Interactions of Transforming Growth Factor-β and Angiotensin II in Renal Fibrosis

Wayne A. Border, Nancy A. Noble

Abstract—Overproduction of transforming growth factor-β clearly underlies tissue fibrosis in numerous experimental and human diseases. Transforming growth factor-β's powerful fibrogenic action results from simultaneous stimulation of matrix protein synthesis, inhibition of matrix degradation, and enhanced integrin expression that facilitates matrix assembly. In animals, overexpression of transforming growth factor-β by intravenous injection, transient gene transfer, or transgene insertion has shown that the kidney is highly susceptible to rapid fibrosis. The same seems true in human disease, where excessive transforming growth factor-β has been demonstrated in glomerulonephritis, diabetic nephropathy, and hypertensive glomerular injury. A possible explanation for the kidney's particular susceptibility to fibrosis may be the recent discovery of biologically complex interactions between the renin-angiotensin system and transforming growth factor-β. Alterations in glomerular hemodynamics can activate both the renin-angiotensin system and transforming growth factor-β. Components of the renin-angiotensin system act to further stimulate production of transforming growth factor-β and plasminogen activator inhibitor leading to rapid matrix accumulation. In volume depletion, transforming growth factor-β is released from juxtaglomerular cells and may act synergistically with angiotensin II to accentuate vasoconstriction and acute renal failure. Interaction of the renin-angiotensin system and transforming growth factor-β has important clinical implications. The protective effect of inhibition of the renin-angiotensin system in experimental and human kidney diseases correlates closely with the suppression of transforming growth factor-β production. This suggests that transforming growth factor-β, in addition to blood pressure, should be a therapeutic target. Higher doses or different combinations of drugs that block the renin-angiotensin system or entirely new drug strategies may be needed to achieve a greater antifibrotic effect. (Hypertension. 1998;31[part 2]:181-188.)

Key Words: transforming growth factor-β • angiotensin II • kidney • fibrosis

The RAS is the prototype of a classic systemic endocrine network whose actions in the kidney and adrenal glands regulate blood pressure, intravascular volume, and electrolyte balance. In contrast, TGF-β is considered to be a prototypical cytokine, a peptide signaling molecule, whose multiple actions on cells are mediated in a local or paracrine manner (TGFB exists in mammals in three isoforms, TGF-β1, -β2, and -β3, that have largely overlapping functions). In this review, we will use the generic term TGF-β because even where there are hints of isoform-specific actions, we believe the results need confirmation. Recently, an explosion of new information has dramatically expanded and altered our understanding of the RAS and TGF-β. For example, while the RAS was long thought to be entirely systemic, new data indicate that there is an intact RAS in many tissues whose actions are entirely paracrine. TGF-β, long thought to have only paracrine and autocrine effects, has now been shown to have wide-ranging systemic (endocrine) effects. Furthermore, there is a biologically rich and complex interaction between the RAS and TGF-β in which both act at various points to regulate the actions of the other. This interaction has great importance for understanding the vital roles that the RAS and TGF-β play in normal organ development, physiology, and tissue repair. However, the interplay between the RAS and TGF-β also has a dark side.

Activation of the RAS and generation of Ang II have long been known to play a role in the pathogenesis of hypertension and renal and cardiac fibrosis. Recently, TGF-β has been shown to be a uniquely powerful fibrogenic cytokine. TGF-β acts to simultaneously stimulate the synthesis of extracellular matrix, to inhibit the actions of proteases that degrade matrix, and to increase the expression of cell surface integrins that interact with matrix components. Through these three effects, TGF-β rapidly causes the deposition of an exuberant extracellular matrix. Recent studies from our laboratory have shown that Ang II infusion strongly stimulates the production and activation of TGF-β in the kidney. Ang II blockade reduces TGF-β overexpression in kidney and heart, and there is now a consensus that TGF-β mediates a good deal of renal and cardiac fibrosis, associated with activation of the RAS.

In this review, we will focus on new information about the interaction of the RAS and TGF-β. We will provide an overview of how the RAS and TGF-β act in the biosynthetic emergency of tissue injury as "911" molecules to maintain...
tissue homeostasis. We will propose that when injury is repeated or continual, this interplay between the RAS and TGF-β causes continued activation that may result in chronic hypertension and progressive tissue fibrosis leading to organ failure. We will provide evidence that the current pharmacological approaches to block the RAS are suboptimal and that, in addition to blood pressure, normalization of TGF-β should be part of the therapeutic goal. Current evidence suggests that a combination of RAS blockade with a separate agent to suppress TGF-β may be superior to RAS blockade alone. Such a combination may be required if progressive fibrotic diseases, such as diabetic nephropathy, are to be truly prevented, instead of just delayed.

**Biological “911” Molecules**

Maintenance of physiological and biochemical homeostasis is a key priority of living organisms. In this regard, the RAS and TGF-β can be viewed as powerful effector molecules that act to preserve systemic and tissue homeostasis. When there is a threat to homeostasis, an emergency “911” signal is sent, and the RAS and TGF-β respond by becoming activated. The concept that the RAS and TGF-β are members of a “911” response team is important because it anticipates the importance of cross-talk between the RAS and TGF-β as they carry out their biological functions.

A wound is a good example of a biological emergency in which the independent “911” actions of the RAS and TGF-β can be easily observed. In a systemic response, the RAS rapidly generates Ang II that acts by vasosconstriction to maintain blood pressure and then later stimulates the secretion of aldosterone, resulting in an increase in intravascular volume. In the wound, TGF-β is rapidly released by degranulating platelets where it does the following: (1) autoduces the production of TGF-β by local cells to amplify the biological effects, (2) chemoattracts monocyte/macrophages that degrade and fibroblasts that begin matrix synthesis, (3) causes the deposition of new matrix by simultaneously stimulating the synthesis of new matrix, inhibiting the proteases that degrade matrix, and modulating the numbers of integrin receptors to facilitate cell adhesion to the newly assembled matrix, (4) suppresses the proinflammatory effects of interleukin-1 and tumor necrosis factor, (5) regulates the actions of platelet-derived growth factor and fibroblast growth factor so that cell proliferation and angiogenesis are coordinated with matrix deposition, and (6) terminates the process when repair is complete and the wound is closed.

What is not apparent in the emergency paradigm of a wound is the significant interconnections (cross-talk) and overlapping properties between the RAS and TGF-β. These interconnections have only been discovered recently and are apparent at both the systemic and molecular levels and are especially apparent in the kidney. In a series of articles, we have shown that TGF-β’s actions in causing matrix deposition in a healing wound are the same actions that make TGF-β a powerful fibrogenic cytokine. Indeed, it is the failure to terminate the production of TGF-β that distiguishes normal tissue repair from fibrotic disease. Evidence now indicates that the RAS and TGF-β coregulate each other’s expression. Thus, this interaction in the kidney may keep both systems activated long after the “911” response should have been terminated. This sustained activity would lead to progressive fibrosis. Transgenic animals, where the TGF-β1 gene is linked to the albumin promoter, were produced as a model of liver fibrosis. However, these animals were found to have high plasma levels of TGF-β and to die of renal fibrosis before they exhibited significant liver fibrosis, indicating that the kidney is particularly susceptible to overexpression of TGF-β. The interrelationships of the RAS and TGF-β, which are the subject of this review, may explain this unique susceptibility of the kidney to TGF-β overexpression and why pharmacological suppression of the RAS and inhibition of TGF-β are both therapeutic in fibrotic diseases of the kidney.

**Interactions of the RAS and TGF-β in the Kidney**

Fig 1 depicts the complex interplay between the RAS and TGF-β in the kidney. Fluid shear stress, due to increased pressure or flow, has been shown to activate both the RAS and TGF-β in endothelial cells. In the glomerular endothelium, activation of the RAS and TGF-β has been shown to play a role in the pathogenesis of glomerulonephritis and hypertensive injury. Volume depletion and restriction of potassium are two classic maneuvers that stimulate renin production and hypertrophy in the JGA in the kidney. It was a complete surprise when it was discovered that these stimuli also strongly induce the production of TGF-β in the JGA. Co-localization of renin and TGF-β in the hypertrophic JGA has also been described in a hypertensive human. Treatment of rats with enalapril, an ACE inhibitor, further increased the expression of renin and TGF-β in the JGA. This result indicates that...
in the JGA, it is not Ang II that is inducing TGF-β, but suggests that the production of renin and TGF-β are coregulated. Indeed, TGF-β stimulates the release of renin from kidney cortical slices and cultured juxtaglomerular cells. In a recent study, prorennin transgenic rats that elevated levels of prorennin, were not hypertensive, and developed renal fibrosis, raising the possibility that stimulation of TGF-β was involved.

There are other fascinating interconnections between the RAS and TGF-β. Ang II strongly induces TGF-β production in cultured cells and vivo. It is thus likely that the fibrogenic effects that have been attributed to Ang II, are actually mediated by TGF-β, as will be described in detail later. Like Ang II, TGF-β stimulates the contraction of vascular smooth muscle cells and glomerular mesangial cells. This suggests that release of TGF-β from the JGA might modulate the glomerular micrcirculation. Indeed, the intravenous injection of high doses of TGF-β into rats that were volume depleted produced a dramatic reduction in glomerular filtration rate. The rat also developed severe retroperitoneal fibrosis. Surprisingly, injection of TGF-β had no effects in euolemic rats. The reason that TGF-β had such an effect in the presence of volume depletion is unclear but could be due to a synergistic effect between TGF-β and an activated RAS.

Another interplay between the RAS and TGF-β is at the level of aldosterone. Ang II stimulates the production and release of aldosterone from the adrenal gland. In contrast, TGF-β suppresses aldosterone production and strongly blocks the ability of Ang II to stimulate aldosterone by reducing the number of Ang II receptors expressed on the adrenal gland. Furthermore, TGF-β acts to block the effects of aldosterone on sodium reabsorption in cultured collecting renal duct cells. It was shown that infusion of aldosterone into rats with a remnant kidney increased blood pressure, proteinuria, and glomerulosclerosis, and neutralized the beneficial effects of Ang II blockade. The mechanism of aldosterone's pathologic effects is unknown but might be due to stimulation of TGF-β production in the kidney.

Angiotensin II and Renal Fibrosis: Insights From Angiotensin II Blockade

As scientific data supporting the central role of TGF-β in fibrotic disease have increased in recent years, so has evidence supporting the role of Ang II in renal and cardiac fibrosis. Much of the Ang II-fibrosis connection has come from animal and recently from human studies on the therapeutic effects of Ang II blockade. As early as 1986, using the remnant kidney model of progressive renal disease, Anderson et al. showed that ACE inhibitors slowed progression of disease. At present, more than 40 publications have shown the efficacy of angiotensin blockade in essentially every animal model of renal disease. In addition, data from human studies are now available that support the animal work. A large number of studies of both normotensive and hypertensive diabetic patients have been published. Other reports, first in renal scleroderma crisis and later in glomerulonephritis, hypertensive renal disease, sickle cell nephropathy, IgA nephropathy, and very recently human immunodeficiency virus-associated nephropathy, all indicate that angiotensin blockade slows progression of renal fibrosis.

While Ang II-mediated hypertension has long been known to lead to glomerular injury and sclerosis, it has not been entirely clear whether angiotensin blockade reduces fibrosis solely through controlling glomerular hypertension and thereby glomerular injury, or whether pressure-independent, as well as pressure-dependent, mechanisms are operating. There were early suggestions that glomerular hypertension and sclerosis did not necessarily coexist in the same glomerulus and that ACE inhibition can reduce sclerosis without altering glomerular pressure. The efficacy of angiotensin blockade in nonhypertensive diabetic subjects also suggested that pressure-independent effects were operating, but did not prove this because systemic blood pressure does not necessarily reflect glomerular capillary pressures. Recent data, however, obtained from in vitro studies in which pressure is not a factor, have provided strong support for pressure-independent actions of Ang II.

How Much Reduction of TGF-β Can Be Achieved With Angiotensin II Blockade?

If one accepts reduction of TGF-β overexpression as a valid and potentially useful therapeutic target, one might ask how much reduction can be achieved with conventional therapies. Specifically, can Ang II blockade normalize TGF-β? Although answering this question in humans has been difficult to date, there is a preliminary report showing reductions in urinary TGF-β in patients treated with ACE inhibition. We are aware of 11 publications in which TGF-β expression has been measured in animals treated with either ACE inhibition or AT1 receptor antagonists. By using numbers or by estimating from graphs presented in these publications, the approximate reduction of TGF-β overexpression achieved by these treatments was determined. The results are shown in the Table. Interestingly, in most of these studies, TGF-β levels remained somewhat elevated and, while disease severity was reduced in all cases, considerable disease remained. These findings are consistent with human studies showing that ACE inhibitors slow, but do not halt, disease. Although there are many possible explanations for these findings, an obvious one is that these therapies have been designed with blood pressure reduction as their target. Based on new data presented above, one can ask whether greater disease reduction can be achieved if TGF-β rather than blood pressure is the therapeutic target.

We have approached this question by using a rat model in which a single injection of ATS is used to induce glomerulonephritis. The ATS recognizes an epitope on the surface of rat mesangial cells and imitates a complement-dependent lysis of a portion of the mesangial cells. This tissue injury is followed by a fibrotic repair process involving increases in TGF-β expression, increased fibronectin, collagen and PAI-1 synthase, and marked deposition of extracellular matrix within the mesangium by 6 days after disease induction. This model has been used by us to demonstrate the importance of TGF-β in tissue fibrosis. Overexpression of TGF-β has been confirmed in essentially every model of renal fibrosis and in a large number of human fibrotic conditions. We recently set out to ask the question of how much reduction of TGF-β is possible with

However, the importance of TGF-β in the human disease has not been as well documented. Although the studies described above are encouraging, more work is needed in human disease to determine if similar reductions in TGF-β can be achieved in patients treated with conventional therapies. If these findings are confirmed in human disease, then TGF-β may be a potential target for future therapeutic intervention.

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Angiotensin Blockade Decreases TGF-β Expression

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<th>Reference</th>
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ACEI indicates angiotensin-converting enzyme inhibitor, AT,RA, angiotensin II type 1 receptor antagonist, DOCA, deoxycorticosterone acetate, SHR, spontaneously hypertensive rat, SP, stroke prone, Uni-Nx, unilateral nephrectomized, and CsA, cyclosporin A

Ang II blockade: We treated animals with increasing doses of either the ACE inhibitor enalapril or the AT, receptor antagonist losartan beginning 24 hours after disease induction with ATS. Interestingly, preliminary data indicate that increasing the dose of either drug above those usually used in such experiments does further reduce TGF-β overexpression. The idea that higher doses of these drugs than used to reduce blood pressure can more effectively reduce fibrosis was also found in several earlier studies. However, the maximum TGF-β reduction possible in our study was only about 50%. These findings are quite consistent with those presented in the Table, although none of those studies specifically tried to maximize TGF-β reduction.

We anticipate that these preliminary data will be confirmed and that similar data will appear using other animal models of renal fibrosis. If this occurs, it will be quite clear that Ang II blockade alone is not sufficient to halt renal fibrosis.

Molecular Interactions Between RAS and TGF-β in the Kidney

Why is it that Ang II blockade is only partially effective in reducing TGF-β and fibrosis? As discussed above, a rapidly increasing body of recent work supports the notion that RAS and TGF-β are far more intimately interconnected than had been previously imagined. The in vitro components of this work have strongly supported earlier suggestions that glomerular pressure is only part of the picture. Studies at the molecular level have already explained some RAS-TGF-β interactions, while new connections provide fascinating questions for future research. The interactions and potential interactions are shown in Fig 2.

Angiotensin II and TGF-β

Early in vitro suggestions that Ang II is a fibrogenic molecule came from demonstrations that when added to cultured murine mesangial cells, an increase in collagen synthesis is seen. That same year, as the studies linking TGF-β overexpression and fibrosis were increasing, it was reported that Ang II upregulates TGF-β production and increases TGF-β activation when added to cultured vascular smooth muscle cells. We then postulated that Ang II upregulates TGF-β, independently of pressure, and that this increase in TGF-β leads to extracellular matrix protein production and deposition. Using cultured rat mesangial cells, we confirmed the work with vascular smooth muscle cells by showing that Ang II induced time- and dose-dependent increases in TGF-β mRNA and TGF-β activation. In addition, lagging slightly behind the TGF-β increases were increases in mRNAs for matrix proteins biglycan, fibronectin, and collagen I, which were shown to result in increased protein production by immunoprecipitation and electrophoresis of newly synthesized, radiolabeled fibronectin and collagen. When neutralizing antibody to TGF-β was added to the cultures, the matrix protein increases were completely blocked in the case of fibronectin and about 77% blocked in the case of collagen I. Thus, the Ang II-mediated increases in matrix protein production are almost certainly mediated by TGF-β. These data provided an important missing link between Ang II and fibrosis. Ang II may also interact with other cytokines that might be involved in pathological processes.

A number of in vivo experiments have confirmed this in vitro work. Using the five sixths nephrectomy, or remnant kidney, model in the rat, in situ hybridization and immunohistochemistry studies were done over time. In early disease when sclerosis is not present, marked elevations in glomerular endothelial cell angiotensinogen mRNA were seen to be co-localized with increases in TGF-β mRNA. Later, TGF-β
mRNA was more widely increased and colocalized with staining for fibrotic matrix components laminin and fibronectin.56 These data suggested a pathological cascade in which reduced renal mass results in hyperperfusion. The hyperperfusion causes glomerular endothelial cell injury, perhaps through shear stress on cell walls. This injury results in induction of Ang II, then TGF-β and increased matrix protein synthesis and deposition. This cascade is supported by the finding that an Ang II receptor antagonist markedly reduces disease.

Although this study is particularly nice because it shows increases in angiotensinogen mRNA at the site of injury, a large number of studies have now shown that Ang II blockade decreases overexpression of TGF-β. These studies, shown in the Table, used animal models with a wide range of etiologies. In all cases, Ang II blockade reduced TGF-β, making it likely that Ang II blockade will reduce TGF-β overproduction at least to some extent in all fibrotic renal diseases. While this does not prove that Ang II causes fibrosis in all of these diseases, it does suggest a strong biological linkage between Ang II and TGF-β, which is very relevant to renal fibrotic diseases.

**Angiotensin II and the Plasmin Protease System**

Plasmin has long been investigated as a fibrinolytic enzyme important in dissolution of clots after wounding. Plasmin is generated from plasminogen by the enzymatic action of plasminogen activators, which are inhibited by PAs. The generation of plasmin, and thus its action as a protease, are determined by the balance of plasminogen activators and PAs.66 Interestingly, the inhibitor of plasmin generation, PAI-1, like Ang II and TGF-β, can be considered a "911" molecule, in that it is rapidly increased at the site of wounding where it acts to stabilize the fibrin clot, which helps to stop hemorrhage, provides a temporary seal against bacterial invasion, and serves as a scaffolding for platelet aggregation and as a temporary matrix. Again, as with Ang II and TGF-β, a relatively new role for this system has been elaborated that is highly relevant to fibrotic diseases.

In addition to degrading fibrin, plasmin acts on a wide range of extracellular matrix proteins. It also cleaves some procollagens to produce active molecules that degrade collagens. The importance of plasmin in turnover of the mesangial matrix was recently shown by studies in which mesangial cells were plated onto radioactive matrices. The turnover of matrix was measured by the appearance of radiolabeled degradation products into the culture supernatant. These studies showed that mesangial matrix turnover is related to plasmin generation and that PAI-1 levels are a major determinant of the plasmin generated.67

Earlier work from our laboratory had indicated that in response to added TGF-β, isolated, cultured glomeruli show a rapid and marked increase in PAI-1 mRNA and protein.68 In addition, using the ATS model of glomerulonephritis in the rat, we showed that by day 3 of disease, glomerular PAI-1 mRNA and protein were greatly elevated compared with levels in normal control rats. The ability of culture supernatant from isolated nephritic glomeruli to degrade casem was also markedly reduced, suggesting that these PAI-1 elevations did in fact lead to decreased plasmin proteolytic activity.68

Marked increases in PAI-1 expression in renal fibrotic diseases have now been shown in a number of animal76,78 and human studies. Increased immunohistochemical staining of PAI-1 in human renal biopsy tissues has now been shown in crescentic nephropathies, acute and chronic transplant rejection, diabetic nephropathy, IgA nephropathy, lupus nephropathy, focal sclerosis, and human immunodeficiency virus-associated nephropathy, making PAI-1 immunostaining a very useful marker of fibrosis.71-74 Also supporting a role for decreased plasmin generation contributing to matrix accumulation in fibrotic diseases in other tissues is a recent report showing that PAI-1-overexpressing mice had more, and PAI-1 deficient mice less, severe fibrosis in response to bleomycin-induced lung injury.78

Beginning in 1991, reports of a linkage between Ang II and PAI-1 have appeared. It has now been shown that Ang II leads to rapid and marked elevations in PAI-1 expression when added to cultured brain astrocytes, vascular endothelial cells, vascular smooth muscle cells, and bovine aortic endothelial cells.66-70

However, the interconnections between Ang II, TGF-β, PAI-1, and fibrosis in renal cells were made very recently in two publications. In experiments similar to those discussed above showing that TGF-β mediates Ang II-induced increases in matrix protein expression by cultured mesangial cells, we showed that addition of Ang II caused a very rapid increase in PAI-1 mRNA, which appeared to precede the increase in TGF-β.69 When antibody to TGF-β was added, the early increase in PAI-1 expression remained, indicating that it was not mediated by TGF-β. However, in the presence of anti-TGF-β, the early increase was not sustained over time. It was concluded that Ang II induces both an early TGF-β-independent increase in PAI-1 and a later TGF-β-mediated increase. In the context of "emergency" molecules, it is interesting to speculate that Ang II produced or delivered to the site of injury early immediately induces PAI-1, which helps to stabilize the fibrin clot. Later, after Ang II has upregulated TGF-β, this TGF-β sustains PAI-1 increases, reducing matrix degradation and thereby enhancing wound closure. The orchestration and timing of PAI-1, PA, and plasmin activity, however, are likely to be complex, since there is ample evidence that plasminogen and plasmin carry out important functions in response to injury.81-83 That the early increase in PAI-1 does, in fact, occur in response to renal injury is suggested by preliminary data from our laboratory, which showed a 10-fold increase in PAI-1 production by glomeruli isolated 6 hours after induction of ATS-glomerulonephritis (W A B, and N A N, unpublished observation, 1997). It will be interesting to determine, by blocking Ang II before injury, whether this increase in Ang II mediates and whether blocking this early PAI-1 increase reduces or worsens the fibrotic disease that follows.

Finally, in a model of radiation-induced renal injury and scarring, dramatic increases in PAI-1 mRNA were seen, which were partially ameliorated by ACE inhibition or AT1 receptor antagonists.61 Whether the PAI-1 overexpression is mediated through TGF-β or is caused directly by Ang II as the authors argue, or is a combination of the two, remains unclear. Whatever the case, this study does, once again, suggest a
relationship between Ang II and PAI-1 that is relevant to human fibrotic renal diseases

**Aldosterone and Renal Fibrosis**

Fascinating new data mentioned briefly above have appeared in which aldosterone overproduction has been linked to hypertension and glomerulosclerosis. In the remnant kidney model, aldosterone levels more than 10-fold above normal were seen. When the ACE inhibitor enalapril and the AT1 receptor antagonist losartan were given in combination, aldosterone levels and disease severity as measured by proteinuria, systemic blood pressure, and glomerulosclerosis were decreased compared with those in untreated remnant kidney animals. However, when aldosterone infusion was added to enalapril and losartan, disease severity increased to the level of untreated rats. These data suggest that aldosterone may have fibrogenic effects independent of Ang II and clearly raise the question of whether aldosterone upregulates TGF-β expression. Because administration of the aldosterone receptor antagonist spironolactone did not block glomerulosclerosis but did transiently reduce proteinuria, arterial pressure, and cardiac hypertrophy, the fibrotic actions of aldosterone and the possible induction of TGF-β from mesangial cells, independent of Ang II, are both increased, independent of Ang II, in the JGA are both increased, independent of Ang II, in the JGA.

**Renin and Prorenin as Fibrogenic Molecules**

As with aldosterone, recent studies suggest that prorenin or renin may have Ang II-independent actions to increase fibrosis. First, transgenic, prorenin-overexpressing rats were found to be normotensive but to develop severe glomerulosclerosis. Second, human recombinant renin, added to human mesangial cells, induces marked upregulation of PAI-1 production, which is not only independent of Ang II but acts through a renin receptor on mesangial cells, independent of the enzyme site used by renin to convert angiotensinogen to angiotensin I. It is tempting to speculate that renin also upregulates TGF-β expression. That this may, in fact, be the case is also suggested by findings that TGF-β and renin staining in the JGA are both increased, independent of Ang II, in response to water deprivation and potassium depletion.

**TGF-β as a Renin Secretagogue**

Finally, several articles have been published suggesting that TGF-β enhances renin release. In an early article using renal cortical slices, TGF-β was shown to cause renin release. More recently, addition of TGF-β to short-term culture of juxtaglomerular cells was shown to increase renin release from these cells. These studies suggest that TGF-β may be an important factor in renin release, however, the relevance of this interaction to fibrotic disease is unknown.

In summary, new data suggest that Ang II exerts pressure-independent effects on renal fibrosis through upregulation of TGF-β which, in turn, leads to tissue fibrosis. In addition, Ang II has direct effects on PAI-1 production, which may also play a role in accumulation of pathological extracellular matrix through decreasing the actions of plasmin to degrade matrix and to activate collagenases. Although far from conclusive, new data suggest that the RAS components aldosterone, prorenin, and renin may be intimately connected with TGF-β production and fibrotic matrix accumulation.

It is interesting to speculate that these remarkable interconnections between the RAS and TGF-β arise evolutionarily from the importance of restoring homeostasis and effecting rapid wound closure to avoid sepsis in response to injury. In that preventing blood loss and sepsis was likely to have been a major evolutionary advantage, it is not surprising that there may be redundancy in these systems, such that if one arm is blocked, others can adequately take over the missing function. Such redundancy appears quite commonly in nature as shown by studies using a great number of mouse lines, where genes thought to have been critical to survival are knocked out, and the animals are born and develop with minimal abnormalities. Therefore, when this "911" system with all its interconnections remains activated, as it may well do in renal fibrotic diseases, therapies aimed at more than one arm will be necessary to effectively halt, rather than merely slow, disease.

**Acknowledgments**

This work was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases DK 49342 to W A B. Preparation of this manuscript is sponsored in part by an unrestricted educational grant from Merck & Co.

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Hypertension. 1998;31:181-188
doi: 10.1161/01.HYP.31.1.181
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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