [49, 50], ACE inhibitors [53–55, 58], and calcium channel blockers (CCBs) [51, 52]. The safety and efficacy of eplerenone have been demonstrated across a wide range of patient populations, including patients with low plasma renin levels [49, 50], blacks [49], elderly patients with isolated systolic hypertension [51], and patients with LVH [54]. Eplerenone has additive effects in

Table 2. Randomized, double-blind, controlled trials of eplerenone in hypertension

Group (first author)	Patient population	Study design	Treatment groups[1]	Study duration
Weinberger, 2002 [46]	Patients with mild-to-moderate hypertension	PBO- and active-controlled, fixed-dose, parallel group	1) EPL 50 mg 2) EPL 100 mg 3) EPL 400 mg 4) EPL 25 mg bid 5) EPL 50 mg bid 6) EPL 200 mg bid 7) Spironolactone 50 mg bid 8) PBO	8 wks
White, 2003 [47]	Patients with mild-to-moderate hypertension	PBO-controlled, fixed-dose, dose-ranging, parallel group	1) EPL 25 mg 2) EPL 50 mg 3) EPL 100 mg 4) EPL 200 mg 5) PBO	12 wks
Saruta, 2003 [48]	Japanese patients with mild-to-moderate hypertension	PBO-controlled, fixed-dose, multicenter, parallel group	1) EPL 50 mg 2) EPL 100 mg 3) EPL 200 mg 4) PBO	8 wks
Flack, 2003 [49]	Black patients or white patients with mild-to-moderate hypertension	Active- and PBO-controlled, titration-to-effect[2]	1) EPL 50–200 mg 2) Losartan 50–100 mg 3) PBO	16 wks
Weinberger, 2002 [50]	Patients with low-renin hypertension	Active-controlled, titration-to-effect[2]	1) EPL 100–200 mg 2) Losartan 50–100 mg HCTZ 12.5–25 mg could be added to either treatment arm after week 8 for DBP ≥90 mm Hg	16 wks
White, 2003 [51]	Patients ≥50 years with elevated systolic hypertension	Active-controlled, titration-to-effect[2]	1) EPL 50–200 mg 2) Amlodipine 2.5–10 mg	24 wks
Williams, 2003 [52]	Patients with mild-to-moderate hypertension	Active-controlled, titration-to-effect[2]	1) EPL 50–200 mg 2) Amlodipine 2.5–10 mg	16 wks
Burgess, 2002 [53]	Patients with mild-to-moderate hypertension	Active-controlled, titration-to-effect[2]	1) EPL 25–200 mg 2) Enalapril 5–40 mg	24[3] 12 mths

Table 2 (continued)

Group (first author)	Patient population	Study design	Treatment groups[1]	Study duration
Pitt, 2003 [54]	Patients with LVH and mild-to-moderate hypertension	Active-controlled, forced-titration	1) EPL 200 mg 2) Enalapril 40 mg 3) EPL 200 mg + enalapril 10 mg Add-on HCTZ 12.5–25 mg and amlodipine 10 mg allowed after week 8 for DBP ≥90 mm Hg or SBP ≥180 mm Hg	36 wks
Epstein, 2002 [55]	Patients with type 2 diabetes, a UACR ≥100 mg/g, and mild-to-moderate hypertension	Active-controlled, forced-titration	1) EPL 200 mg 2) Enalapril 40 mg 3) EPL 200 mg + enalapril 10 mg Add-on HCTZ 12.5–25 mg and amlodipine 10 mg allowed after week 8 for DBP ≥90 mm Hg	24 wks
Krum, 2002 [56]	Patients with mild-to-moderate hypertension on a fixed dose of either an ACE-I or ARB	PBO-controlled, titration-to-effect[2]	1) EPL 50–100 mg 2) PBO Either placebo or EPL was added to background antihypertensive therapy with an ACE-I or ARB	8 wks
Willenbrock et al, 2003	Patients with mild-to-moderate hypertension on a fixed dose of either a CCB or BB	PBO-controlled, titration-to-effect[2]	1) EPL 50–100 mg 2) PBO Either placebo or EPL was added to background antihypertensive therapy with a CCB or BB	8 wks

Table 2 (continued)

Group (first author)	Patient population	Study design	Treatment groups[1]	Study duration
Epstein, 2003 [58]	Patients with type 2 diabetes, a UACR ≥50 mg/g, with or without hypertension	Enalapril – run-in, active-controlled, fixed-dose	1) EPL 50 mg + enalapril 20 mg 2) EPL 100 mg + enalapril 20 mg 3) PBO + enalapril 20 mg After week 4, add-on amlodipine 2.5–10 mg was allowed for uncontrolled SBP/DBP ≥130/8 mm Hg	12 wks
Burgess, 2003 [59]	Patients with mild-to-moderate hypertension	Long-term, open-label, titration-to-effect[2]	1) EPL 50–200 mg After week 6, add-on therapy of investigator's choice allowed for BP control (SBP/DBP <140/90 mm Hg)	14 mths

ARB = Angiotensin II receptor blocker; ABPM = ambulatory blood pressure monitoring; ACE-I = angiotensin-converting enzyme inhibitor; BB = β-blocker; CCB = calcium channel blocker; DBP = diastolic blood pressure; EPL = eplerenone; HCTZ = hydrochlorothiazide; LVH = left ventricular hypertrophy; LVM = left ventricular mass; PBO = placebo; PP = pulse pressure; QD = daily; SBP = systolic blood pressure; UACR = urinary albumin:creatinine ratio.

[1]All regimens were QD unless otherwise noted.

[2]Criterion for up-titration was: DBP ≥90 mm Hg [50, 52, 53, 56, 57]; SBP ≥140 mm Hg [51]; and DBP ≥90 mm Hg or SBP ≥140 mm Hg [49, 59].

[3]The primary efficacy endpoint was assessed at week 24; secondary efficacy endpoint was assessed at month 12 after forced down-titration at week 24 in patients responding to therapy at week 24.

reducing blood pressure when used in combination with other antihypertensive medications, such as ARBs, ACE inhibitors, CCBs, and β-blockers [56, 57]. The antihypertensive effects of eplerenone are seen as early as 4 weeks of therapy and are maintained over time.

Recent studies indicate that aldosterone blockade induced by eplerenone is an effective treatment for Japanese patients with hypertension [48]. As detailed

in a recent comprehensive review [60], non-Caucasian ethnic groups, including Asian patients, differ in their responsiveness to various classes of antihypertensive agents. Furthermore, such patients are underrepresented in the majority of randomized, clinical trials. Consequently, it is necessary to investigate the efficacy of each antihypertensive agent in different ethnic and racial groups. It recently has been shown that an increased frequency of certain polymorphisms in CYP11B2, an aldosterone synthase gene, among Japanese patients may predispose them to low-renin hypertension, a form of hypertension in which there is a high ratio of aldosterone to plasma renin activity [61, 62]. This observation supports a role for inappropriate aldosterone synthesis in the development of hypertension, highlighting the importance of aldosterone blockade as a rational therapeutic strategy for blood pressure control in Japanese hypertensive patients. Building on these considerations, Saruta et al. [48] recently conducted a randomized, double-blind, placebo-controlled study of eplerenone in Japanese patients with hypertension and demonstrated significant blood pressure reduction with excellent tolerability in these patients.

Aldosterone Blockade Mitigates Renal Disease: Clinical Studies

Recent clinical studies have extended the preclinical observations to the clinical arena, and have indicated that aldosterone blockade may confer an antiproteinuric effect in diabetic patients. Although the current standard of practice entails blockade of the renin-angiotensin system with either an ACE inhibitor or an ARB [63–66], such a strategy may be fraught with difficulties for long-term therapy. Although, initially, ACE inhibition attenuates the release of aldosterone, over time, aldosterone rebounds from this control [67]. Such 'rebound' should theoretically countervail the beneficial effects on the kidney.

Recently, several studies have investigated the effectiveness of aldosterone blockade on albumin excretion. Sato et al. [68] investigated the role of aldosterone rebound in 45 patients with type 2 diabetes and early nephropathy treated with an ACE inhibitor for 40 weeks. With treatment, there was a 40% reduction in average urinary albumin excretion, although urinary albumin excretion in patients with aldosterone rebound (18 patients) was significantly higher than that in patients without rebound (27 patients). Of the 18 patients with rebound, spironolactone (25 mg/day) was added to ACE inhibitor treatment in 13 patients. After a 24-week study period, urinary albumin excretion and left ventricular mass index were significantly reduced without blood pressure change. This study emphasizes that aldosterone rebound might occur in 40% of patients with type 2 diabetes with early nephropathy, despite the use of ACE inhibitors, and that aldosterone blockade can abrogate the detrimental effects on the heart and kidney.

Recently, Epstein et al. [58] extended these observations to assess the role of selective aldosterone blockade on protein excretion using eplerenone in

patients with type 2 diabetes mellitus. This 12-week, double-blind study tested the hypothesis that eplerenone, coadministered with the ACE inhibitor enalapril, would attenuate proteinuria in patients with type 2 diabetes. Following open-label run-in with enalapril 20 mg daily (ENAL), type 2 diabetic patients with proteinuria (urinary albumin:creatinine ratio (UACR) ≥50 mg/g) with or without mild-to-moderate hypertension (diastolic blood pressure, 90–110 mm Hg; systolic blood pressure, 140–180 mm Hg) were randomly assigned to additionally receive 1 of 3 daily treatments: eplerenone 50 mg, eplerenone 100 mg, or placebo. After week 4, open-label amlodipine was allowed for adequate blood pressure control. The results of this trial demonstrated that treatment with eplerenone 50 mg/ENAL or eplerenone 100 mg/ENAL, but not placebo/ENAL, significantly reduced albuminuria from baseline. By week 12, the placebo/ENAL group demonstrated a 12.9% reduction in UACR, compared with a 52.2% reduction in the eplerenone 50 mg/ENAL group, and a 54.9% reduction in the eplerenone 100 mg/ENAL group (both eplerenone groups, p < 0.001 vs. placebo/ENAL).

In light of earlier observations that a higher dose of eplerenone (200 mg) reduces proteinuria in patients with type 2 diabetes but is associated with an elevated incidence of hyperkalemia in this population [55], it is important to note that the above study demonstrated a similar antiproteinuric effect with lower doses of eplerenone (50 mg and 100 mg) while obviating the hyperkalemia observed previously; the rates of sustained and severe hyperkalemia were low and were not statistically different among all three treatment arms.

In summary, these studies suggest that aldosterone blockade may constitute an effective strategy for abrogating aldosterone rebound in the course of ACE inhibitor or ARB therapy. The combination of renin-angiotensin-modulating drugs in concert with selective aldosterone blockade might constitute a rational intervention for retarding progressive renal disease. Larger, randomized trials are needed to corroborate and extend these promising findings.

Are SABs Preferable to Non-SABs?

Multiple studies have demonstrated that the major adverse effect of the currently available aldosterone antagonist, spironolactone, is inhibition of the action of other steroids, primarily androgens. This action is mediated by a direct effect on the testes, by altering the conversion of androgens to estrogens in peripheral tissues, and most importantly, by blocking the androgen receptor. The effect on conversion is augmented when liver function is abnormal, such as occurs in the setting of heart failure. This antiandrogenic effect of spironolactone has limited its use in males, particularly when administered over the long term or in doses >25 mg/day [69]. Preliminary data suggest that the SAB eplerenone is free of the antiandrogenic adverse effects associated with spironolactone therapy [46]. In contrast to spironolactone, eplerenone has little effect on the

androgen or progesterone receptors and does not modify the metabolism of testosterone [70].

Summary and Future Perspectives

Our knowledge of the role of aldosterone in the development of cardiovascular and renal injury has expanded markedly during the past few years. Increasing evidence indicates that aldosterone mediates vascular remodeling and collagen formation, hypertensive nephrosclerosis and progressive renal disease, and has a nongenomic action to modify endothelial function. Recent studies have clearly demonstrated that the newly developed SAB, eplerenone, is an efficacious agent for blood pressure control. The safety and antihypertensive efficacy of eplerenone have been demonstrated across a wide range of patient populations. Aside from the antihypertensive efficacy of aldosterone blockade, several important clinical trials (RALES and EPHESUS) support the cardioprotective effects of aldosterone blockade in patients with heart failure. Because eplerenone is highly selective for the mineralocorticoid receptor and exhibits little affinity for other steroid receptors, it is not associated with sexual adverse effects including gynecomastia and impotence. Consequently, eplerenone constitutes a rational approach for achieving blood pressure control and target organ protection, including cardioprotection, with a desirable side effect profile that will enhance patient compliance.

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Clinical Implications of Blockade of the Renin-Angiotensin System in Management of Hypertension

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Hypertension is the most common cardiovascular condition in the world. In the USA alone, it affects 50 million people (1 in 4) and accounts for approximately 10% of deaths [1]. Despite its prevalence, data from the Third National Health and Nutrition Examination Survey III (NHANES III) demonstrate that only 27% of hypertensive subjects achieve the blood pressure (BP) goal of <140/90 mm Hg [2], although the more recent NHANES IV report indicates that the control levels for those aged 18–74 have increased to 34%, which is an improvement but still far below that seen in clinical trials wherein control rates are 65–85%.

The kidney plays a crucial role in various forms of hypertension. Hypertension is both a cause and consequence of renal disease, a common diagnosis in many patients starting maintenance dialysis. Management of hypertension in kidney dysfunction is challenging and generally requires at least three different and complementary acting medications to achieve the recommended BP goal of <130/80 mm Hg. The Seventh Report of the Joint National Committee (JNC 7) recommends that angiotensin-converting enzyme (ACE) inhibitors and angiotensin-receptor blockers (ARBs) be used [2] in concert with diuretics as first-line therapy to reduce BP in patients with hypertension and renal disease (table 1).

Renin-Angiotensin System (RAS)

The afferent arteriole of each glomerulus contains specialized cells, called juxtaglomerular cells, which secrete renin under the major physiologic stimuli of renal hypoperfusion and increased sympathetic activity [3]. Renin initiates a

Table 1. JNC-7 guidelines for compelling indications for individual drug classes [data from 2]

High-risk conditions with compelling indication*	Recommended drugs					
	diuretic	β-blocker	ACE inhibitor	ARB	CCB	aldosterone antagonist
Heart failure	•	•	•	•		•
Post-myocardial infarction		•	•			•
High coronary disease risk	•	•	•		•	
Diabetes	•	•	•	•	•	
Chronic kidney disease			•	•		
Recurrent stroke prevention	•		•			

*Compelling indications for antihypertensive drugs are based on benefits from outcome studies or existing clinical guidelines. The compelling indication is managed in conjunction with BP.
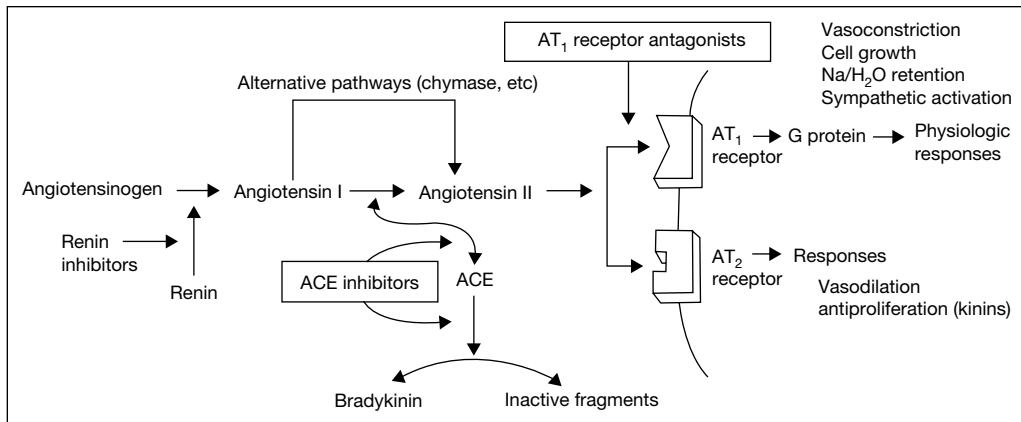
Fig. 1. The renin-angiotensin system and the mechanism of ACE inhibitors and angiotensin-receptor blockers.

biochemical cascade that starts with cleavage of the renin substrate angiotensinogen to angiotensin I, which is later converted into the vasoactive peptide angiotensin II via the ACE. Angiotensin II has two major systemic effects: vasoconstriction and sodium as well as water retention, mediated via specific receptors AT1 and AT2 (fig. 1). RAS also exists in a variety of extrarenal sites, including the lungs, vascular endothelium, and adrenal gland and brain [4].

Blockade of both circulating and tissue RAS to reduce the systemic and renal hemodynamics effects of angiotensin II are a focus in recent antihypertensive therapy. ACE inhibitors and ARBs are two drug classes that effectively block the actions of the RAS, demonstrating unique capabilities and mechanisms of action that delay the progression of diabetic and nondiabetic renal diseases, decrease proteinuria, and provide renoprotective qualities independent of BP control [5–9]. However, the effective doses of ACE inhibitors and ARBs have been determined primarily by BP reduction rather than by renal and/or cardiovascular outcomes.

ACE Inhibitors

ACE inhibitors act upon the RAS by blocking the production of angiotensin II and increasing the concentration of bradykinin, which acts as an endogenous antihypertensive (fig. 1). In older male subjects, treatment with ACE inhibitors demonstrates better outcomes compared to that for diuretics, despite similar reductions of blood pressure [10]. When used in conjunction with diuretics, these agents have been shown to decrease mortality from cardiovascular events in the setting of prior myocardial infarction, heart failure, and left ventricular dysfunction.

Additionally, this therapeutic class is protective against progression of renal disease. In the management renal dysfunction, guidelines from both JNC-7 and National Kidney Foundation indicate that pharmacologic agents with the ability to reduce BP and micro- or macroalbuminuria are preferred [2, 11]; ACE inhibitors, dose-titrated to the moderate-high range, are the logical first choice (table 2).

ARBs

As depicted in figure 1, angiotensin II can be synthesized via an alternate biochemical pathway involving chymase [12]. ARBs selectively compete with angiotensin II at the level of the AT-1 receptor thus attenuating vasoconstriction, hypertrophy, sodium and water retention as well as sympathetic activation while the AT-2 receptor is unaffected, resulting in a lower BP [13]. ARBs can serve as suitable substitutes for ACE inhibitors if not tolerated. Similarly when used with diuretics, these agents have been shown to reduce mortality from cardiovascular events in the setting of left ventricular hypertrophy and microalbuminuria or nephropathy while protecting against the progression of renal disease [8, 9, 14]. In light of promising evidence, the American Diabetes Association has recently recommended that ARBs be the treatment of choice in type-2 diabetes with diabetic nephropathy [15].

Table 2. Dose and price comparisons of ACE inhibitors and ARBs

Drug	Trade name	Dose range, mg/day[†] (frequency)	Cost[††]
ACE inhibitors			
Benazepril	Lotensin	20–40 (1)*	$27.60
Captopril	Capoten	25–150 (2–3)	$62.40
	Generic		$19.50
Enalapril	Vasotec	10–40 (1)*	$43.50
Fosinopril	Monopril	20–40 (1)*	$29.70
Lisinopril	Prinivil, Zestril	20–40 (1)	$30.00
Moexipril	Univasc	7.5–30 (1)*	$20.40
Perindopril	Aceon	4–8 (1)*	$29.40
Quinapril	Accupril	20–80 (1)*	$33.90
Ramipril	Altace	2.5–20 (1)*	$33.30
Trandolapril	Mavik	2–4 (1)	$22.80
Angiotensin II receptor antagonists			
Losartan	Cozaar	50–100 (1)*	$44.40
Valsartan	Diovan	80–320 (1)	$40.20
Irbesartan	Avapro	150–300 (1)	$40.50
Telmisartan	Micardis	40–80 (1)	$38.57
Candesartan	Atacard	16–32 (1)	$38.70

*The drug may be given in divided doses at the higher dose levels.

[†]The dose range refers to the treatment of patients with hypertension. Doses as low as one-half or one-quarter the lowest dose may be used initially in high-risk patients such as those with congestive heart failure.

[††]Data from Med Lett Drugs Ther 1998;40:19, 1998;40:109, and 1999;40:105; cost represents that to pharmacist for 30 days' treatment at the usual lowest dose.

Renal Benefits of ACE Inhibitors and ARBs

At the onset of hypertension, the kidney is initially able to autoregulate renal blood flow and glomerular filtration via a process involving tubuloglomerular feedback from the macula densa and stretch receptors [16, 17]. With time, nephron loss occurs and the remaining nephrons undergo hypertrophy with a concomitant decrease in renal arteriolar resistance and an elevation in glomerular plasma flow [18, 19]. Angiotensin II mediates hypertension through arteriolar vasoconstriction, sodium and water retention as well as sympathetic hyperactivity [12].

Additionally, angiotensin II also affects renal hemodynamics by constricting both afferent and efferent arterioles, preferentially increasing efferent resistance with a resultant elevation in glomerular capillary hydraulic pressure and increased nephron filtration work [20]. In essence, the residual nephrons function at a relatively higher baseline pressure to maintain stable renal function. Ultimately, these 'compensatory' changes result in further renal demise. In the setting of preexisting renal insufficiency, aggressive BP control without RAS blockade is likely to lead to a rise in serum creatinine level due to loss of renal reserve and autoregulatory ability [21].

Interference of angiotensin II in the glomerulus likely improves glomerular permselectivity, a process that may be partially independent from changes in glomerular pressure [22]. In the hypertensive patient, ACE inhibitors and ARBs mechanistically effect the renal function via the glomerular actions of angiotensin II and the autoregulation of the glomerular filtration rate [23]. This can be seen in the AIPRI trial whereby patients randomized to the ACE inhibitor benazepril achieved a greater improvement in renal function compared to placebo, with a 66% risk reduction in the disease progression [24]. In one particular subgroup of hypertension, diabetics with advanced nephropathy and macroalbuminuria, the largest renoprotection is derived from treatment involving ACE inhibitors, with similar result for ARB therapy [8, 14, 15, 25].

Dosing

Proteinuria defined as >300 mg albumin/g creatinine in a spot-morning urine signifies renal disease and is strongly associated with an increased risk for kidney disease progression. Proteinuria despite adequate BP control is indicative of progressive renal disease and is itself a marker and independent risk factor for major cardiovascular (CV) events [26]. The dose titration of ACE inhibitors and ARBs beyond BP control to maximally reduce proteinuria and albuminuria has recently been advocated as a strategy to optimize CV risk profiles [27–29], especially in patients with diabetes [8, 14, 29, 30].

Interventions that completely inhibit RAS are more likely to provide to better long-term outcomes in reducing CV and renal outcomes. These approaches include maximal ACE inhibition or angiotensin-receptor blockade as well as combination therapy of either an ACE inhibitor or an ARB used in conjunction with either diuretics or sodium restriction. In a substudy of HOPE, ramipril at 10 mg/day demonstrated a slower rate of carotid atherosclerosis at a lower dose of 2.5 mg/day [31]. Likewise, the stark contrast between submaximal and higher dosage ARBs, as seen with the comparison of OPTIMAAL and
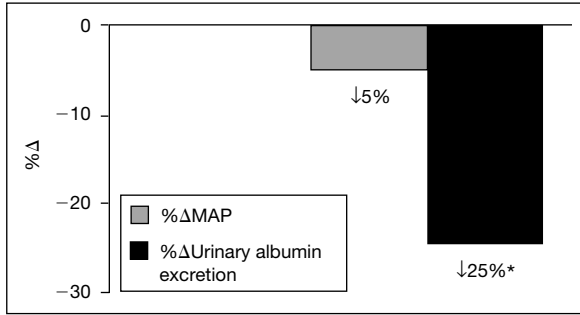
Fig. 2. Percent change of BP and albuminuria when high dose ARB is added to the maximally recommended dose of ACE inhibitor for diabetic nephropathy. *Dual blockade + ARB (300 mg irbesartan) was added to existing ACE inhibitor (100 mg captopril or 20 mg lisinopril) therapy. n = 24 patients; Rx = 8 weeks; baseline BP = 131/74 (Δ 8/4) mm Hg; albuminuria = 519 mg/24 h.

ELITE II (losartan 50 mg/day) to RENAAL and LIFE (losartan 100 mg/day), suggests that higher doses may confer a greater advantage through a more complete RAS blockade [9, 14, 32, 33]. Nevertheless, conflicting data exists that indicate neutral to modest benefits from high-dose treatment using ACE inhibitor or ARB [34–36].

The alternative pathway synthesis of angiotensin II may likely be responsible for the continued progression of renal disease despite chronic high-dose ACE inhibitor therapy [37]. Concomitant use of ACE inhibitors with ARBs has been postulated to further reduce proteinuria and has now proven been beneficial for both nondiabetic kidney disease as well as for diabetic nephropathy [38–41] (fig. 2). Sodium intake can strongly affect response to ACE inhibitors or ARBs by activating or inhibiting RAS activity; in another combined ACE inhibitor + ARB study, changes in urinary protein excretion were increased beyond that seen with monotherapy and was greatly influenced by sodium restriction in a subset of subjects [42]. Unfortunately, recent outcomes from concurrent ACE inhibitor and ARB therapy for heart failure and coronary artery disease do not provide similar results [43, 44] (fig. 3).

Safety

Adverse reactions from ACE inhibitors include cough, angioedema, skin rashes and dangerous functional renal insufficiency with resultant renal failure in patients with undiagnosed renal artery stenosis. Cough, described as dry, and persistent, occurs typically in 5–20% of patients, usually beginning within the
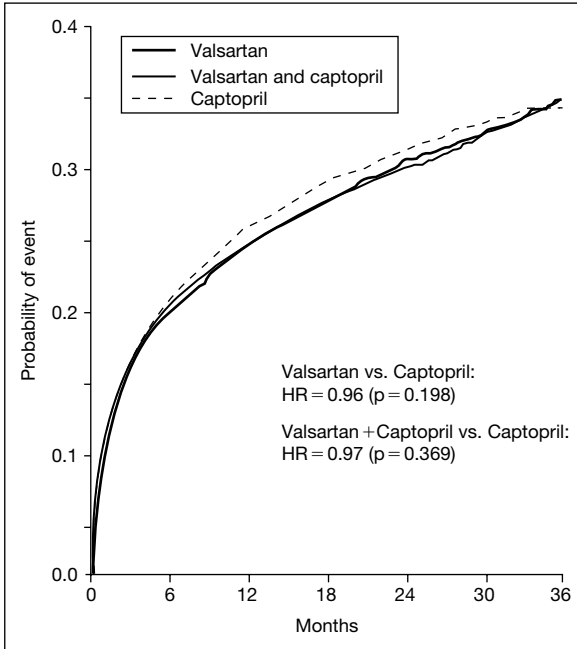
Fig. 3. Combined cardiovascular endpoint.

first 2 weeks of therapy, but can be delayed up to 6 months, with a strong predilection for women [45]. Angioedema occurs in 0.1–0.7% of patients, appearing within hours or weeks, but can occur as late as more than 1 year and is not related to ACE inhibitor-induced cough. It is characterized by a well-demarcated swelling of the mouth, tongue, pharynx and eyelids, and occasionally laryngeal obstruction. Patients with a prior history of idiopathic angioedema may be at increased risk for developing angioedema when using an ACE inhibitor. Management of either cough or angioedema involves discontinuing therapy; cough frequently resolves within days while angioedema may require hospital observation.

Cough is uncommon with ARBs; the frequency of cough for patients on telmisartan or losartan is significantly lower than lisinopril and comparable to that with diuretics or placebo [46, 47]. Some cases of angioedema have been reported with ARB [46, 48]. Moreover, patients who develop angioedema on an ACE inhibitor have, in a minority of cases, also had it recur with an ARB.

Both ACE inhibitors and ARBs are contraindicated during pregnancy, due to the association with an increased incidence of fetal complications.

In patients with renal insufficiency, serum potassium is maintained within the normal range, despite reduced nephron mass, by an increase in potassium excretion by the remaining distal nephrons via the mechanism of increased aldosterone production. Therapy with ACE inhibitors and/or ARBs reduces aldosterone secretion, thereby impairing urinary potassium excretion with the resultant development of hyperkalemia. ACE inhibitors and ARBs generally raise the plasma potassium concentration by <0.5 mEq/l in patients with relatively normal renal function [49]. Independent factors conducive to hyperkalemia are elevated creatinine level (>1.6 mg/dl), congestive heart failure and an increase in serum urea nitrogen level >18 mg/dl [50]. Once modest hyperkalemia has been identified, predictors of severe hyperkalemia (>6.0 mmol/l) include age >70 years and a serum urea nitrogen level >25 mg/dl. Use of diuretics has been shown to ameliorate hyperkalemia.

As depicted in the AIPRI trial, patients on an ACE inhibitor develop an increased serum creatinine concentration during the first 2 months of therapy [24]; this is likewise inferred for ARBs. A 30% increase in serum creatinine (baseline <3 mg/dl) within the initial 4 months of starting therapy or achievement of goal BP $<130/80$ mm Hg are prognostic signs that correlate with reduction in the progression of renal disease. (It is assumed that serum potassium is maintained <6.0 mEq/l and the rise in Cr stabilizes after this 4-month period.) In the absence of heart failure, if serum Cr rises by more than 30% and continues to rise within the first 2 months of starting ACE inhibitors or ARBs, chronic volume depletion or bilateral renal artery stenosis need to be ruled out.

Recommendations

Evidence from clinical trials reveal that the maximum approved doses for ACE inhibitors and ARBs should be used in patients with either diabetic or nondiabetic renal disease and/or proteinuria [51–53]. In patients wherein proteinuria persists despite high-dose RAS single-therapy blockade, an alternative and appropriate strategy is the combined use of ACE inhibitor and ARB although it is difficult to determine the effectiveness of this approach as there is a tendency to combine low to moderate doses of ACE inhibitors and ARBs together without first titrating the initial agent to its maximum [54, 55].

The 'maximal dosing strategy' is characterized by inherent complexities due to large variable individual responses to RAS blockade. It is difficult to ascertain the most effective approach to maximize the reduction in proteinuria and CV events. In a recent small but promising study of hypertensive patients with proteinuria, candesartan cilexetil dosed at five times its therapeutic

maximum (160 mg/day) effectively reduced the rate of kidney disease progression without adverse effects or significant changes in plasma potassium and creatinine [56].

In summary, ACE inhibitors and ARBs, either as monotherapy or in combination, have evolved into accepted first-line agents for management of hypertension in patients with diabetes and/or renal dysfunction largely via their effects upon the RAS. Clinical evidence indicates that the beneficial blockade of RAS extend beyond the realm of BP control. Nevertheless, the question exists regarding the optimal dosage of ACE inhibitors or ARBs for preventing individual CV and renal outcomes. Given that ARBs have no dose-limiting side effects, and to a lesser extent ACE inhibitors, it is very possible to safely adjust patients to supramaximal therapeutic doses of these medications to determine the greatest benefit.

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Renal Renin-Angiotensin System

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The cardinal role of the intrarenal renin-angiotensin system (RAS) in the control of sodium excretion and the pathophysiology of hypertension continues to receive increased attention. In addition to its very powerful vasoconstrictor action, angiotensin (Ang) II exerts important actions on tubular transport function and several recent studies have emphasized the potential importance of actions of angiotensin peptides on receptors localized to the luminal membranes of both proximal and distal nephron segments. Furthermore, a strong case is being developed supporting the importance of local mechanisms regulating the activity of the RAS. This is due to the fact that all components of the RAS are strongly expressed in the kidneys.

Intrarenal Localization of Components of the RAS

Angiotensinogen

In situ hybridization studies have demonstrated that the angiotensinogen gene is specifically present in the proximal tubules [1]. Angiotensinogen mRNA is expressed largely in the proximal convoluted tubules and proximal straight tubules, and only small amounts are expressed in glomeruli and vasa recta as revealed by reverse transcription and polymerase chain reaction [2]. In addition, immunohistochemical studies have showed that renal angiotensinogen protein is specifically located in the proximal convoluted tubules by immunohistochemistry [3–5]. There is strong positive immunostaining for angiotensinogen protein in proximal convoluted tubules and proximal straight tubules, and weak positive staining in glomeruli and vasa recta; however, there is no perceptible staining in distal tubules or collecting ducts [6].

Renin

The juxtaglomerular apparatus (JGA) cells have abundant expression of renin mRNA [7] and protein [8, 9], and renin is primarily generated in and secreted by the JGA to the circulating system [10]. The circulating renin acts on systemic angiotensinogen and also can enter organs and contribute to the activation of the local RAS [11]. Renin mRNA and renin-like activity have also been demonstrated in proximal and distal tubular cells [12–14]. In addition, low but measurable renin concentrations in proximal tubule fluid have been reported in rats [15]. Renin has been localized to collecting duct cells as well suggesting a role in the activation of angiotensin in the distal nephron. Thus, local renin may contribute to the activation of the local RAS as a pracrine/autocrine factor.

Angiotensin-Converting Enzyme (ACE)

In addition to its localization on endothelial cells of the renal microvasculature, there is abundant expression of ACE mRNA and protein in brush border of proximal tubules [16, 17]. ACE has also been measured in proximal and distal tubular fluid but is more plentiful in proximal tubule fluid [18].

Angiotensin II Receptors

There are two major types of angiotensin II receptors type 1 (AT1) receptors and type 2 (AT2) receptors, but there is much less AT2 receptor expression in adult kidneys [19, 20]. AT1 receptor mRNA has been localized to proximal convoluted and straight tubules, thick ascending limb of the loop of Henle, cortical and medullary collecting duct cells, glomeruli, arterial vasculature, vasa recta, and juxtaglomerular cells [2]. In rodents, AT1 subtypes (AT1A and AT1B receptor subtypes) mRNAs have been demonstrated in the vasculature and glomerulus and in all nephron segments [20]. The AT1A receptor mRNA is the predominant subtype in nephron segments, whereas the AT1B receptor is more abundant than AT1A receptor in the glomerulus [21].

Studies using polyclonal and monoclonal antibodies to the AT1 receptor demonstrated that AT1 receptor protein is localized on vascular smooth muscle cells throughout the vasculature, including the afferent and efferent arterioles and mesangial cells [22], and on brush border and basolateral membranes of proximal tubules, thick ascending limb epithelia, distal tubules, collecting ducts, glomerular podocytes, and macula densa cells [19, 20, 22]. A recent study using confocal laser microscopy has shown the immunohistochemical localization of AT1 and AT2 receptors in isolated juxtaglomerular cells containing renin granules [9]. Both AT1 and AT2 receptors were detected not only on the cell surface but also in the cytoplasm, however, AT2 receptor signals indicated a lower expression level compared to AT1 receptor signals under normal

conditions. These results suggest an important role of AT receptors in the functions of the JGA.

Effects of Angiotensin II on Juxtaglomerular Apparatus

In addition to its direct vasoconstrictor effects, the RAS exerts an important modulatory influence on the magnitude of the tubuloglomerular feedback (TGF) mechanism with high angiotensin levels causing increased TGF sensitivity. Enhanced TGF activity is associated with the development of systemic hypertension in several models of hypertension including two-kidney, one-clip Goldblatt hypertension [23], one-kidney, one-clip hypertension [24], hypertensive *ren*-2 transgenic rats [25] and genetic hypertensive rats [26]. It has also been observed in the remnant kidneys of prehypertensive rats [27] and the kidneys of spontaneously hypertensive rats whose perfusion pressure is normalized by aortic coarctation [28]. In these animals, administration of ACE inhibitors or AT1 receptor blockers significantly reduce the sensitivity of the TGF mechanism [23–27], and peritubular infusions of Ang I and II enhanced the TGF activity [29]. Thus, Ang II contributes to the development of hypertension through its positive modulating effects on the TGF mechanism.

Recent studies have demonstrated the presence of neuronal nitric oxide synthase (nNOS) [30], cyclooxygenase-2 (COX-2) [31], and cytochrome P_{450} [32] in macula densa and adjoining ascending loop of Henle cells. Nitric oxide (NO), and arachidonic acid metabolites have been shown to exert modulating roles on the TGF mechanism as shown in figure 1. NO derived from nNOS counteracts afferent arteriolar constriction during enhanced TGF activity [33]. In addition, eNOS-derived NO and nNOS-derived NO respectively inhibit the afferent and efferent arteriolar responses to exogenous Ang II [34]. In Ang II-induced hypertension, the ability of nNOS-derived NO to counteract the TGF-mediated afferent arteriolar constriction is reduced [35]. Ang II stimulates superoxide production in vascular smooth muscle cells through phospholipase D-dependent pathways [36], and superoxide scavenges NO to form peroxynitrite ($ONOO^-$), a short-lived and less potent vasorelaxant than NO [37]. Thus, superoxide may mediate the decreased ability of nNOS-derived NO to counteract the TGF-mediated afferent arteriolar constriction in Ang II-induced hypertension. This concept is supported by recent evidence that the superoxide dismutase mimetic, tempol, restores the reduced bioavailability of nNOS-derived NO in spontaneously hypertensive rats [38]. In addition, the expression of the nNOS gene and protein in the macula densa are upregulated in angiotensinogen-gene-knockout mice [39] and AT1 receptor-deficient mice [40]. Interestingly, the enhanced ability of nNOS-derived NO to counteract the TGF-mediated afferent arteriolar constriction observed in
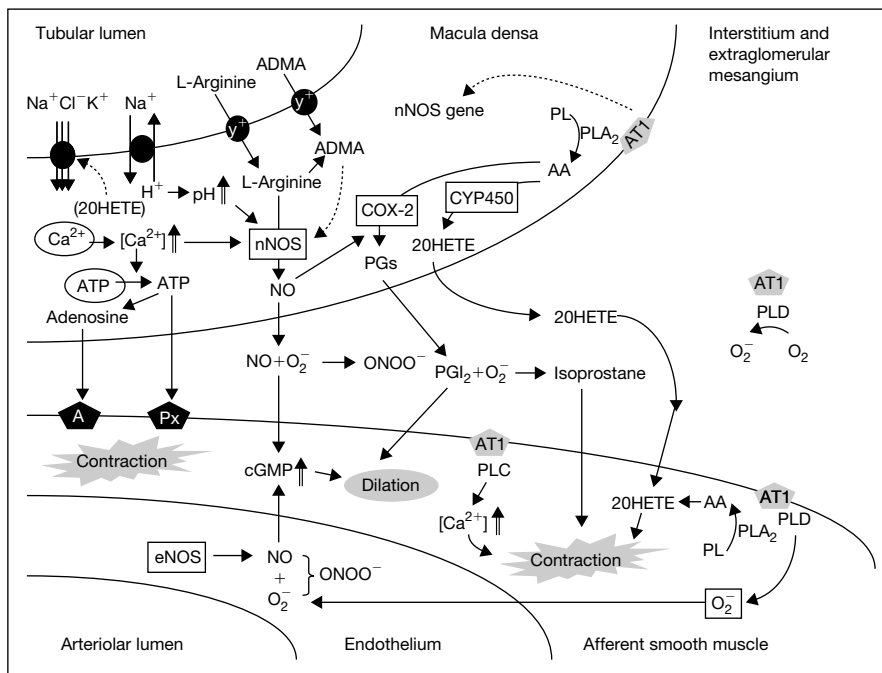
Fig. 1. Pivotal roles of angiotensin type 1 (AT1) receptors in the modulation of tubuloglomerular feedback responses. AA, arachidonic acid; ADMA, asymmetric, N^G-dimethyl-L-arginine; ATP, adenosine 5′-triphosphate; cGMP, guanosine 3′,5′-cyclic monophosphate; COX-2, cyclooxygenase-2; CYP450, cytochrome P_{450}; eNOS, endothelial nitric oxide synthase; 20HETE, 20-hydroxyeicosatetraenoic acid; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; ONOO⁻, peroxynitrite; PGs, prostaglandins; PL, phospholipid; PLA₂, phospholipase A₂; PLC, phospholipase C; PLD, phospholipase D.

AT1 receptor-deficient mice [41], suggests that the modulation of TGF responses by Ang II is partially due to decreased activity of macula densa nNOS.

Ang II generates arachidonic acids from phospholipids by stimulating phospholipase A_2. The cyclooxygenase pathway is a major route of arachidonic acid metabolism in the kidney [42], and the arachidonic acid metabolites generated by the COX-2 adjacent to the macula densa counteracts the TGF-mediated afferent arteriolar constriction directly and indirectly through interacting with nNOS-derived NO [43, 44]. In addition, superoxide interacts with arachidonic acid metabolites to form the vasoconstrictor, isoprostane. Because Ang II stimulates superoxide production in vascular smooth muscle cells, part of the effect of Ang II to modulate the TGF responses may be through the COX-2-dependent pathways.

The cytochrone P_{450} pathway is also a route of arachidonic acid metabolism in the kidney [42], and 20-HETE generated by cytochrome P_{450} has a constrictor effect on afferent arterioles [45] and thus may also contribute to the exaggerated TGF response in Ang II-dependent hypertension.

Ang II inhibits renin secretion in primary culture of juxtaglomerular cells [8, 46]. A recent study demonstrated unique roles of AT1 and AT2 receptors in renin synthesis and secretion from juxtaglomerular cells [9]. Increased Ang II levels in the culture medium inhibited renin secretion from juxtaglomerular cells without affecting total intracellular renin content. In the presence of AT1 receptor blockers, however, increased medium Ang II levels reduced intracellular active renin content without affecting renin secretion or total intracellular renin content, and the reduced intracellular active renin content was resumed by add-on administration of AT2 receptor blockers. In addition, AT2 receptor decreased intracellular active renin content without affecting intracellular total renin content. These results suggest that AT1 receptors inhibit renin secretion from juxtaglomerular cells, while AT2 receptors inhibit conversion of inactive to active renin (prorenin processing) in juxtaglomerular cells.

Renal Hemodynamic Regulation by Intrarenal Angiotensin II

In Ang II-induced hypertension, acute administration of the AT1 receptor blocker, losartan, significantly increases cortical blood flow, total renal plasma flow, glomerular filtration rate, and urinary sodium excretion [47]. Thus, intrarenal Ang II plays an important role in regulating renal hemodynamics. Of interest, the renal responses to acute losartan were also observed in Ang II-infused rats treated chronically with losartan, although acute losartan decreased blood pressure only slightly, demonstrating adequate systemic vascular blockade, in these animal models [47]. Therefore, substantive intrarenal actions of Ang II can be maintained even when the systemic vascular AT1 receptors are effectively blocked.

When intrarenal Ang II influences renal hemodynamics, NO plays an important role in the modulation of renal responses to intrarenal Ang II. In Ang II-induced hypertension, intrarenal production of NO is similar to that in normotensive control rats [48] with an enhanced expression of eNOS and nNOS [49]. Both endogenous and exogenous NOs counteract afferent and efferent arteriolar constrictor responses to Ang II [48]. Although afferent arteriolar responses to Ang II were enhanced in Ang II-induced hypertension [50], the buffering effects of endogenous NO on the Ang II-induced vasoconstriction was greater in afferent arterioles than in efferent arterioles [48] and thus contributes to maintaining the renal circulation under conditions of elevated systemic Ang II levels [51].

Renal Interstitial Function of Angiotensin II

The intrarenal content of Ang II is not distributed in a homogenous manner but is compartmentalized in both a regional and segmental manner [52]. It has been reported that Ang II is present at high concentrations in renal interstitial fluid (RIF) thus contributing to the disproportionately high total Ang II levels in the kidney [52]. Earlier measurements of renal lymph suggested that RIF Ang II concentrations were much higher than arterial or renal venous plasma concentrations [53]. More recent studies assessed RIF concentrations of Ang peptides using microdialysis probes implanted in the renal cortex [54–58]. Using this procedure, several studies demonstrated that RIF concentrations of Ang I and II are much higher than the corresponding plasma concentrations [54, 55] (fig. 2a). We also found that acute ACE inhibition failed to lower the RIF Ang II concentrations significantly or decreased it only a small percentage, suggesting that much of the RIF Ang II may be derived from sites not readily accessible to ACE inhibitors [54, 55] (fig. 2b,c). In addition, acute extracellular volume expansion lowered the plasma Ang I and II levels but failed to lower the RIF Ang I and II concentrations. Interestingly, interstitial infusion of Ang I significantly increased the RIF Ang II concentration, and this conversion was blocked by the addition of enalaprilat to the perfusate [54]. These results demonstrate that there is ACE activity in the interstitial compartment. However, the failure of ACE inhibitors to reduce endogenous RIF Ang II concentrations substantially suggests that much of the RIF Ang II is formed at sites not readily accessible to ACE inhibitors or is formed via non-ACE-dependent pathways such as cathepsin, chymase or tonin [52]. It was also demonstrated that the RIF Ang II levels in Ang II-infused hypertensive rats are augmented above control [56] (fig. 2d). These results suggest that at least part of the augmented Ang II content in the kidney from Ang II-infused hypertensive rats is distributed to the RIF. Zhuo et al. [59] showed that Ang II levels in renal cortical endosomes and intermicrovillar clefts are markedly increased in Ang II-infused hypertensive rats and that the increases in endosomal and intermicrovillar cleft Ang II levels were prevented by concurrent administration of candesartan. These results suggest that intracellular trafficking or accumulation of circulating and/or intrarenally formed Ang II into cortical tubular endosomes are enhanced during Ang II-dependent hypertension, and that this process is mediated by AT1 receptors. It is possible that accumulation of endosomal Ang II levels may result in translocation of part of the intracellular Ang II into the renal interstitial space during the development of Ang II-induced hypertension. In addition, Ang II-induced hypertension has also been associated with increased angiotensinogen (AGT) formation in the kidney [6]. Therefore, this pathway could also lead to de novo Ang II formation and secretion into the renal interstitial space. Treatment
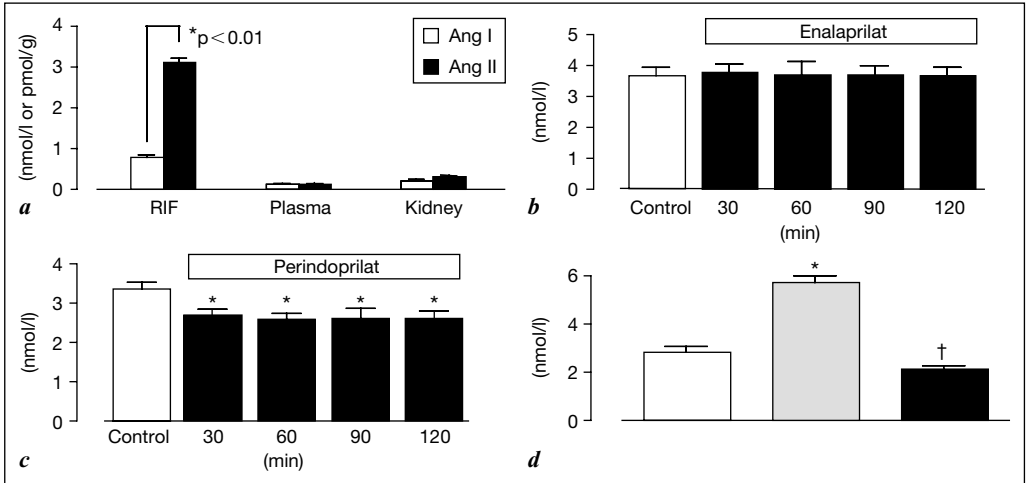
Fig. 2. a Comparison of Ang I and II levels in plasma, total kidney and RIF. RIF concentrations of Ang I and II were calculated based on equilibrium rates determined in vitro [data derived from 55]. ***b*** Effects of intra-arterial infusion of enalaprilat (7.5 μmol/kg/min) on RIF concentrations of Ang II [data derived from 55]. ***c*** Effects of interstitial infusion of perindoprilat (10 mmol/l in perfusate) on RIF concentrations of Ang II [data derived from 54]. *p < 0.05 vs. ***c***. ***d*** RIF concentrations of Ang II in normal rats (open bars), Ang II-infused hypertensive rats (hatched bars), and Ang II-infused rats treated with candesartan cilexetil (solid bars). *p < 0.05 vs. vehicle-infused rats. †p < 0.05 vs. Ang II-infused rats [data derived from 56].

with AT1 receptor blockers prevents the cascade involving ligand-receptor activation and internalization [59] with subsequent stimulation of angiotensinogen synthesis and release [60], leading to reductions in RIF concentration of Ang II [56] (fig. 1d).

Several studies have indicated that RIF Ang II exerts biological effects, including the regulation of microvascular tone, tubular sodium reabsorption and the TGF mechanism [42]. In Ang II-dependent hypertension, the elevated Ang II concentrations acting on vascular and tubular basolateral receptors may contribute to enhanced sodium transport and impaired pressure natriuresis as well as the increased vascular resistance [42, 61, 62]. Micropuncture studies by Mitchell and Navar [63] demonstrated that peritubular capillary infusion of 10–7 mol/l Ang II resulted in increases in fractional proximal tubular fluid reabsorption and decreases in tubule fluid flow, stop-flow pressure, and single nephron glomerular filtration rate. These results indicate that increases in the postglomerular interstitial Ang II concentration can enhance proximal tubular reabsorption and increase preglomerular resistance. Studies using the juxtamedullary

nephron preparation demonstrated that in Ang II-infused hypertension, afferent arteriolar responsiveness to Ang II administered from the interstitial side is significantly enhanced [50]. In addition, RIF Ang II levels may play an important role in the pathogenesis of tubulointerstitial changes when levels are inappropriately elevated [64]. It is likely that elevated RIF Ang II levels contribute to Ang II-dependent hypertension via multiple effects on the vasculature and the tubules leading to vasoconstriction, sodium retention and long-term proliferative and inflammatory responses.

Tubular Function of RAS

Angiotensin II

Ang II plays an important role in regulating proximal tubular reabsorptive function primarily via activation of AT1 receptors on both basolateral and luminal membranes [65]. This effect is mediated mainly by influencing the proximal sodium-hydrogen exchanger on the luminal membrane and the sodium-bicarbonate cotransporter on the basolateral membrane. Studies in isolated proximal tubular cells showed that Ang II stimulates Na^+/H^+ exchanger via AT1 receptors [66]. Although some AT2 receptors have been confirmed on proximal tubules [20], most functional studies suggest that the major effects of Ang II on proximal tubules are via AT1 receptors [67]. Recent studies have also indicted that Ang II contributes to the regulation of distal tubular sodium reabsorption rate [68]. This effect was blocked by either saralasin or losartan indicating that this effect also involves AT1 receptor activation [68]. These findings, together with the demonstration that AT receptors are present on the luminal membranes of distal nephron segments [22, 69] indicate that luminal Ang II plays an important role in the regulation not only of proximal reabsorption rate but also of distal tubular reabsorptive function [52]. Peti-Peterdi et al. [70] demonstrated that Ang II directly stimulates epithelial sodium channel activity in the cortical collecting duct via AT1R. Furthermore, epithelial sodium channel gene expression in the cortical collecting duct is upregulated by chronic infusion of Ang II [71]. Thus, Ang II exerts important effects on tubular transport rate in distal as well as in proximal tubular segments via its action on both basolateral and luminal receptors [65].

Angiotensinogen

It has been clearly established that the liver is the primary source of circulating angiotensinogen and angiotensinogen is constitutively secreted into the circulation yielding concentrations much higher than the free Ang I and Ang II concentrations. Thus, in most species including primates and rodents, the rate

of Ang I formation in the systemic circulation is determined primarily by the plasma renin activity. While angiotensinogen of liver origin may be an important source of substrate for intrarenal formation of the Ang peptides, it is now well recognized that angiotensinogen mRNA and protein are present in the kidney in proximal tubule cells. This finding, together with studies showing angiotensinogen in proximal tubule fluid and in the urine have led to the concept that much, if not all, of the angiotensinogen in the tubule and urine is of renal origin.

Lalouel and colleagues [72, 73] have focused on intratubular RAS. Confluent monolayers of conditionally immortalized cells of murine proximal tubules were grown on semipermeable membranes separating apical and basolateral compartments. In monolayers with verified integrity, angiotensinogen was reproducibly detected in the apical but not in the basolateral compartment [72]. In two mouse strains, angiotensinogen protein has been detected in urine. Water deprivation induced significant activation of tubular expression of angiotensinogen [73]. These data support the hypothesis that the intratubular angiotensinogen functionally exists and is regulated by pathophysiological conditions.

Sigmund and colleagues [74–76] developed a series of interesting models of inducible hypertension. Transgenic mice were generated in which a fragment of the kidney-specific androgen-regulated protein (KAP) promoter was fused to the human angiotensinogen (hAGT) gene. Renal expression of the transgene in female mice was undetectable under basal conditions but was strongly induced by administration of testosterone. In situ hybridization demonstrated that expression of hAGT mRNA in males and testosterone-treated females was restricted to proximal tubule epithelial cells in the renal cortex. Although there was no detectable hAGT protein in plasma, it was shown in the urine, consistent with a pathway of synthesis in proximal tubule cells and release into the tubular lumen [75]. Mouse angiotensinogen and hAGT have a similar structure; however, mouse renin is species-specific and cannot cleave hAGT [77]. Therefore the transferred hAGT was inactive in these mice, and these KAP-hAGT mice were normotensive. In double transgenic mice harboring both human renin gene and kidney-specific hAGT gene or systemic hAGT gene, plasma Ang II was elevated in the systemic model but not in the kidney-specific model. Nevertheless, blood pressure was markedly elevated in both transgenic mice. Acute administration of an AT1 receptor antagonist, losartan, into the circulation lowered blood pressure in the systemic model but not in the kidney-specific model [74]. These data support the hypothesis that the tissue-specific intratubular RAS can participate in the regulation of blood pressure independently of circulating RAS.

Kobori et al. [6] recently reported that Ang II-infused rats have increases in renal angiotensinogen mRNA and protein [78], and an enhancement of urinary excretion rate of angiotensinogen [79]. Chronic Ang II infusion to normal

rats significantly increased urinary excretion rate of angiotensinogen in a time- and dose-dependent manner which was associated with elevation in kidney Ang II levels. Urinary excretion rate of angiotensinogen was closely correlated with systolic blood pressure and kidney Ang II content, but not with plasma Ang II concentration. Urinary protein excretion in volume-dependent hypertensive rats was significantly increased more than in Ang II-dependent hypertensive rats; however, urinary angiotensinogen excretion was significantly lower in volume-dependent hypertensive rats than in Ang II-dependent hypertensive rats [80]. Rat angiotensinogen was detected in plasma and urine before and after the injection of hAGT. However, hAGT was detected only in the plasma collected after the administration of hAGT but was not detected in the urine in Ang II-dependent hypertensive and or sham-operated normotensive rats. The failure to detect hAGT in the urine suggests limited glomerular permeability and/or tubular degradation [80]. These data support the hypothesis that urinary angiotensinogen provides a specific index of intrarenal angiotensinogen production in Ang II-dependent hypertension.

Renin

In addition to renin formed by the JGA cells, renin is synthesized by principal cells of connecting tubules and cortical collecting ducts [72, 73]. Renin expression in connecting tubules was increased by sodium restriction [72]. Water deprivation induced significant activation in the tubular expression of renin [73]. These data support the hypothesis that intratubular renin functionally exists and is also regulated by pathophysiological conditions.

Prieto-Carrasquero et al. [81] recently reported that renin or renin-like immunoreactivity was enhanced in distal nephron segments of Ang II-dependent hypertensive rats while it was decreased in JGA. Concomitant with the enhancement of proximal tubular angiotensinogen, as described above, the increases in distal renin expression may help to explain the continued intrarenal formation of Ang II, which is observed in Ang II-dependent hypertension. This continued expression may contribute to the development and progression of high arterial pressure in this model. Such changes demonstrate the differential regulation of renin expression in distal tubular cells from that in cells of the JGA.

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Kidney and Blood Pressure Regulation

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Diabetes mellitus is a major cause of morbidity and mortality across the world, and its incidence continues to increase at alarming rates in all parts of the world. The microvascular and macrovascular complications of diabetes have become a major public health concern because of the extremely high rates of morbidity and premature death among diabetics. Diabetic renal disease is by far the main cause of end-stage renal disease (ESRD) in the USA, accounting for 44% of all new cases of ESRD in 2001 [1, 2]. It is the leading cause of morbidity and mortality among patients with chronic renal failure approaching the need for renal replacement therapy, and the main cause of death in patients undergoing dialysis treatment and recipients of a successful kidney transplant.

Until recent years, it was thought that type 1 diabetes mellitus was the main form responsible for kidney disease in diabetics. But with the explosive increase in type 2 diabetes among all age ranges, the total number of new dialysis patients with type 2 diabetes now far surpasses the number of type 1 diabetics. The classic hallmarks of diabetic nephropathy, both types 1 and 2, are progressive proteinuria (ranging from microalbuminuria to overt proteinuria), increasing blood pressure, and finally loss of glomerular filtration rate (GFR) leading to ESRD. These factors are also the main predictors of progression of diabetic kidney disease, with poor blood sugar control also contributing to the initiation of diabetic nephropathy. In the last decade, the medical community has witnessed major advances in reducing the risk of new diabetic renal disease and in delaying progression of established nephropathy by tight control of blood pressure and by blockade of the renin-angiotensin-aldosterone system (RAAS) using angiotensin-converting enzyme inhibitors (ACEinhs) and angiotensin II receptor blockers (ARBs). Use of these drugs has been demonstrated to delay progression of kidney disease not only in patients with overt hypertension, but
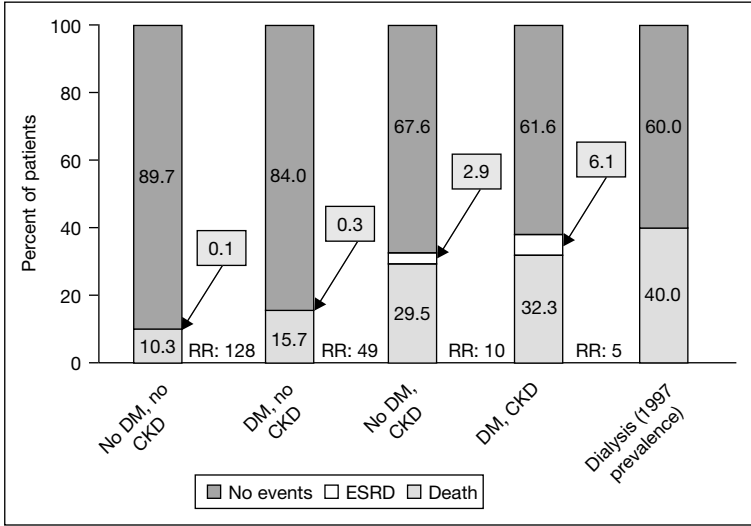
Fig. 1. Outcomes for Medicare patients during a 2-year follow-up and comparison with prevalent dialysis patients: risk of death vs. ESRD. CKD = Chronic kidney disease; DM = diabetes mellitus; ESRD = end-stage renal disease [adapted from 2, with permission].

appears to have stabilized or be slowly decreasing, the incidence of ESRD among younger non-white patients and older patients of all races (thought mostly to be type 2 diabetes) has increased over the 10 years from 1991 to 2001 at an average rate of 12% per year. Fortunately in the most recent data, there is some suggestion that the above rate of increase may have slowed somewhat.

ESRD is not the only adverse consequence of diabetes. Among patients in the Medicare population, the 2-year mortality rate among all patients with neither diabetes nor CKD was only 10.3% [2]. Among patients with CKD, the 2-year death rate was 29.5%, and among patients with CKD due to diabetes the death rate was 32.3% in 2 years, close to the death rate of 40% among ESRD patients (fig. 1). Indeed, despite the high 6.1% chance of ESRD within 2 years among diabetics with CKD, the chance of death is five times greater. The age-adjusted death rate in the entire dialysis population is 40-fold higher than in the general population [6], and it is higher (22% per year in 2001) among the diabetics than among any other group of ESRD patients (13–17% per year in 2001) [1]. Specifically, death from cardiovascular causes is 20-fold higher in ESRD patients compared to the general population. Strategies to prevent diabetic nephropathy and delay the progression of established diabetic kidney disease are critical both to improve cardiovascular outcomes in the general population and decrease the number of diabetics in the dialysis population.

Diabetic Nephropathy: Factors Affecting Progression and Pharmacological Intervention

Diabetic nephropathy is defined as a clinical syndrome characterized by persistent and increasing proteinuria, progressive hypertension, and progressive decline in renal function. Its rate of progression varies, and blood pressure control as well as rates of proteinuria plays a critical role. More than 20 years ago, Mogensen [7] reported successful slowing of the rate of progression of renal dysfunction in a patient with type 1 diabetes, achieved by lowering blood pressure with β-blockers and diuretics. They also observed that proteinuria had decreased with lowering of the blood pressure. This study, for the first time, suggested that blood pressure control alone is renoprotective in diabetics and can also decrease proteinuria. At that time, it was neither clear whether proteinuria played a role in the progression of diabetic nephropathy nor if it could be used as a surrogate marker for intervention in diabetic renal disease. The benefits of tight blood pressure control were subsequently confirmed by Parving et al. [8] in a small series of type 1 diabetic patients with nephropathy (fig. 2). These investigators were able to track the progress of their patients in the 2-years prior to presentation at the investigators' clinic. During this period the patients' proteinuria was increasing, they had become overtly hypertensive, and they were losing GFR at a rate of 11 ml/min/year. After control of their blood pressure to a target of about 130/80 mm Hg, using agents other than ACEinhs, their rate of loss of renal function slowed by two-thirds, and their proteinuria declined. After several years of good blood pressure control, their rate of loss of GFR slowed to close to the normal 1 ml/min/year seen in all adults. There has never been a controlled trial confirming that blood pressures below 140/90 mm Hg slows loss of renal function in diabetics with overt nephropathy, but, as discussed below, there is now strong confirmatory observational data from large studies of patients with type 2 diabetic nephropathy, and tight blood pressure control has become one of the cornerstones of treatment of diabetic nephropathy.

Following seminal experimental studies in animals with diabetes and kidney disease, comparing blockade of the RAAS with ACEinhs versus use of conventional antihypertensive drugs (see chapters 9 and 10), pilot trials in humans were initiated to evaluate whether RAAS blockade would have a renoprotective effect in human diabetic renal disease. A pioneering clinical study evaluating reduction in proteinuria in diabetic human disease was published by Marre et al. [9] in the 1980s. Studying 20 diabetic, microalbuminuric patients (type 1 and type 2) for 1 year, they observed that the ACEinh enalapril reduced blood pressure and microalbuminuria. In the early 1990s, Lewis et al. [10] published the results of a large, randomized, prospective study using captopril in type 1
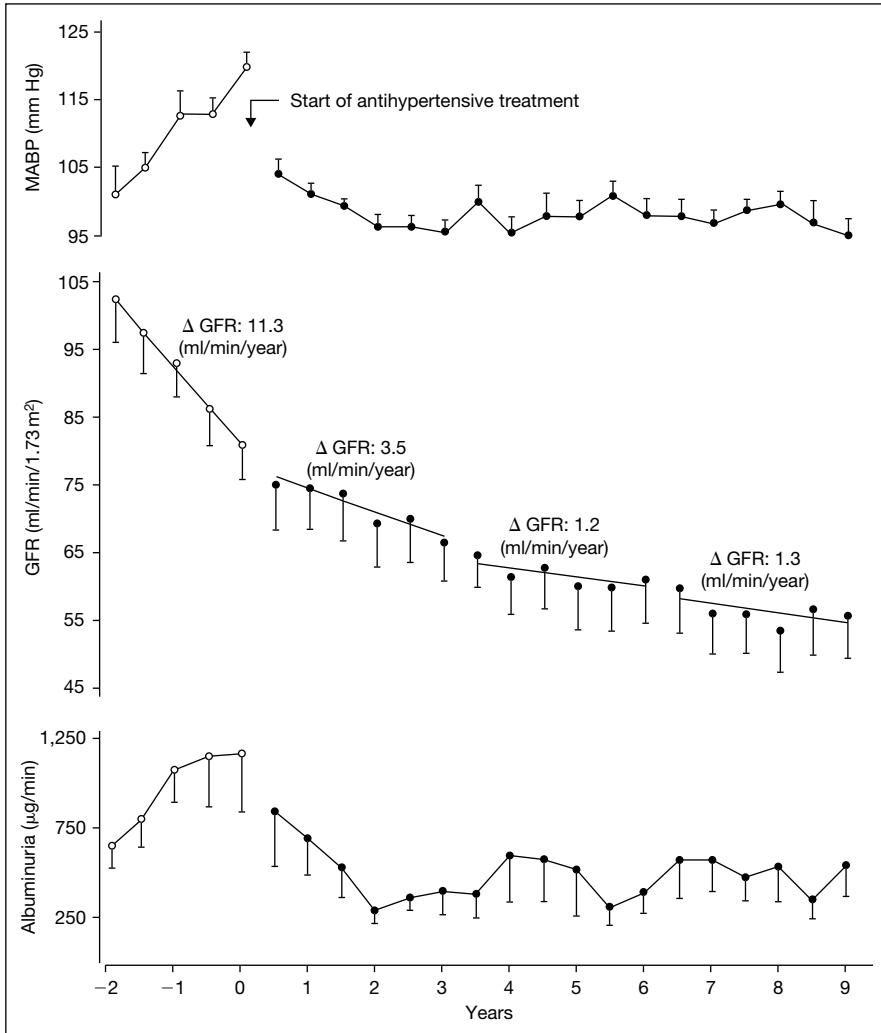
Fig. 2. Average course of mean arterial blood pressure, GFR, and urine albumin excretion before (○) and during (●) long-term effective antihypertensive treatment on 9 patients suffering from type 1 diabetic nephropathy [reprinted from 8, with permission].

diabetics with overt nephropathy. This study showed a clear advantage for the group receiving captopril, in whom the risks of doubling of baseline creatinine and of progression to ESRD during the observational period were significantly lower compared to the group of patients receiving a placebo, despite similar target blood pressure reduction in both groups (fig. 3).
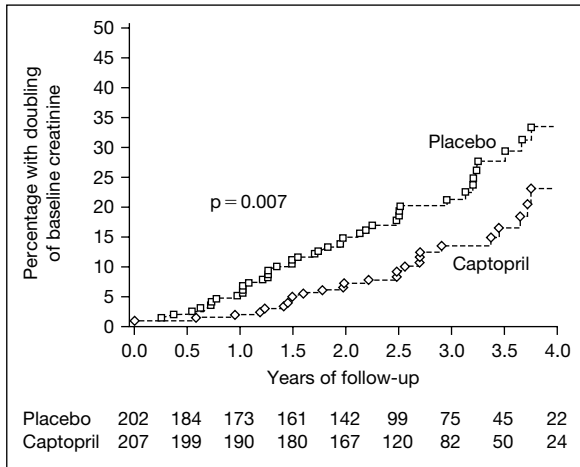
Fig. 3. Lewis Captopril Study: Cumulative incidence of doubling of baseline serum creatinine events in patients with type 1 diabetic nephropathy in the captopril and placebo groups. The numbers at the bottom of the panel are the numbers of patients in each group at risk for the event at baseline and after each 6-month period [reprinted from 10, with permission].

More recently a newer class of antihypertensive agents blocking the RAAS, the ARBs, has been demonstrated to have similar renoprotective effects to ACEinhs on progression of overt diabetic kidney disease. Two large, prospective randomized trials testing ARBs in patients with overt type 2 diabetic nephropathy have been published in the last 2 years and warrant discussion in detail.

The Irbesartan Diabetic Nephropathy Trial (IDNT) [11] was a randomized, double-blind, placebo-controlled prospective trial in hypertensive patients with overt nephropathy due to type 2 diabetes. Patients were randomized to receive the ARB irbesartan 300 mg/day, a calcium channel blocker, amlodipine, at 10 mg/day, or placebo. Blood pressure in the three groups was controlled to the same goal of 135/85 mm Hg using conventional antihypertensives other than ACEinhs, ARBs, and calcium channel blockers. The primary endpoint was time to a composite outcome of doubling of baseline creatinine, the development of ESRD, or death from any cause. Patients were followed for a mean duration of 2.6 years. Patients assigned to the irbesartan arm had a 20% (p = 0.024) reduction of the risk of the primary outcome compared with patients assigned to placebo, and a 23% (p = 0.006) reduction compared with the patients assigned to amlodipine (fig. 4). The risk of a renal endpoint (doubling of serum creatinine or ESRD) was 26% lower in the irbesartan group compared to the placebo (p = 0.012) and 37% lower compared to the amlodipine group (p = 0.003)
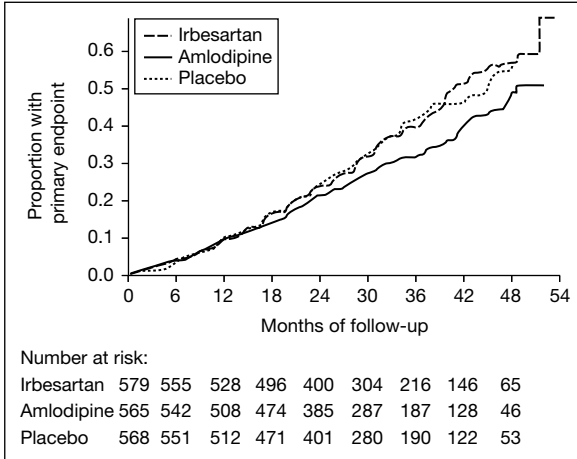
Fig. 4. IDNT: Cumulative proportions of type 2 diabetic nephropathy patients with the primary composite endpoint (doubling of baseline serum creatinine, ESRD, or death from any cause). The numbers at the bottom of the panel are the numbers of patients in each group at risk for the event at baseline and after each 6-month period [reprinted from 11, with permission].

[16]. There was no difference among the three groups in the risk of all cause mortality. A secondary analysis of the IDNT data [12] focused on the impact of achieved systolic blood pressure during follow-up on the risk of a renal endpoint. This analysis revealed that the study patients achieving the lowest quartile of systolic blood pressures (<132 mm Hg) had the lowest risk of a renal endpoint. These data reinforce the conclusion that both RAAS blockade and blood pressure reduction are critical targets in preventing progression of diabetic kidney disease.

The Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan study (RENAAL) compared the ARB losartan (100 mg/day) to placebo in another double-blind, randomized trial in hypertensive patients with type 2 diabetic nephropathy [13]. In this study, too, blood pressure was controlled to the same target in both treatment groups using antihypertensive drugs other than ACEinhs or ARBs. This study used the same composite endpoint as in the IDNT – occurrence of doubling of serum creatinine, ESRD, or death from any cause. These authors demonstrated 16% reduction of the risk of this composite endpoint in the losartan group compared with the placebo group ($p = 0.022$) (fig. 5). The risk of doubling of serum creatinine was reduced by 25% and of ESRD by 28%. Again, no change in rate of death was observed during the observational period.
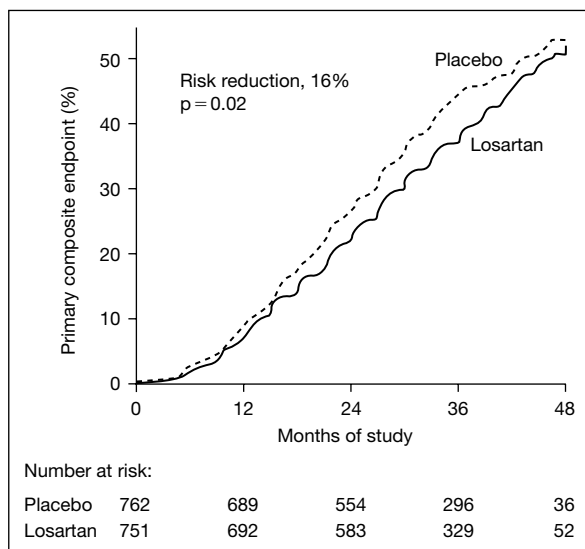
Fig. 5. RENAAL: Cumulative percent of type 2 diabetic nephropathy patients with the primary composite endpoint (doubling of baseline serum creatinine, ESRD, or death from any cause). The numbers at the bottom of the panel are the numbers of patients in each group at risk for the event at baseline and after each 12-month period [reprinted from 13, with permission].

Proteinuria as a Surrogate for Later Renal Events, and Management of Early Diabetic Kidney Disease

Proteinuria was reduced in both the IDNT (by 30%) and the RENAAL (by 35%) studies in patients assigned to the ARBs irbesartan and losartan. There is increasing evidence that the reduction of proteinuria in response to blockade of the RAAS is a predictor of, and therefore an early surrogate for, ultimate renal outcomes. Atkins et al. [14], in another secondary analysis from the IDNT trial, studied the effect of baseline proteinuria and change in proteinuria on the risk of renal outcomes. This study showed that reduction in proteinuria from baseline was strongly correlated with protection against progression of renal disease. Among the 1,261 patients with proteinuria at both baseline and 12 months of follow-up, reduction of proteinuria at 12 months was associated with a significant reduction of risk of a renal endpoint (RR = 0.52 for each halving of proteinuria, $p < 0.0001$).

Several long-term follow-up studies in both types 1 and 2 diabetics have confirmed that microalbuminuria is also the single most important predictor of development of overt diabetic nephropathy and ESRD among diabetics that do

not yet have overt kidney disease. The prevalence of microalbuminuria and macroalbuminuria is approximately 30–35% in both types of diabetes. It appears that the risk of developing overt nephropathy is higher for proteinuric type 1 diabetics compared to proteinuric type 2 diabetics, but this is likely due to the increased competing risk of death in type 2 diabetics. Since it is not practical to follow patients in a clinical trial from first presentation with microalbuminuria to final ESRD, the above observations suggest that reduction of proteinuria itself might be an appropriate surrogate by which to evaluate the efficacy of RAAS blockade in the earlier stages of diabetic kidney disease.

In fact, ACEinhs and ARBs have also been administered to normotensive patients with diabetes normal urinary albumin or microalbuminuria to see whether this intervention would prevent progression to overt diabetic nephropathy in these patients. Pedersen et al. [15] was the first group to observe reduction of albuminuria at 3 months of treatment with ACEinhs. These patients were normoalbuminuric at baseline but had a lower rate of excretion at 3 months. A larger, long-term study was conducted by Ravid et al. [16] using enalapril in normotensive, normoalbuminuric type 2 diabetics and followed these patients for 6 years. They were able to demonstrate that enalapril reduced the absolute risk for development of microalbuminuria by 12.5% compared to placebo, despite same mean blood pressure in both groups. Furthermore, the rate of decrease in creatinine clearance was significantly lower in the enalapril group. Zandbergen et al. [17] treated normotensive, normoalbuminuric type 2 diabetics with losartan for 10 weeks with different doses. They observed a reduction in albumin excretion of 25 and 34% using 50 and 100 mg of losartan respectively. This was a short-term study and progression of renal disease could not be evaluated.

The most definitive study of the impact of blockade of the RAAS in protection from progression of early diabetic nephropathy (microalbuminuria) has been the Irbesartan in Microalbuminuric Type 2 Diabetic Nephropathy (IRMA-2) study. Parving et al. [18] studied the effects of the ARB irbesartan, in patients with type II diabetes, hypertension, and microalbuminuria. This was a large, randomized, double-blind, placebo-controlled study testing two different doses of irbesartan, 150 and 300 mg daily. The primary endpoint was time to onset of diabetic nephropathy, defined by persistent albuminuria $>200\,\mu g/min$ with a final value at least 30% higher than baseline level. During the 24-month study, the group of patients receiving 300 mg daily had a statistical significantly lower rate of progression to overt diabetic nephropathy compared to the placebo group (5.9% for 300 mg irbesartan and 14.9% for the placebo group; $p < 0.001$). The group taking 150 mg of irbesartan also had lower incidence of diabetic nephropathy compared to the placebo group, but this difference did not reach statistical significance (9.7% for irbesartan and 14.9% for the placebo group; $p = 0.08$) (fig. 6).
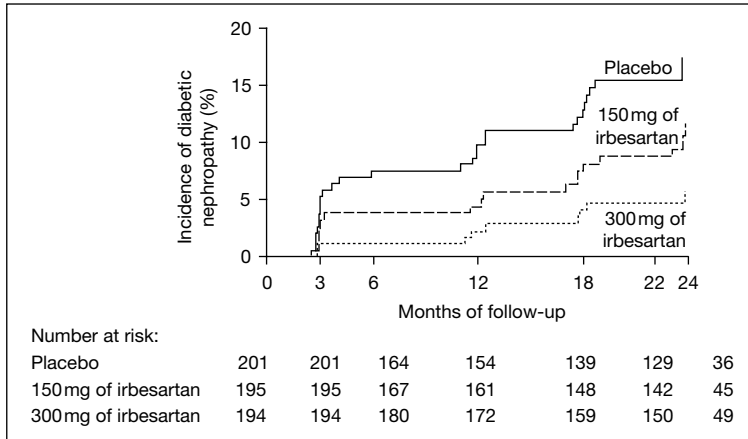
Fig. 6. IRMA2: Cumulative percent of microalbuminuric type 2 diabetic patients with progression to overt diabetic nephropathy during treatment with 150 or 300 mg of irbesartan daily or with placebo (150 mg irbesartan vs. placebo: p = 0.08; 300 mg irbesartan vs. placebo: p < 0.001). The numbers at the bottom of the panel are the numbers of patients in each group at risk for the event at baseline, at 3 months and after each 6-month period [reprinted from 18, with permission].

Proteinuria, RAAS Blockade, and the Risk of Cardiovascular Disease in Diabetics

As noted in the introduction, progression of renal disease and ESRD are not the only adverse outcomes associated in diabetics with proteinuria and CKD. Earlier studies by Borch-Johnsen et al. [19] revealed an association between proteinuria and cardiovascular mortality in patients with type 1 diabetes mellitus. In recent, large populational studies using correlating urinary albumin excretion with cardiovascular mortality, Hillege et al. [20] found albuminuria as a risk indicator of mortality with a clear dose-response relationship (fig. 7). Moreover, a recent publication from the American Heart Association Council on Kidney in Cardiovascular Disease has extensively reviewed the status of proteinuria with and without diabetes mellitus as a risk factor for cardiovascular outcomes [6]. Given the association of proteinuria with cardiovascular disease and the effectiveness of agents blockading the RAAS in reducing proteinuria, it is reasonable to ask whether these agents may provide protection against cardiovascular mortality in diabetics, in addition to delaying progression of kidney disease. The IDNT and RENAAL studies discussed above did not show a survival advantage in patients randomized to irbesartan or losartan during the relatively short (2.6–3.4 years) follow-up periods of the trials. But
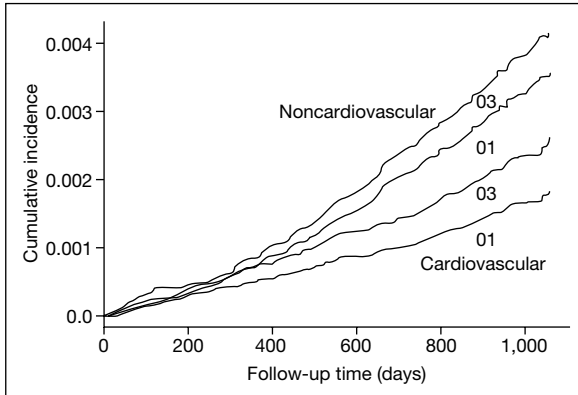
Fig. 7. Cause-specific cumulative incidence of CV and non-CV mortality for non-hypertensive, nonhyperlipidemic, nondiabetic, nonsmoking 50-year-old males with no history of myocardial infarction or stroke, with urinary albumin concentration levels set equal to the 25th percentile (quartile 1 – Q1: 3.8 mg/l) and 75th percentile (quartile 3 – Q3: 9.8 mg/l) [from reprinted 20, with permission].

given the fact that the incidence of ESRD was lower in the ARB-treated groups, it is reasonable to expect that there may be a longer term survival advantage by decreasing the number of patients reaching the ESRD endpoint, a major marker of accelerated cardiovascular morbidity and mortality. Conversely, the Steno group [21] has recently prospectively studied the reduction in proteinuria in diabetics with nephrotic range albuminuria and its potential role in cardio-vascular protection. They studied 126 patients with type 1 diabetes mellitus and a 24-hour albuminuria >2.5 g and followed them for 3 years or until death. Remission of nephrotic range albuminuria was defined as sustained urinary excretion <0.6 g/24 h for at least 1 year. Endpoints were death or ESRD. Remission was achieved in 28 patients (22%). Most of them (n = 21) were on ACEinhs. At the end of the observational period, adverse outcomes were observed in 6 of 28 (21%; 2 ESRD and 4 deaths) patients in the remission group, and 58 of 98 (59%; 24 ESRD and 34 deaths) were observed in the group that did not achieve sustained remission.

A possible mechanistic basis for an association between albuminuria and cardiovascular mortality has been provided by studies evaluating endothelial function in patients treated with ACEinhs. Endothelial function in these studies was assessed by measuring flow-mediated dilation of the brachial artery, mediated by endogenous generation of nitric oxide and assessed using high-resolution Doppler. Impaired endogenous nitric oxide production is well docu-mented in the early phases of atherosclerosis and precedes structural changes in

the coronary arteries. There is a strong correlation between vasodilatory function in the peripheral arteries, and coronary vasodilatory function. Several studies [22–25] have demonstrated that impaired endothelial function is an excellent predictor of cardiovascular events in asymptomatic patients. The mechanisms by which blockade of the RAAS reduces proteinuria and improves endothelial function may be related, providing further rationale for aggressive treatment of proteinuria in diabetics.

Dual Blockade (ACEinhs and ARBs) of the RAAS in Kidney Disease

Because the ACEinhs and the ARBs block the RAAS at different points, it has been suggested that combined treatment with agents of both classes may be more effective in RAAS blockade and in renal protection. Several recent studies have addressed this issue. In a randomized controlled trial of dual blockade with candesartan and lisinopril in microalbuminuric type 2 diabetic patients (CALM), Mogensen et al. [26] found a better reduction in albumin excretion and a lower blood pressure compared to a single agent. Similarly, Jacobsen et al. [27] found that in the short term a combination of benazepril and valsartan resulted in a greater reduction in proteinuria than use of either agent alone. This hypothesis has been most strongly corroborated in a large prospective, randomized study conducted in Japan in nondiabetic (mostly IgA nephropathy) proteinuric disease (the COOPERATE trial) [28]. Patients were randomized either to receive trandolopril 3 mg/day, losartan 100 mg/day, or a combination of the two and were followed both for proteinuria and for evolution of their renal disease. Despite virtually identical follow-up blood pressures in the three randomization groups, these investigators found a significantly greater reduction in proteinuria and a proportionately greater protection against progression to doubling of serum creatinine or ESRD in patients receiving concomitant treatment with the combination therapy as compared to either single agent alone. The combined use of these two classes of agents in order to maximize RAAS blockade and the antiproteinuric effect in diabetics needs to be studied in further prospective clinical trials, to confirm the benefits seen in the COOPERATE Trial and to extend the observations to patients with diabetes.

In summary, aggressive blood pressure control, blockade of the RAAS, and reduction in proteinuria should be considered as major clinical tools to treat and follow patients with diabetes and kidney disease. Aggressive control of blood pressure and maximal reduction of proteinuria/albuminuria by pharmacological blockade of the RAAS with ACEinhs and ARBs not only delay progression of renal disease in diabetics, but may also improve cardiovascular outcomes.

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Clinical Strategy for the Treatment of Hypertension in Non-Diabetic and Diabetic Nephropathy in Japan

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Characteristics of Patients with Hypertension in Japan

The number of hypertensive patients in Japan is estimated to be 33 million, with 51.9% of males and 39.1% of females having pressures of $\geq 140/90\,mm\,Hg$. This frequency increases even more with age [1]. However, the increase in people in their 30–50s is striking when looking at annual changes. This is thought to be caused by the increase in recent years of increased salt intake and the increased frequency of obesity. This has been shown in numerous epidemiological studies in Japan, including the Hisayama Study. The Hisayama Study was a long-term epidemiological study carried out in 1961 on 80% of the citizens of Hisayama town with a population of 8,000. There is an enormous amount of information in this study, making it important for understanding the characteristics of disease in Japan. Many reports, focusing mainly on cardiovascular disease, have been published [2, 3].

Another epidemiological study from Okinawa, Japan, is frequently cited [4, 5]. Okinawa differs from other areas of Japan with respect to race and daily customs, especially diet, and has a higher than average life expectancy. The data from this study is thus useful for making hypotheses about factors of daily life related to disease in comparison to other areas in Japan. The results of these and other epidemiological studies have shown that cerebrovascular complications are more common and ischemic heart disease less common than in the West [6], but in recent years, with the westernization of Japanese life, cerebrovascular disorders are decreasing and ischemic heart disease is increasing [7].

There have been several reports on factors particular to hypertensive patients in Japan. Komiya et al. [8] studied 3,222 Japanese subjects with normal blood pressure and 741 Japanese subjects with essential hypertension and found the distribution curve of serum sodium was shifted to the right in subjects with essential hypertension compared to those with normal blood pressure. The serum sodium concentration of these patients was correlated to the level of blood pressure. However, there was no significant difference found in the frequency of gene polymorphism in relation to the epithelial sodium channel between those with normal blood pressure and patients with essential hypertension [9]. Other factors have been studied with a genetic approach, but no consensus has been reached on which genetic factors have an effect on essential hypertension in Japan [10, 11].

Another unique feature of hypertensive treatment in Japan is the widespread use of calcium antagonists [12]. The reasons for this are numerous, including their lack of adverse effects and reliable antihypertensive effect. Calcium antagonists work well in hypertensive patients, many of whom are elderly or have high salt intake and low renin. Other advantages include their large combined effect with other antihypertensive drugs, their protective effect in cardiovascular disorders and the development of numerous new drugs with long action.

Proving the efficacy of drugs now requires large-scale studies involving many institutions, but this type of large-scale research in Japan is not common due to monetary and insurance issues, ethical reasons and resistance toward the use of placebos. However, such worldwide large-scale studies as RENAAL [13] and PROGRESS [14] have registered approximately 100 and 800 Japanese patients, respectively. It is highly significant that such a large number of Japanese patients participated in the international, multicenter clinical trials and it is possible that through this process, large-scale studies will gradually be conducted in Japan as well.

Antihypertensive Treatment for Patients with Renal Dysfunction in Japan

There are not as many studies on the renoprotective effect of antihypertensive treatment in non-diabetic nephropathy as there are on diabetic nephropathy, but Kumagai et al. [15] carried out a comparative study on the use of amlodipine and enalapril in 72 hypertensive patients with renal disorders. They followed the patients for 1 year, primarily observing kidney function, and obtained equivalent levels of antihypertensive effect with both drugs. Decreased kidney function was seen in both groups, with no significant difference between

groups. However, adverse effects, such as hyperkalemia, were found in the group that was administered enalapril, requiring 33% of the cases to cease usage of the drug.

Hayashi et al. [16], from the same institution, conducted a comparative study of the suppressive effect on proteinuria of the calcium antagonist, efonidipine, which acts to dilate efferent and afferent arterioles, and ACE inhibitors. They studied the effect of efonidipine or ACE inhibitors 48 h after drug administration in 68 hypertensive patients with proteinuria of 1 g or more. The daily amount of urinary protein found in the efonidipine group decreased from 2.7 ± 0.3 to 2.1 ± 0.3 g and in the ACE inhibitor group from 3.0 ± 0.4 to 2.0 ± 0.5 g, an equivalent level of decrease. The blood pressure control was kept to equivalent levels, but there were cases in the efonidipine group in which a remarkable decrease in proteinuria was seen without an antihypertensive effect. This shows that calcium antagonists have a direct renoprotective effect other than decreasing systemic blood pressure.

In the National Intervention Cooperative Study in Elderly Hypertensive (NICSEH) trial, a double-blind comparative trial, the calcium antagonist, nicardipine, or the thiazide diuretic, trichloromethiazide, were used for patients 60 years or older with mild or moderate hypertension. An analysis of 414 cases was made. The frequency of occurrence of cardiovascular disease in the nicardipine group was 27.8/1,000 people per year and 26.8 in the diuretic group, with no difference found between groups [17, 18]. The ability of calcium antagonists and diuretics to decrease the risk of cardiovascular disease at equivalent levels is highly meaningful in a trial carried out in Japan, a country with a high level of cerebrovascular disease.

Reports of this type from various institutions led to the widespread use of calcium antagonists in Japan. To compare this calcium antagonist and ARB, which are recently expected to have organ-protective effects, JLIGHT trials (Japanese Losartan Therapy Intended for Global Renal Protection for Hypertensive Patients) are currently finished. But the results were not presented except the just published interim report [19].

Hypertensive patients with mild kidney function disorders (serum creatinine, 1.5–3.0 mg/dl) and proteinuria of 0.5 g or more per day were randomly assigned to receive either ARB or calcium antagonists and their course is being followed. Equivalent levels of hypertensive control were found in a comparison of the 58 cases of the ARB group and the 59 cases of the calcium antagonist group that could be followed up after 1 year. A significant reduction in proteinuria was found in the ARB group, but not in the calcium antagonist group. This shows some evidence for the renoprotective action of ARB in non-diabetic nephropathy in Japanese. The CASE-J study of 4,000 patients comparing candesartan and amlodipine is currently being conducted.

Nakao et al. [20] focused on the renoprotective effect of ARB. Since there is a limit to the renoprotective effect of ACE inhibitors alone, they conducted a prospective 3-year study on the combined effect of ARB and ACE on renoprotection, administering losartan (100 mg) and trandrapril (3 mg) in 263 cases with non-diabetic nephropathy. This study, named COOPERATE, eventually followed approximately 90 patients in each of three groups. The number of patients in the group with combined therapy who achieved the primary endpoint of doubling of serum creatinine and end-stage renal insufficiency was half of either of the groups that used only one of the two drugs. Proteinuria was also significantly reduced in the combined therapy group compared to either of the single drug groups. The additive effect of the combined use of two renin-angiotensin suppressants was clearly demonstrated.

In Japan, as in the West, diabetic nephropathy accounts for most patients who have end-stage renal insufficiency. Diabetic nephropathy occurs across different races, genders and lifestyles, but its progression is thought to be affected by gender and race. The risk of renal disease progression associated with hypertension or hyperlipemia is even greater, making the control of hypertension in diabetic nephropathy extremely important [21].

There are many reports indicating that by controlling blood pressure, proteinuria can be suppressed and the speed of deterioration of renal function can be slowed [22]. The suppressant effect of controlling blood pressure on increased proteinuria has been confirmed in Japan in the elderly [23]. The ideal blood pressure of the JNC-7 guidelines [24] and of the Japanese Society of Hypertension is 130/85 mm Hg. The ADA and the Japan Diabetes Society guidelines recommend a blood pressure of 130/80 mm Hg. Since ACE inhibitors [25, 26] and ARB [13] have been shown to have a renoprotective effect in diabetic nephropathy apart from their lowering blood pressure, the treatment guidelines of the Japanese Society of Hypertension recommend them as the first drugs of choice when microalbuminuria is present.

A multicenter study on prevention of the progression of diabetic nephropathy in Japanese patients with insulin-dependent diabetes mellitus (IDDM) also demonstrated the efficacy of ACE inhibitors [27]. Long-acting dihydropyridine CCB is also mentioned as a drug of choice because of the results of the J-MIND study [28], but this is a special characteristic of these guidelines as they do not appear elsewhere.

Antihypertensive Treatment for Dialysis Patients in Japan

Renal replacement therapy in Japan is characterized by the large number of patients undergoing hemodialysis and the excellent long-term prognosis of

patients after beginning dialysis [29]. Life expectancies of 20 years or more can be expected after beginning dialysis in the case of chronic renal failure due to non-diabetic nephropathy. Antihypertensive control must be considered in order to prevent cardiovascular events, an important risk to life expectancy in dialysis patients.

There have been two clinical research studies on blood pressure in dialysis patients in Japan, and while both trials were non-interventional, the results obtained do show clearly that controlling blood pressure reduces long-term complications [30, 31].

An important factor affecting blood pressure in dialysis patients is body fluid volume control, which becomes even more important as years on dialysis increase and spontaneous urine production decreases. Thus, setting limits on salt intake and setting an appropriate dry weight level is of basic importance. Also, as years on dialysis increase, atherosclerosis and calcification progress, making control of not just salt, but also cholesterol, calcium, and phosphorous intake important considerations. In addition, in dialysis patients, there are large variations in blood pressure by season [32], and since there are problems with hypertension associated with erythropoietin [33], it is necessary to always deal actively with blood pressure adjustment.

Calcium antagonists are used often in Japan as antihypertensive agents, as they would be for conservative phase renal failure, but in general the reality is that many drugs are used in combination. While some support the use of ACE inhibitors, ARB and β-blockers for their organ-protective effect [34, 35], our experience, albeit short, shows that ARB [36], ACE inhibitors or both combined are effective against regression of left ventricular hypertrophy [37]. There is no large-scale clinical research related to the choice of drugs either overseas or in Japan [34], making it necessary to use guidelines for renal function in normal people, even with regard to antihypertension targets. There is still no consensus on which measurement value to actually set as the blood pressure value [38]. There are reports that blood pressure control improved due to daily dialysis [39], but perhaps due to the limited spread of dialysis in Japan, no data has been collected and no research has been reported here.

Antihypertensive Treatment for Elderly Patients in Japan

As the population of Japan ages, the percentage of elderly patients with hypertension is increasing. There are those who believe so-called guidelines should be followed in antihypertensive treatment for the elderly, but when anti-hypertensive control is followed in practice exactly according to the guide-lines, many patients complain about the decrease of their quality of life. Since

Organ Protection by Blood Pressure Control

Calcium antagonists have reliable and excellent antihypertensive effects for organ protection. We studied their organ protection mechanism during treatment in combination with α,β-blockers. After controlling blood pressure in 13 patients with accelerated hypertension using the intravenous administration of nicardipine at an early stage, long-acting nifedipine at 60–80 mg/day and arotinolol at 20 mg/day were administered with a blood pressure target of 140/90 mm Hg. Guanabenz was added when blood pressure was not lowered adequately. Blood pressure went from an average of (233 ± 8)/(144 ± 3) mm Hg at the time of hospitalization to (162 ± 4)/(102 ± 4) mm Hg after 3 days and to (148 ± 3)/(89 ± 2) mm Hg after 1 month. Hemodialysis was needed in 7 patients, but most of the cases could be weaned so that after 1 year only 1 case was still undergoing hemodialysis [42]. Treatment with nifedipine and arotinolol was continued and the patients were followed through 3 years. One of the patients died of myocardial infarction and 1 case still required hemodialysis. Serum creatinine was 4.5 ± 0.7 mg/dl at the time of hospitalization, 2.9 ± 0.9 mg/dl after 1 year and 2.2 ± 0.4 after 3 years. Left ventricular hypertrophy involuted during this period, demonstrating clearly that combined use of nifedipine and arotinolol brings about reliable antihypertensive and organ-protective effects [43].

In this way, the main focus of antihypertensive therapy from the late 1980s to the early 1990s was which antihypertensive drug had the most protective effect on the organs. Expectations were high in relation to renal function for ACE inhibitors, which were thought to have a mechanism for improvement of glomerular hyperfiltration, and numerous reports from various institutions were carried out. However, as was already mentioned, in Japan calcium antagonists are frequently used for reasons of safety and reliable antihypertensive effect. We conducted a comparative study on amlodipine and benazepril in 49 hypertensive patients with IgA nephropathy (average age 39 ± 9 years). We compared renoprotective effects using a reduction in creatinine clearance as an index. No difference was found between groups after 3 years (fig. 2) [44]. A similar study was also carried out on patients in a different age group. We conducted a comparative study on cilnidipine and benazepril in hypertensive patients (average age 62 ± 4 years) with mild renal function disorders due to underlying benign nephrosclerosis (serum creatinine 1.40 ± 0.2 mg/dl). The same type of and same level of antihypertensive control and renoprotective effect were obtained as in the younger group [45]. The same results were obtained in patients with polycystic kidney disease [46] demonstrating that calcium antagonists have reliable antihypertensive action as the main mechanism and the same renoprotective effect as ACE inhibitors.
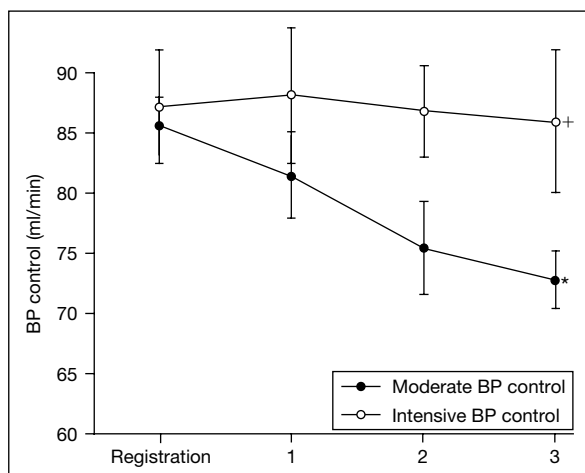
Fig. 2. Changes in creatinine clearance of the patients in two groups. *p < 0.05 vs. registration respectively and [+]p < 0.05 vs. moderate BP control group at each year point [from 44].

Morning Blood Pressure Control

As a result of these various basic and clinical research studies, in recent years the focus of antihypertensive therapy for organ protection has been on lowering blood pressure itself [47]. Interventional trials on such propositions as how much to lower pressure and what control indices of blood pressure measurement to use are being carried out. But the widespread use of 24-hour blood pressure measurement and home blood pressure devices has turned the discussion towards how to incorporate these factors into antihypertensive therapy that no longer takes place only in the outpatient clinic as in the past. A 7-year study called the Hypertension Objective Treatment Based on Measurement by Electrical Devices of Blood Pressure (HOMED-BP) is currently underway in Japan to follow 9,000 cases [48].

With this discussion in mind, we focused on the importance of early morning blood pressure measurement in the household and conducted two studies on its relation to organ protection. A study on the effect of antihypertensive treatment with amlodipine, benazepril, furosemide or guanabenz on renal function in 113 hypertensive patients with renal disorders in non-diabetic nephropathy was carried out, with the goal of maintaining blood pressure ≤130/85 mm Hg as measured at the outpatient clinic. Patients were instructed to measure their blood pressure at home in the morning and at night, and their course was followed for 3 years. Decreased renal function over the 3 years was evaluated
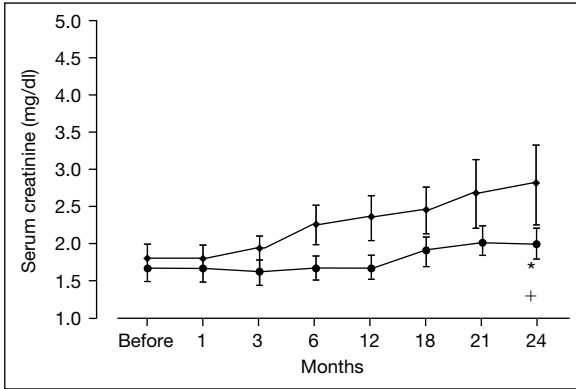
Fig. 3. Changes in serum creatinine in patients with coexisting chronic renal failure and left ventricular hypertrophy treated with guanabenz (•) and placebo (•). *p < 0.01: compared to the baseline value. +p < 0.05: compared to placebo group [from 50].

by changes in glomerular filtration rate. Many of the cases in which renal function was preserved had early morning systolic blood pressure measurements that did not go over 140 mm Hg. None of the other measurements showed a correlation between blood pressure and the degree of decreased renal function. There was a correlation only with early morning systolic blood pressure [49].

Another study on renal disorders associated with non-diabetic nephropathy in 34 hypertensive patients (average age 52.6 ± 3.5 years; serum creatinine 1.72 ± 0.15 mg/dl) was carried out with blood pressure controlled by amlodipine and benazepril as the base medications. In 17 of these cases, guanabenz was administered before going to sleep to suppress early morning blood pressure. In another 17 patients, a placebo was administered, but no significant difference was found between groups for outpatient blood pressure values. However, early morning blood pressure in the home was significantly lower in the group taking guanabenz (134 ± 4 mm Hg). Renal function was suppressed in the guanabenz group and involution of left ventricular hypertrophy was found (fig. 3). In the placebo group however, there was a reduction in renal function and progression of left ventricular hypertrophy. This research demonstrated clearly that an enhanced organ-protective effect could be achieved by controlling early morning hypertension, not only for outpatients [50].

Combination of Antihypertensive Agents

The ideal blood pressure control level differs by guideline, but is gradually being lowered [24, 51, 52]. In order to look at the effect of hypertension on organ

protection, we studied 46 hypertensive cases that were associated with heart failure (ejection fraction of 55% or less) or renal failure (glomerular filtration rate of ≤50 ml/min). They were divided into two groups – group 1 had a blood pressure control target of 120/75 mm Hg and group 2 a target of 130/80 mm Hg – and followed for 2 years. The antihypertensive drugs used were amlodipine, benazepril, guanabenz and furosemide. Both groups met their blood pressure control targets, but after 2 years, it became clear that the more blood pressure was lowered the more both renal and cardiac function were protected [53].

In general, if the most effective way to strictly control blood pressure is to use calcium antagonists, how should the selection of secondary drugs be made? Historically, β-blockers have been considered to be compatible with calcium antagonists [54]. However, in recent years there have been reports of the various protective effects on organs of ACE inhibitors [55]. There are also reports of the efficacy of the actual combined use of calcium antagonists and ACE inhibitors in suppressing the progression of renal disorders [56], thus making it necessary to change conventional methods of drug administration.

We carried out a 2-year interventional trial on hypertensive patients with non-diabetic nephropathy associated with left ventricular hypertrophy [57]. Amlodipine (5 mg) was the primary medication for all patients, with benazepril (2.5 mg) administered as the secondary medication in one group (n = 32) and arotinolol (20 mg) administered as the secondary medication in another group (n = 33). Equivalent degrees of blood pressure control were obtained in both groups, (130 ± 11)/(75 ± 9) mm Hg, and no difference in renal function as indexed by serum creatinine levels was found between groups. However, while ACE inhibitors merely suppressed the progression of the left ventricular mass index, α,β-blockers lowered it significantly. That is, the results suggested that α,β-blockers improve cardiac function in patients with non-diabetic nephropathy and in hypertensive patients associated with left ventricular hypertrophy. In our study, ACE inhibitors did not demonstrate the same effect on cardiac function, but it is possible that the dosages were insufficient.

The treatment of cases with decreased renal function in Japan involves either the use of reduced dosages of ACE inhibitors or ARB, or the recommendation not to use them at all [54]. This is to avoid the dangers of hyperkalemia or acute renal failure, and is one of the reasons calcium antagonists are frequently used in Japan. However, since it is clear that the long-term prognosis of cardiac failure is greatly improved by the suppression of the renin-angiotensin system, there is a dilemma in deciding whether to use ACE inhibitors or ARB in cases with diabetic nephropathy, which are often associated with cardiovascular complications.

We conducted an interventional trial for 1 year on 36 cases of diabetic nephropathy associated with left ventricular hypertrophy and a control group

with the same number of cases of diabetic nephropathy not associated with left ventricular hypertrophy [58] in order to clarify the effect of ACE inhibitors on the progression of renal failure. The 36 cases were divided into three groups – one group was administered benazepril at 5 mg/day, one group was administered benazepril at 2.5 mg/day, and one group was administered a placebo. Blood pressure was controlled to the target of 140/90 mm Hg in each group and evaluations of left ventricular hypertrophy by echocardiography and decreases of renal function were made. A significant suppression of decreased creatinine clearance was seen in the 36 cases with left ventricular hypertrophy administered 5 mg of benazepril compared to the other two groups and suppression of decreased ejection fraction was also found. Increases in serum potassium levels, which we were worried about, were not found, and proteinuria decreased compared to the levels seen at the beginning of the trial. Renal function decreased significantly in the placebo group. Hyperkalemia occurred possibly as a result of decreased renal function. In addition, proteinuria increased and the ejection fraction was significantly decreased.

However, in the 36 cases without left ventricular hypertrophy, cardiac function and renal function both showed protective effects in the group administered 2.5 mg of benazepril compared to the other groups.

The results above make us think that active suppression of the renin-angiotensin system is necessary for patients with diabetic nephropathy since progressive decreases in both cardiac and renal function are found when ACE inhibitors are not administered, regardless of whether left ventricular hypertrophy is present.

Conclusion

We discussed the treatment of hypertensive patients associated with disorders of renal function with a focus on Japan and our department. As advocates of 'total nephrology', active treatment to reduce blood pressure is useful for renoprotection at all levels of renal function disorders. But it is important to use treatments that are appropriate for each individual patient by understanding the mechanisms of organ protection of all types of hypertensive drugs, beside their effect of lowering blood pressure.

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Angiotensin Type 1 Receptor Blockers in Chronic Kidney Disease

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Angiotensin (Ang) II plays a pathophysiological role in the progression of chronic kidney disease and its inhibition and control is, without doubt, crucial in the control of its damaging effects. Angiotensin-converting enzyme (ACE) inhibitors block the production of Ang II and have been shown to have beneficial effects in patients with hypertension, congestive heart failure, diabetes mellitus, as well as chronic kidney disease. Proteinuria is a major predictor of decline in renal function in patients with both diabetic and non-diabetic renal disease and ACE inhibitors reduce both micro- and macroalbuminuria in many types of renal diseases and prevent the progression of chronic kidney disease [1, 2]. Although there have been no large-scale clinical studies of angiotensin type 1 receptor blockers (ARBs) comparing with ACE inhibitors, recent clinical trials with ARBs have shown impressive results in reducing progression of microalbuminuria to overt nephropathy and in prolonging the time to doubling of serum creatinine [3–5]. ARBs have a different mode of action than ACE inhibitors in that they block the angiotensin type 1 (AT1) receptor itself [6]; however, all known Ang II-related effects are mediated via the AT1 receptor. In the kidney, these effects include modulation of renal blood flow, glomerular filtration rate, tubular epithelial transport, renin release, and cell growth [7, 8]. On a theoretical basis, ARBs seem to be superior to ACE inhibitors with respect to effectiveness and tolerability [6, 9–11]. Hollenberg [12] showed a different response to salt intake in renal blood flow between ACE inhibitors and ARBs; however, it remains unknown whether ARBs are possibly less susceptible than ACE inhibitors to the effects of dietary salt in reversing their antihypertensive and antiproteinuric effects. A short-term trial using a crossover design has demonstrated identical antihypertensive and antiproteinuric effects of an ARB

and an ACE inhibitor in patients with hypertension, non-diabetic chronic kidney disease, and macroproteinuria [13]. In contrast to ACE inhibitors, all ARBs undergo a significant degree of hepatic elimination with the exception of candesartan, olmesartan, and the E-3174 metabolite of losartan, which are 40, 60 and 50% hepatically cleared, respectively. Irebesartan and telemisartan undergo the greatest degree of hepatic elimination with each having more than 95% hepatic clearance. Valsartan and eprosartan are each approximately 70% hepatically cleared. From these data, dose adjustment in chronic renal failure is not advocated. When the ARBs are administered to the patients with chronic renal failure, the major adverse consequences of ARB accumulation therein are prolonged blood pressure reduction, an extended fall in glomerular filtration rate, and/or hyperkalemia. The mere fact that these physiologic and biochemical sequelae occur does not mandate permanent discontinuation of an ARB; rather, cautious reintroduction of the offending ARB is recommended, albeit at lower doses or with less frequent administration.

Recently, four large-scale clinical trials demonstrated that ARBs are superior in preventing the progression of diabetic kidney disease. In the IRMA (Irbesartan for Microalbuminuria in Type 2 Diabetes) study [5], 590 patients with microalbuminuria, normal renal function, and blood pressure $>135/85\,mm\,Hg$ were randomized to treatment with either placebo or irbesartan (150 or 300 mg/day). At the end of 2 years, progression to microalbuminuria occurred in 14.9% of patients receiving placebo and 9.7 and 5.2% in the groups given irbesartan, 150 and 300 mg/day, respectively. The levels of blood pressure were similar among the three groups. A dose-dependent reduction in the incidence of progression from microalbuminuria to macroalbuminuria was clearly demonstrated.

In the IDNT (Irubesartan Diabetic Nephropathy Trial) study [4], 1,715 patients with type 2 diabetes mellitus, proteinuria ($>0.9\,g$ daily), and serum creatinine levels between 1.0 and 3.0 mg/dl were randomized to treatment with irbesartan (300 mg/day), amlodipine (10 mg/day) or placebo. The primary end points were the duration of the doubling of serum creatinine, reaching end-stage renal disease or death. At the end of 2.6 years, patients in the irbesartan group had a 20% reduction in reaching the primary end point compared with the placebo group, and 23% lower than in the amlodipine group.

In the RENALL (Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan) study [3], 1,513 patients with type 2 diabetes mellitus, urine albumin excretion $>300\,mg/g$ creatinine and serum creatinine levels between 1.2 and 3.0 mg/dl were randomized to receive either placebo or losartan. The primary end points were the duration of the doubling of serum creatinine, reaching end-stage renal disease or death. At the end of 3.4 years, proteinuria decreased by 35% with losartan and did not change with placebo, while the levels of blood

pressure were nearly identical in both groups. In the losartan group, patients had a 16% reduction in the primary end point.

In the MARVAL (MicroAlbuminuria Reduction with Valsartan) study [14], 332 patients with type 2 diabetes mellitus and microalbuminuria were randomized to receive 80 mg/day valsartan or 5 mg/day amlodipine for 24 weeks. A target blood pressure of 135/85 mm Hg was aimed for by dose-doubling followed by addition of bendrofluazide and doxazosin whenever needed. The primary end point was the percent change in urine albumin excretion from baseline to 24 weeks. The urine albumin excretion was 56% of baseline with valsartan and 92% of baseline with amlodipine. Blood pressure reductions were similar between the two treatments. These trials provide compelling evidence that in patients with type 2 diabetes mellitus with either microalbuminuria or overt diabetic nephropathy, ARBs are effective in inhibition of progression of renal disease. Moreover, this class of drugs has shown a blood pressure-independent antiproteinuric effect in these patients.

Evidence of the Dual Blockade in Nephropathy

Non-Diabetic Nephropathy

A large number of trials with dual blockade in nephropathy are currently being conducted. Russo et al. [15] first demonstrated that in 8 patients with biopsy-proven IgA nephritis with massive proteinuria (>1 g daily), treatment with an ACE inhibitor and losartan (50 mg daily) in combination reduced proteinuria and blood pressure. Proteinuria was reduced over 4 weeks to a mean of 0.5 g daily. Moreover, an additional reduction in proteinuria was observed when combined therapy doses were doubled. The reduction in proteinuria was not correlated with clinical blood pressure; however, reductions in diastolic and mean ambulatory blood pressures significantly correlated with the decrease in proteinuria, as well as creatinine clearance. These findings were confirmed by Woo et al. [16] who demonstrated a significant reduction in proteinuria in 21 patients with IgA nephropathy with dual blockade treatment. In a similar study that included 60 patients with chronic kidney disease in a randomized crossover design, combination treatment of 8 mg of cadesartan and ACE inhibitors was associated with a greater reduction in blood pressure and proteinuria than ACE inhibitors alone [17]. Moreover, when combination treatment was continued beyond the initial 12 weeks of the study, further reductions in proteinuria were observed at between 6 and 9 months versus 12 weeks. Ruilope et al. [18] treated 108 patients with chronic kidney disease in a three-arm design for 5 weeks. One group received valsartan 160 mg daily, a second group received 80 mg of valsartan and 5–10 mg of benazepril, and a third group

received 160 mg of valsartan and 5–10 mg of benazepril. In groups 2 and 3, benazepril was given in addition to valsartan after the first week of treatment with valsartan. Systolic blood pressure was significantly lowered in both dual blockade treatment groups. Diastolic blood pressure was significantly lowered in all three groups. Proteinuria was only significantly reduced in the dual blockade treatment with high-dose valsartan. Serum creatinine and potassium increased in all three groups. In addition, both dual blockade treatments resulted in reduction of proteinuria. The total number of patients with adverse effects were 10 (45.5%), 14 (33.3%) and 11 (25%) in groups 1, 2 and 3 respectively.

Campbell et al. [19] conducted a prospective, randomized, crossover study of 24 patients with non-diabetic, chronic nephropathies. They compared the effects on proteinuria, renal hemodynamics and glomerular permselectivity with comparable blood pressure control achieved by benazepril (10 mg/day) and valsartan (80 mg/day) combined therapy with those achieved by benazepril (20 mg/day) or valsartan (160 mg/day) alone for 8 weeks. Dual blockade treatment decreased proteinuria more than benazepril and valsartan. There was a bigger decease in filtration fraction and renal vascular resistance with dual blockade or benazepril than with valsartan. Renal vascular resistance changes, adjusted for glomerular filtration rate changes, were associated with the degree of proteinuria. Changes in glomerular permeability were comparable and did not predict different changes in proteinuria in the three groups.

Compared to these small trials, more recently, Nakao et al. [20] performed a study in which over a 4-year period, 245 patients with non-diabetic chronic kidney disease and proteinuria were randomized to receive trandolapril, losartan or both. There was a highly significant reduction in doubling of serum creatinine and end-stage renal disease in the double treatment group compared with either monotherapy, independent of disease stage inclusion. Only 13 patients reached the end point in the dual blockade treatment group, compared with 25 patients in the trandolapril group and 24 in the losartan group. Evidence of the effect of dual blockade in nephropathy seems stronger than any other disease entity. In all cases, proteinuria was significantly reduced independently of the type of agent, follow-up time, and etiology behind the nephropathy.

Diabetic Nephropathy

Studies have sought to examine the renoprotective potential of dual blockade treatment. Hebert et al. [21] were the first to apply dual blockade to 7 diabetes patients with hypertension and macroalbuminuria. They found a significant decrease in blood pressure when 50 mg losartan was given concomitantly with ACE inhibitors without a significant reduction in proteinuria.

Jacobsen et al. [22] showed that in 21 type 1 diabetes mellitus patients with proteinuria exceeding 1 g daily and hypertension, despite ACE inhibitors

treatment the addition of 300 mg of irbesartan resulted in a mean 8/5 mm Hg reduction in blood pressure and a further 37% reduction in albuminuria. Rossing et al. [23] conducted a trial of 18 type 2 diabetes patients with hypertension and massive proteinuria. By adding 8 mg of candesartan to ACE inhibitors, both blood pressure and proteinuria were significantly reduced. The same group has recently performed a study in which 20 type 2 diabetes mellitus patients with hypertension and nephropathy were randomized to receive 16 mg of candesartan or placebo added to existing treatment with lisinopril/ enalapril 40 mg daily or captopril 150 mg daily. During dual blockade, there was a mean reduction in albuminuria compared with ACE inhibitors alone. There was a modest reduction in systolic and diastolic blood pressure. Changes in albuminuria did not correlate with the change in ambulatory blood pressure. No significant reduction in glomerular filtration rate was observed in the patients with dual blockade treatment.

In the CALM (Candesartan and Lisinopril Microalbuminuria) study, 199 hypertensive type 2 diabetes mellitus patients were randomized to receive 20 mg of lisinopril, 16 mg of candesartan, or both drugs in combination [24]. After 4 weeks of placebo treatment, patients continued with either monotherapy or the dual blockade treatment for an additional 12 weeks. All three treatments significantly reduced blood pressure from baseline with dual blockade being the most effective. The study also found greater reductions in the urine-albumin-creatinine ratio with dual blockade (50%) compared with lisinopril alone (39%) and candesartan alone (24%). However, when adjusted for diastolic blood pressure, baseline values and weight, the differences were not significant.

In contrast to these studies, one study to date failed to show a greater lowering of blood pressure or proteinuria with dual blockade treatment versus ACE inhibitors among diabetes patients [25]. In the latter study, 12 patients with type 2 diabetes mellitus out of a total number of 16 severely obese patients with nephropathy were assigned to receive lisinopril 40 mg daily along with other antihypertensive therapy in one period and to receive losartan 50 mg daily in another period. The study failed to show any effects of dual blockade treatment over a 1-month treatment period, with a 2-week washout between periods.

Are There Any Significant Differences Among ARBs? (Personal View)

ARBs share the same mechanism of action. However, they have different pharmacokinetic profiles, which may account for potential differences in efficacy. In addition, the selected starting dose may have been chosen using different criteria, thus resulting in noncomparable degrees of blockade of the RA system. The relative antihypertensive efficacy of ARBs was evaluated in a recent meta-analysis of 43 randomized, placebo-controlled trials. This analysis suggests comparable antihypertensive efficacy within the ARB class.

In my personal view, valsartan might be more potent in patients with salt loading. A total of 736 patients who had a mean sitting diastolic blood pressure of >95 and <115 mm Hg were randomized into five treatment groups to receive a once-daily oral dose of placebo or valsartan 20, 80, 160, or 320 mg for 8 weeks. All doses of valsartan produced statistically significantly greater reduction in mean sitting both diastolic and systolic blood pressure. Reductions of greater magnitude from baseline in mean sitting both diastolic and systolic blood pressure were seen for doses of 80 mg and above. However, there was only a small incremental decrease in blood pressure with doses of valsartan 160 and 320 mg [26]. More interestingly, valsartan reduced blood pressure in elderly and young patients, men and women, and whites and blacks. These clinical data will provide assumption that valsartan reduces blood pressure irrespectively of salt dependency, since previously it is indicated that the blacks and the elderly people are prone to be more salt sensitive compared to the white and young people. In addition to this evidence, de Gasparo et al. [27] reported that treatment of spontaneously hypertensive rats treated with inhibitors of nitric oxide synthase with valsartan, alone or in combination with enalapril, improved survival rate. In this study, a high dose of valsartan showed a reduction of blood pressure, increases in urinary excretion of sodium and prevention of elevation of serum creatinine. Combining these clinical and experimental data, it would be suggested that valsartan may have natriuretic action in salt loading conditions. This would be more strengthened by the study conducted by Bakris et al. [2] that compared the effects on blood pressure reduction and changes in serum potassium and plasma aldosterone in patients with impaired renal function between the ACE inhibitor and valsartan. Valsartan did not reduce the levels of plasma aldosterone while a significant reduction in plasma aldosterone was noted in those taking the ACE inhibitor. In spite of no changes in plasma aldosterone levels in those treated with valsartan, no significant increases in serum potassium were found. Why does valsartan have a natriuretic action? Since potassium is mainly regulated by the amount of sodium delivered to the collecting tubules in the medulla and the levels of aldosterone, a larger amount of sodium delivered to the ducts may produce more secretion of potassium. It is therefore likely that valsartan produces natriuresis producing potassium excretion in patients with impaired renal function.

Losartan may have antiatherogeic action since low doses of losartan significantly improved aortic atherosclerosis in apolipoprotein-E-deficient models and similarly losartan prevented development of atherosclerosis induced by fatty food in rhesus monkey. Additional studies would be needed to assess whether these differences are really clinically relevant when examining end points such as morbidity and mortality.

A Look into the Future

Dual blockade treatment efficiently reduces blood pressure and proteinuria in non-diabetic and diabetic patients with nephropathy and hypertension. Since many of the mentioned studies were conducted with few patients, an overall conclusion may be unreliable. Large clinical trails still need to be conducted before general recommendations can be formulated. Hopefully, future studies will elucidate whether dual blockade treatment has the predicted benefits. Until then, aggressive control of blood pressure with a regimen that includes either an ACE inhibitor or ARBs should be undertaken in all patients with chronic kidney disease.

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