Molecular Biology of Renal Injury: Emphasis on the Role of the Renin-Angiotensin System

Julie R. Ingelfinger1 and Victor J. Dzau

J.R. Ingelfinger, Division of Pediatric Nephrology, Massachusetts General Hospital, Boston, MA
V.J. Dzau, Stanford University School of Medicine, Division of Cardiovascular Medicine, Falk Cardiovascular Research Center, Stanford, CA
(J. Am. Soc. Nephrol. 1991; 2:S9–S20)

ABSTRACT

The developments in molecular biology of the past decade have created a powerful technology with important, if not revolutionary, clinical applications. This review discusses the molecular biology of renal injury focusing on the renin-angiotensin system as a model, first considering the molecular physiology of the renin-angiotensin system within the kidney and then considering its abnormalities in renal injury. All of the components of the renin-angiotensin system are present within the kidney and are involved in modulation of glomerular microcirculation, in proximal tubular reabsorptive function, in control of glomerular/tubular balance, in modulation of medullary blood flow, and in growth and repair of the renal tubule. A new understanding of these multiple roles of the renin-angiotensin system within the kidney is made feasible by combining physiological studies with techniques such as mRNA analysis (e.g., Northern and slot blots, in situ hybridization, and RNA protection assays), transgenic animal studies, transfection studies, and restriction fragment length polymorphism analysis. The ways in which such approaches have been used to examine the role of the renin-angiotensin system in acute renal failure, proteinuric states, renal hypertension, and diabetes mellitus are discussed.

Key Words: Molecular biology, mRNA, angiotensins, Intrarenal renin angiotensin system, transgenic, restriction fragment length polymorphism

1 Correspondence to J.R. Ingelfinger, Division of Pediatric Nephrology, Massachusetts General Hospital, Hull Street, Boston, MA 02114.
1046-6673/02-00S9-$03.00/0
Journal of the American Society of Nephrology
Copyright © 1991 by the American Society of Nephrology

The developments in molecular biology of the past decade have created a powerful technology with important, if not revolutionary, clinical applications. Present advances permit identification of molecular abnormalities, pinpointing alterations in gene structure and/or expression, thereby allowing identification of specific mechanisms for particular disease entities. This brief review discusses the molecular biology of renal injury, focusing on the renin-angiotensin system (RAS) as a model. We will first discuss the molecular physiology of the RAS within the kidney and will then discuss its abnormalities in renal injury.

AN INTRARENAL RAS: MOLECULAR BIOLOGY AND PHYSIOLOGY

All of the components of the RAS are present within the mammalian kidney (1–7). The proteins and mRNA for renin, angiotensinogen, and angiotensin converting enzyme (ACE) have all been demonstrated in the kidney. In situ hybridization studies demonstrate that renin mRNA is largely expressed in the juxtaglomerular apparatus in the adult animal, though during development it may be found in the arterioles and arteries (6). Immunohistochemical studies have shown renin present in those structures, as well as within the proximal tubule, implying that reabsorption from tubular fluid is the means by which renin is available to the renal tubule (1). Angiotensinogen mRNA is found primarily in the proximal tubule, with considerably smaller amounts in the glomerulus (primarily in mesangial cells) and vasculature (7); angiotensinogen protein is found in congruent locations (2). ACE mRNA localization has been reported preliminarily in proximal tubule and in the vasculature which are the sites where immunohistochemical studies demonstrate the mature protein (1). Binding studies have shown that angiotensin II (Ang II) receptors are present within multiple intrarenal structures (5). A myriad of studies now amply demonstrate that Ang II is produced within the kidney (8–10) where it has the potential to exert local autocrine, paracrine, or intracrine influences in addition to its influence on the circulating RAS. Indeed, the intrarenal RAS may have several roles, acting as a modulator of glomerular microcirculation, an enhancer of proximal tubule reabsorptive functioning, a controller of glomerular/tubular balance, a
modulator of medullary blood flow, and a factor in the growth and repair of the renal tubule. The importance of this system to renal homeostasis is demonstrated by the fact that both ACE inhibitors (ACE I) and Ang II receptor antagonists appear to favorably influence the course of renal disease or injury.

**Role of the RAS in the Renal Vasculature and Mesangium**

Angiotensins appear to exert important influences in the vasculature and mesangial cells in general (11–14). Ang II is not only a potent contractile agonist but also acts as a growth factor in vascular smooth muscle cells and mesangial cells (15–18). Thus, this octapeptide may play an important role in vascular remodeling and in mesangial proliferation. Ang II induces the expression of mRNAs for proto-oncogenes such as c-fos and c-jun, as well as for growth factors such as platelet-derived growth factor in these cells (16–18), suggesting a means for its role in repair and proliferation. Thus, angiotensin may play a role in vascular structural changes in hypertension, renal ischemia, and other conditions of activated RAS. Furthermore, it may be an important pathogenic factor in the mesangial hyperplasia of glomerular diseases.

The source of Ang II is of much interest. In addition to the circulating peptide, evidence suggests that angiotensin may be produced locally within the renal vasculature and/or the glomerulus. We have described the presence of the RAS within the vasculature (11–14). Angiotensinogen mRNA, ACE, and angiotensin receptors can be demonstrated in cultured smooth muscle cells, as well as in situ by immunohistochemical and molecular biological techniques. Within the normal adult kidney, renin is synthesized and released by specialized smooth muscle cells—the juxtaglomerular (JG) cells, which are located in the afferent arteriole at its entrance into the glomerulus. In normal fetal development—at least as studied in the rat—renin mRNA is more widely expressed and is also found in the walls of arcuate and interlobular arteries (6,19). As the animal matures, renin is found in more familiar locations. That renin is important in the normal sequence of vascular development is typified by the finding that renal vascular development is disordered in the presence of an abnormal renin gene. For instance, in transgenic mice in which the production of renin is altered by the presence in the transgenic genome of a fusion transgene consisting of the promoter for the renin gene and the SV-40 large T antigen (20), events which stimulate renin lead to abnormal renal expression. In the affected mice, there is severe vascular hyperplasia of intrarenal smooth muscle.

It is unclear why renin gene and protein expression appears so widely distributed in the vasculature of the fetus but appears to be confined more towards the JG apparatus in the adult. However, under certain pathophysiological circumstances such as ischemia and ACE inhibition, other renal vascular smooth muscle cells appear to be recruited to express renin again (19). Thus, the potential to express renin exists in a multiplicity of renal vascular smooth muscle cells. The ability to recruit renin-expressing cells may potentially be important not only in modulating intrarenal blood flows, but also in vascular remodeling. Though direct evidence is still needed to demonstrate the role of the RAS in such vascular remodeling or in the proliferation of the neovasculature in the kidney, it is of interest that ACE inhibition appears to ameliorate the course of a variety of renal diseases.

The glomerulus contains angiotensin receptors, angiotensinogen mRNA, ACE, and immunoreactive renin (1–7). We have previously reported that cultured mesangial cells contain renin (21). Indeed, Gomez et al. observed high quantities of immunoreactive renin in the glomeruli of sodium-depleted rats (19). The importance of the angiogenic action of Ang II in the glomerular growth of maturing kidneys has been considered indirectly by Fogo and colleagues (22) who studied the effect of various antihypertensive agents upon the rapid growth of glomerul which occurs during maturation. In this study of glomerular size, young Munich-Wistar rats were given ACEI, verapamil, or hydralazine for 6 wk starting at age 4 to 5 wk. The ACEI, but not the other agents, caused glomerular growth retardation. Additional evidence that Ang II is angiogenic within the glomerulus comes from the work of Homma et al. (23) who reported that Ang II causes hypertrophy and stimulates collagen production in cultured rat mesangial cells. It would appear that, in such a setting, the angiogenic effect of Ang II is distinguishable from its hemodynamic effects (24,25) as the animals are otherwise healthy and normal.

In models such as experimental hypertension, a decrease in renal mass and glomerulopathy alterations of the RAS have been reported. The use of ACEI often appears to limit renal injury in these settings (26,27). Likewise, the blockade of Ang II receptor delays the onset of renal disease, strokes, and death (28) and ameliorates diabetic glomerular hypertension in salt-sensitive (SS) Dahl rats (29). Further studies will be required to delineate more completely the role of the RAS in disease states.

**The Role of the RAS in the Proximal Tubule**

The proximal tubule appears to be a major site for an intrarenal RAS, as all components appear to be present (1–2,7,30). Ang II has multiple effects in this segment of the nephron, including sodium and bicarbonate resorption (31,32) and volume resorption.
Although a number of studies recently have implicated Ang II as having direct effects on the proximal tubule, the most direct evidence comes from data of Seikaly et al. who demonstrated endogenous production of Ang II within the proximal tubule (10).

Interestingly, there are proximal tubule Ang II receptors both in brush border and basolateral membranes. At the luminal surface, Ang II is rapidly hydrolyzed, whereas at the basolateral surface, alterations in transport likely occur. Studies using techniques such as isolated perfused tubules or micro puncture have shown that Ang II in the proximal tubule produces a bimodal effect on transport (35), causing inhibition of sodium transport at high concentrations and enhancement at lower concentrations. It would appear that these differential effects may be due to the means by which Ang II is coupled to its receptors (5,36,37). At physiological (nanomolar) concentrations, Ang II increases sodium reabsorption via phospholipase C-induced calcium transients, whereas, at higher concentrations, it may act via cAMP-activated inhibitory G protein. Furthermore, there is now growing evidence that Ang II may also act through phospholipase A2 in the kidney (36,37).

Within the proximal tubule, it appears that Ang II induces hypertrophy but not hyperplasia (38,39). Studying a murine proximal tubule cell line, Wolf and Neilon (39) found that a low concentration of Ang II induced cellular hypertrophy, with cells remaining in the G1 phase of the cell cycle. The manner in which such an in vitro observation is integrated into growth and repair of renal tubules is as yet unknown. It is known that EGF-induced mitogenesis is potentiated by Ang II (40). Interestingly, stimulation of Na"/H" antiporter is an early event in the hypertrophy of proximal renal tubule cells and Ang II stimulates the antiporter (41).

In addition to its role in transport phenomena, the proximal tubule RAS may have other functions, such as involvement in protein resorption (42). ACE, which is present in large amounts in the proximal tubule, is characterized not only by this widespread distribution but also by its lack of substrate specificity: in addition to converting Ang I to Ang II and degrading kinins, it hydrolyzes a variety of biologically active peptides, such as enkephalins (43), neurotensin (44), the B chain of insulin, and amidated peptides such as substances P and K. ACE has been shown to inactivate bradykinin within the tubule lumen (45). Generally, ACE is colocalized with other components of the RAS, making it likely that its key function is within that system. However, within the small intestine, ACE is present in largest amounts in the jejunum where few, if any, Ang II receptors are found (46,47). Furthermore, there is evidence that ACE within the brush border of the small intestine is involved in protein reabsorption (46,47). Though all components of the RAS are present within the renal proximal tubule, ACE does not appear to be rate limiting for the effects of Ang II within that structure, where Ang II leads to increased sodium, water, and bicarbonate reabsorption. We have recently shown increases in both ACE activity and mRNA in Phase I of puromycin nephrosis, during which there is heavy proteinuria (see below). During Phase II of puromycin nephrosis, in which proteinuria abates, there is less ACE activity and mRNA levels are lower. Thus, it would appear that, in Phase I of puromycin nephrosis, ACE might be increased in order to process proteinuria. Indeed, increased ACE levels in urine appear in clinical situations with renal injury. It is thus extremely attractive, in view of our finding of increased ACE in experimental nephrosis, to conjecture that ACE has a role in protein processing within the proximal tubule.

Role of the RAS in the Renal Medulla

Little attention has yet focused on the molecular biology of the RAS in the renal medulla. Copious numbers of Ang II binding sites have been demonstrated in the renal medulla (48), where physiological data demonstrate that Ang II modulates medullary blood flow in both the rat and the dog (49–51). In the normal rat, either Ang II-receptor blockade or ACE inhibition increases descending vasa recta blood flow whereas intrarenal infusion of Ang II reduces medullary blood flow. Given that medullary blood flow has an effect on renal water and solute excretion, the effects of Ang II in the medulla may participate in the modulation of urinary salt and water excretion. Both angiotensinogen and renin mRNA have been described in the renal medulla (52,53), though in lesser amounts than within the renal cortex. Preliminary study suggests that ACE mRNA is also present in the renal medulla, presumably within vascular endothelium (J.R. Ingelfinger, unpublished data). However, studies examining the regulation of the intrarenal medullary RAS are, at this juncture, few in number.

MOLECULAR BIOLOGY OF THE RAS IN RENAL INJURY

It appears to be likely that Ang II production is modified by a number of pathways within the kidney. Such a scenario would, in part, explain why ACE inhibition alone may not ameliorate the progression of renal diseases. Alternatively, ACE inhibition, as mentioned earlier, affects other pathways, such as the rate of vasodilatory kinin breakdown. Thus, it is not surprising that although a variety of studies suggest that ACE inhibition improves the outcome of
acute and chronic renal disease, contradictory results have also been found. In models of renal disease such as diabetes (54–56), renal ablation (27,57), uninephrectomy in the spontaneously hypertensive rat (SHR) (58), and late puromycin nephrosis (59), converting enzyme inhibition ameliorates progression. Yet, there are other models of renal failure, such as Adriamycin nephropathy, in which ACE inhibition has few effects on glomerular hemodynamic changes (60,61), presumably because converting enzyme inhibitors possess nonangiotensin-mediated effects, for example, inhibition of kinin breakdown (62,63). In other words, there might be nonhemodynamic, nonangiotensin mechanisms which could counterbalance the effects of reductions in transglomerular pressure effected by the use of ACE antagonists.

The Role of the RAS in Acute Renal Failure

A large body of data suggests that the RAS is involved in the pathogenesis of acute renal failure, and this area has been receiving increasing attention in recent years. A central tenet in support of the RAS involvement in acute renal failure is that sustained and severe vasoconstriction is the strongest event which interrupts renal function. Some of the most persuasive data concerning vasoconstriction come from clinical studies that used xenon transit (64,65). Although renal blood flow in toto is reduced to roughly one-third normal in many individuals with renal failure, the regional intrarenal perfusion demonstrates that renal cortical perfusion, and, thus, glomerular perfusion, is markedly decreased (65). There are several explanations for this finding. Active vasoconstriction would reduce blood flow. Passive vasoconstriction, as with vascular compression due to interstitial edema (66), or swelling of capillaries within the kidney (67), mesangial proliferation (68), intraglomerular capillary thrombosis (69), or shunting of blood to the renal medulla (70) have also been suggested. The improvement in renal perfusion by acetylcholine or hydralazine (71) in humans and by the use of various vasodilators (72) in animals would suggest an important pathophysiological role for active vasoconstriction.

Renin levels tend to be elevated in patients and animals with acute renal failure of many etiologies. A variety of models of acute renal failure in which the circulating RAS has been examined support the importance of increased renin and Ang II as mediators of acute renal failure. Some studies suggest that renin enzyme levels are increased in the kidney in rats with acute renal failure (73,74). Furthermore, manipulations such as the use of antibodies to components of the RAS or blunting of the RAS with high NaCl intake ameliorate the course of renal failure (75–78). The timing and general conditions of salt loading or salt deprivation may be important in acute renal failure; high sodium intake either just before or just after injury seems important (79). The importance of salt intake—and probable suppression of the intrarenal RAS—was shown in some provocative cross-transplant studies. Silber et al. (80–82) found that the most severe renal failure occurred in salt-deprived rats which received salt-deprived donor kidneys; conversely, salt-loaded rats receiving salt-loaded donor kidneys were relatively protected. Dehydration, which stimulates the RAS, either is necessary in some forms of acute renal failure or worsens it (66,83). There is evidence that saralasin and SQ 20881, a peptide ACEI, both increase renal blood flow in glycerol-induced acute renal failure in the rat (84). Other studies have shown that ACEI (65–66), beta blockade (87,88), and clonidine (89) have been effective in ameliorating the course of experimental acute renal failure.

The RAS involvement in the pathogenesis of acute renal failure depends on the concept that Ang II, generated either in peripheral blood or locally within the kidney, constricts the afferent arteriole, decreasing glomerular filtration to the point of initiating acute renal failure. Alternatively, Ang II within the kidney may decrease $K_f$, the ultrafiltration coefficient, causing decreased single nephron glomerular filtration rate (87,90). Furthermore, Ang II may be involved in glomerular tubular feedback (73) in acute renal failure. Although there is evidence that Ang II is generated in the circulation (78,91–95), both within the renal circulation (10,96–98) and extravascular portions (96,99–100) of the kidney, the localization of changes in RAS components in acute renal failure is largely unstudied. Those studies that have been published to date focus on biochemical levels of various RAS components. Few studies have examined gene expression, mRNA expression, or protein processing of RAS components in acute renal failure.

The Intrarenal RAS and Proteinuria

Little is known about the modification of RAS expression in proteinuric states. Heeg et al. (101) reported that ANG II did not reverse the antiproteinuric effects of ACE inhibition in humans in a short-term study of consecutive 2-h Ang II infusions at doses of 5, 10, and 20% of a pressor Ang II dose in proteinuric subjects who had been taking lisinopril (10 mg/day) for 3 months. The role of the RAS in various intrarenal compartments during proteinuria is largely unexplored, so that results such as those of Heeg et al. are difficult to explain. ACE levels are reported elevated in the urine of individuals with renal disease and in experimental renal disease. Ingelfinger et al. studied regulation of the intrarenal RAS in puromycin nephrosis, a disease characterized...
by heavy proteinuria in the rat. Animals were examined 4 days, 2 wk, and 12 wk after a single injection of 150 mg/kg of puromycin and were compared with control animals (102, 103). In the affected nephrotic animals, glomerular filtration rate was decreased, blood pressure was normal, and plasma volume was elevated, as previously reported by Anderson et al. (59). Although there were no differences between control and nephrotic animals with respect to plasma or kidney renin concentrations, renin and angiotensinogen (ang-n) mRNA levels, or to serum ACE, kidney ACE activity and mRNA were elevated in nephrosis and proportional to the degree of proteinuria. Although it is attractive to postulate a role of altered local RAS in the hemodynamic abnormality of puromycin nephrotoxicity, the overall data suggest that renal ACE, a nonspecific carboxypeptidase, may within its proximal tubule location play an additional role in handling heavy proteinuria.

RAS in High-Protein Diet

It has been noted for some time that plasma renin activity varies directly with the amount of protein in the diet. However, the relationships of protein intake to components of the intrarenal RAS and to the development of renal disease have only recently begun to be investigated. Rosenberg and colleagues (104, 105) examined the effect of dietary protein on the intrarenal expression of renin and angiotensinogen mRNA levels in normal male Sprague Dawley rats exposed to a high-protein diet (50%) compared with a low-protein diet (6%). Renal renin mRNA was higher in animals on a high-protein intake for either 3 or 21 days. Furthermore, when high- or low-protein diets were compared with standard protein diet (20%), the rats on the high-protein diet had a higher renal renin mRNA and the rats on the low-protein diet had a lower renal renin mRNA level than did those on a standard diet. In contrast, neither renal nor hepatic angiotensinogen mRNA levels changed with dietary protein intake. To compare the influence of renal hypertrophy on renal renin mRNA levels, the investigators performed uninephrectomy on a group of rats and compared this with protein-induced renal hypertrophy. There was no increase in renal renin mRNA level with uninephrectomy. Rosenberg and colleagues suggest that the level of dietary protein intake is a "novel and specific stimulus" for changes in renal renin mRNA and that increased plasma renin activity (PRA) seen on a high-protein diet is, at least in part, due to increased renal renin synthesis.

The Intrarenal RAS and Diabetes

Both glucose and insulin have potent effects within the kidney (106–108), and the potential interactions of insulin, glucose, and the RAS have been an area of interest for several groups (29, 54–56). For example, insulin per se may influence renal hemodynamics, though data are inconsistent as to direction. In the isolated perfused kidney, insulin attenuates Ang II-induced vasoconstriction (109). It has also been shown to stimulate sodium resorption and to suppress renin release (110). In nondiabetic dogs subjected to euglycemic clamp techniques, insulin appears to enhance the pressor response to Ang II (111). In normal subjects, it increases PRA and plasma Ang II levels. Diabetic ketoacidosis is associated with increased PRA and plasma Ang II levels. Less marked elevations are seen in nonketotic hyperglycemia, and the value for these RAS components in the circulation normalizes when euglycemia is achieved (112). Thus, the effects of insulin on the RAS would appear to be unclear, possibly because of the various techniques employed.

Very early in experimental diabetes, glomerular Ang II receptor density is reduced, perhaps from enhanced local RAS activity (113). With insulin therapy, the receptor density normalizes, suggesting an interaction between RAS, insulin, and/or glycemic control in diabetes (113). A spectrum of hemodynamic patterns has been observed at various levels of blood glucose. Renal vasoconstriction accompanies untreated diabetes mellitus (114). Intensive insulin therapy normalizes hemodynamics (115–116), whereas moderate hyperglycemia is associated with renal vasodilation and hyperfiltration (115, 117–118).

A distinct role for the RAS in diabetes is suggested by the salutary effect of ACEI administration in experimental diabetes, where ACEI normalizes glomerular capillary permeability (Poc) and ultrafiltration coefficient (Kf) and prevents glomerular injury even when there is plasma volume expansion and low plasma renin concentration (55). Chronic treatment with ACEI appears to be superior to treatment with either insulin alone or insulin with other antihypertensive treatment (55).

Although a variety of studies have examined the activities of the circulating RAS in diabetes, data have been inconsistent, perhaps because of the different circumstances of study conditions with respect to sodium state, volume status, glycemic control, and the like. In early diabetes, PRA and plasma Ang II levels are generally low or normal. Plasma inactive renin (prorenin) and serum ACE levels are normal, and PRA suppresses normally (118–122). In long-standing diabetes, some studies (119, 120), though not all (123, 124), suggest that PRA, serum ACE, and prorenin levels increase. Indeed, plasma prorenin has been proposed as a marker for the presence or future development of microvascular complications of diabetes (119, 120). A variety of studies suggest that lower doses of Ang II are suffi-
cient to increase blood pressure in diabetes and that suppression of PRA by salt or blood pressure is impaired (118). Such observations imply that vascular responsiveness to Ang II may increase as diabetes progresses.

Even less is known about the intrarenal RAS in diabetes than about the circulating system. Although insulin is clearly a growth factor within the kidney, its influence on the gene expression and posttranslational processing of components of the RAS is largely unexplored. Although PRA is not high, the glomerular hemodynamic responses to converting enzyme inhibitor suggest the possibility of localized increases in renal Ang II formation and/or action in experimental diabetes.

In streptozotocin diabetic rats, Anderson et al. (125) found plasma renin concentration in diabetic (DM) rats did not differ from that in nondiabetic control rats. However, renal renin concentration was markedly increased (2.3 fold) in DM rats and the increased protein activity level was associated with a significant increase in renal renin mRNA. Thus, in spite of the hyperphagia and increased salt intake in these animals, the diabetic rats did not exhibit decreased plasma renin concentration. Furthermore, increased renal renin mRNA levels suggest increased renal renin synthesis, which might contribute to the increase in renal renin concentration. In addition to increases in renal renin mRNA, there was a significant increase in angiotensinogen mRNA in DM kidneys compared with that in controls. Such findings of increases in both angiotensinogen and renin mRNA strongly suggest the possibility of increased renal Ang II formation in diabetic kidneys. These findings are especially striking, because this model is characterized by increased sodium intake and plasma volume expansion, which should reduce rather than increase renal renin and angiotensinogen levels (55,125). Thus, diabetic rats exhibit inappropriate renin and angiotensinogen gene expression, which was interpreted to indicate disordered (and possibly autonomous) regulation of the intrarenal RAS. These data suggest that the intrarenal RAS is autonomously regulated in diabetes, dissociable from plasma activity, and, with excess angiotensin and renin present, enhanced Ang II formation may occur. Renal ACE appears to be reduced, but not to a level that would prevent Ang II formation. The reduced renal tubular ACE, however, may contribute to proximal tubular dysfunction as manifested by the "tubular" proteinuria of diabetes.

Everett et al. examined renal renin and angiotensinogen expression during the evolution of diabetes in the adult biobreeding (BB) spontaneously diabetic rat (126). They observed an initial increase in kidney renin protein and gene expression followed by a decrease in both renin and angiotensinogen mRNA levels, as well as in the number of cells containing renin. They suggested that the decrease observed in renin gene expression over time was partly due to a decrease in the number of JG cells expressing the renin gene. In still another study, Sechi et al. (127) found no change in the intrarenal expression of RAS genes in rats with streptozotocin diabetes studied 12 days after disease induction. Ang II receptors studied at that time were decreased, providing a possible explanation for the glomerular hyperfiltration which is seen early in diabetes.

The changes in the gene expression of RAS components in diabetes mellitus remain important to examine, yet confounding variables including sodium state, volume homeostasis, and sympathetic nervous system activity make conclusions difficult. Even more significant, the mechanism(s) by which components of the RAS are altered in diabetes are important in that they may shed light on either the pathogenesis of diabetes or the pathophysiology of its complications.

The Kidney RAS in Hypertension

In studies of two-kidney, one-clip renovascular hypertension, involvement of the RAS has long been considered to play a major role in the pathophysiology of the model. In recent years, several studies have examined the changes in renin mRNA in this entity. Moffet and co-workers first demonstrated that in rats where renal artery constriction was performed, renin mRNA expression increases initially, but is then followed by a decrease to levels at or below the control levels (128). Samani et al. also studied renin mRNA expression in Goldblatt hypertension in rats. After 4 wk of clipping, there was a sixfold increase in renal renin mRNA levels in the clipped kidney as compared with those in control animals. In contrast, the unclipped kidney evidenced an eightfold reduction in renal renin mRNA. Twenty weeks after induction of renal artery stenosis, there was a 16-fold reduction in the unclipped side and only a fourfold increase in the clipped side compared with that in sham-operated, age-matched controls (129). Morishita and co-workers recently examined renal renin mRNA and liver angiotensinogen mRNA in 2K1C rats (130). Four weeks after clipping, both plasma renin and Ang II concentrations were elevated and renal renin mRNA levels were 2.6-fold increased in hypertensive as compared with sham rats. However, there were no increases in either plasma angiotensinogen concentrations or liver angiotensinogen mRNA levels at this time. In contrast, 16 wk after clipping, the increases in renal renin level were still higher than in shams and both plasma angiotensinogen concentration and
angiotensinogen mRNA level were elevated. Taken together, these various studies suggest that the intrarenal RAS may act as a modulator in this form of hypertension (128–130). Short term, renin is stimulated in the clipped kidney, but, with the chronic long-term increase in Ang II in this model, the peptide may act to suppress renin mRNA expression in both kidneys, resulting in a relative attenuation of the renin production on the clipped side.

The intrarenal RAS appears to be aberrantly regulated in another form of hypertension than in the SHR (131). Although mild sodium depletion stimulates renal angiotensinogen mRNA levels in normotensive WKY rats, the usual control rat for SHR, the latter kidney shows no change in renal angiotensinogen mRNA levels with alteration in sodium diet. We have shown that, in addition to failure to modulate levels of angiotensinogen mRNA, the SHR has lower steady-state renal angiotensinogen mRNA levels (131). In contrast, renal renin mRNA levels are not significantly different between these two rat strains and appear to be similarly regulated as well. Thus, a factor in the hypertension seen in the SHR, often taken as analogous to essential hypertension, may be abnormal renin substrate regulation within the kidney.

RAS IN URETERAL OBSTRUCTION

Obstructive uropathy may be accompanied by changes in renal plasma flow and glomerular filtration rate. The participation of the RAS in obstructive uropathy has been recognized for some time (131–135). Acute ureteral obstruction has been associated with increased renin release (132,133). Indeed, elevated renin has been reported in clinical hypertension (134,135) and elevated renal renin mRNA has been reported in experimental chronic unilateral ureteral obstruction (136,137). Furthermore, it appears that either mechanical or chemical denervation attenuates this increase, which suggests that the sympathetic nervous system mediates these changes.

"Annealing" Physiology and Molecular Biology

The techniques of molecular biology have clarified many issues concerning the synthesis, fate, and function of the RAS components. However, the ultimate usefulness for the tools of molecular biology in the understanding of renal physiology and pathophysiology requires combining or joining these disciplines. Much of the work done so far provides powerful yet still circumstantial evidence for the molecular pathobiology of the intrarenal RAS.

Physiological techniques such as whole-animal cardiovascular and renal clearances may be performed and followed by determination of mRNA and protein study in homogenates of those organs, either in the same animals or a matched cohort. Such studies compare various treatment groups, for instance, high- and low-salt diets. However, in a heterogeneous organ such as the kidney, the information obtained is limited. Other possibilities include the use of focused physiological and molecular biology techniques. For example, micropuncture studies may be performed, followed by molecular biology studies. Because the anesthesia and preparation involved in micropuncture may themselves affect the findings, study on a parallel cohort may be advisable. Though nucleic acid degradation is a potential issue, techniques such as isolated, perfused kidney and isolated, perfused tubule have been combined with subsequent molecular biology studies, for instance, the demonstration of renin mRNA response to sodium depletion (138,139). Renal cells in culture provide a unique opportunity to study cellular and subcellular physiology in concert with molecular biology. For example, as shown by Wolf and Neilson, mouse proximal tubule cells respond to Ang II with cellular hypertrophy (39).

Cells transfected with genes of interest may soon provide direct demonstration of intracellular mechanisms of the RAS physiology. For instance, Chan et al. (140) showed that the proximal tubule-like opossum kidney cells in culture transfected with the 5' flanking region of the angiotensinogen gene and the reporter gene chloramphenicol acetyltransferase (CAT) respond to thyroxine with an increase in CAT expression. Studies of cellular physiology in such transfected cells will help to explain the cellular role of Ang II.

Transgenic animals may provide a unique model in which to examine the difference from normal physiology. For example, mice transgenic for the rat angiotensinogen gene appear to develop hypertension (141) and rats harboring the mouse ren-2 gene develop fulminant hypertension (142). Furthermore, transgenic mice expressing the renin regulatory region with the large T antigen of simian virus 40 develop abnormal renin expression and a disordered vascular development (20).

On the molecular biology side of the technology equation a variety of new developments hold the potential for studying gene expression in very small amounts of tissue (e.g., obtained from biopsy), which may ultimately have clinical relevance. In situ hybridization studies permitting the identification of cells synthesizing mRNA of interest have, as described above, definitively localized the components of the RAS to specific areas of the kidney. Additionally, the increases in expression of renin (19) and angiotensinogen (6) on low-salt diet have been demonstrated by in situ study. Recently, the technique of polymerase chain reaction has been applied to
single glomeruli and nephrons (138,139) to demonstrate the presence of renin mRNA in small bits of tissue. The use of polymerase chain reaction and in situ techniques have been used to demonstrate Epstein-Barr virus in clinical renal biopsy tissue (143). The role of the intrarenal RAS in human renal disease should be approachable by such techniques.

Other molecular biology techniques such as restriction fragment length polymorphism (RFLP) and restriction fragment melting polymorphism (RFMP) (144) are applicable to the study of renal disease. In humans, RFLP technology is currently being used as a genetic marker for human polycystic kidney disease. RFLP studies have determined polymorphism in both the renin (145,146) and angiotensinogen (R.E. Pratt and V.J. Dzau, unpublished data) genes in several species, including the human. Unfortunately, association of these RFLP with human hypertension or renal disease is difficult to prove. However, studies linking polymorphism with sequence differences by using RFMP may be a more powerful technique, as yet unreported, for RAS genes.

The techniques and studies summarized above lend additional support to the concept that the RAS serves important roles within the kidney. As molecular biology techniques become increasingly refined, it may be possible to obtain new and important information concerning the regulation and function of the RAS and its role in the pathogenesis of renal disease.

ACKNOWLEDGMENTS

The work was supported by grants HL35610, HL35252, HL42663, and HL40210 from the NIH, a grant from the University of California Tobacco Related Disease Program (RT215), and an unrestricted gift from Bristol-Myers Squibb for Cardiovascular Research.

REFERENCES


24. Jose PA, Siovkoff LM, Montgomery S, Cal-


58. Dowkin LD, Grosser M, Feiner H, Ullman M, Randazzo J, Parker M: Renal vascular effects of antihypertensive therapy in uninephrecto-


76. Thiel G, McDonald FD, Oken DE: Micropunc-


