Hypertension induces blood vessels and thereby causes end-organ damage. The mechanisms are complicated and, although studied for decades in experimental animal models, are only currently being elucidated. From the efforts of many investigators, we are now in the position of constructing a chain of events from the endothelium to the underlying matrix, to the vascular smooth muscle cells, and beyond to the adventitia, and surrounding tissues. The endothelial layer acts as a signal transduction interface for hemodynamic forces in the regulation of vascular tone and chronic structural remodeling of arteries. Effects of mechanical forces on signal transduction and gene expression in endothelial cells have been demonstrated. Mechanical stress initiates numerous pathways including ion channels, integrin interaction between cells and matrix, activation of various tyrosine kinases, autocrine production, and release of growth factors. Increased flow through small arteries has been shown to increase connective tissue production and promote medial hypertrophy, probably through proliferation of both endothelial and vascular smooth muscle cells. Increased pressure is also capable of inducing early response genes in the arterial wall. Microvascular endothelium in hypertensive animals has been shown to exhibit increased oxyradical production attributable to xanthine oxidase. Oxyradical production by endothelial cells can result in leukocyte-endothelial adhesion responses that involve transcription-independent and -dependent surface expression of different endothelial cell adhesion molecules. Infiltration of the permeabilized endothelium by leukocytes sets the stage for an inflammatory cascade, involving cytokines, chemokines, growth factors, and matrix metalloproteinases. Altered integrin signaling, the production of tenacin, epidermal growth factor signaling, tyrosine phosphorylation, and activation of downstream pathways culminate in vascular smooth muscle cell proliferation. Evidence is accumulating that matrix molecules provide an environment which decreases the rate of programmed cell death.
Mechanical forces alone are capable of initiating complex events resulting in vascular remodeling and subsequent end-organ damage. However, hypertension is not merely a process of mechanical events. All forms of hypertension involve mediators, which elicit their own responses, independent of arterial pressure. The first practicable model introduced by Goldblatt11 fascinated Wilson and Byrom,1 who appreciated much of which we regard as angiotensin (Ang) II-mediated damage today. Our group is interested in hypertension-induced and Ang II-mediated injury in the kidney and the heart. We have concentrated on a unique, double transgenic model in rats (dTGR) harboring the human renin and human angiotensinogen genes.12 This model was developed by the combined efforts of Ganten et al13 and collaborators from the laboratory of Murakami.14 This model permits studying local vascular effects of blood pressure and Ang II, while permitting use of human renin inhibitors that otherwise would not function in a rat model. All animal studies reported here were conducted according to American Physiological Association guidelines and were duly approved. Similar models have been developed in double transgenic mice by Shimokama et al15 and by Merrill et al.16 The mouse model exhibits characteristics also found in our rat model and is equally suitable. The models provide an opportunity to study a cascade of events, in part briefly mentioned above, which results in vascular and subsequently end-organ damage.

**Hypertensive Mechanisms in dTGR**

The relationship between sodium chloride intake–excretion and systemic blood pressure (pressure-natriuresis) is shifted rightward in all forms of hypertension.17 Roman and Cowley have developed a method which allows the determination of pressure-natriuresis mechanisms intrinsic to the kidney, thereby separating these mechanisms from extrarenal regulators, which can also shift pressure-natriuresis.18 We studied 6-week-old dTGR and found that pressure-natriuresis was shifted rightward and that only intrinsic renal mechanisms accounted for the shift. The high expression of both transgenes within the kidney suggested Ang II acting locally may be responsible.19 We tested for Ang II-related effects by blocking the action of Ang II at the Ang II (AT1) receptor and by inhibiting the generation of Ang II through the actions of angiotensin converting enzyme (ACE).20 We found that both AT1 blockade and ACE inhibition lowered blood pressure and shifted pressure-natriuresis leftward, but both did so only incompletely. When both agents were given together, blood pressure could be normalized and pressure-natriuresis restored to normal levels. More importantly, we also observed that the action of ACE inhibition involved restoring renal blood flow and glomerular filtration rate to normal, while AT1 receptor blockade diminished tubular sodium reabsorption. Thus, two Ang II-related mechanisms appeared operative: hemodynamic effects and tubular sodium reabsorption. Both human and rat renin and angiotensinogen genes were downregulated in dTGR and increased by AT1 blockade and ACE inhibition, whereas no changes in the expression of rat ACE and AT1 receptor genes were observed. We believe these observations are novel because they point to two distinct Ang II-related intrarenal mechanisms accounting for the shift in pressure-natriuresis and increase in blood pressure. The ACE inhibitor effects can be explained by kinin-related mechanisms. AT1 blockers are resistant to degradation and reuptake following filtration and thus may affect AT receptors at the tubular lumen. Our findings differ somewhat from a recent report by Ots et al.21 who studied combination therapy with enalapril and losartan on the rate of progression of renal injury in a 5/6 nephrectomy renal mass ablation rat model. They found similar degrees of blood pressure reduction with enalapril and losartan. However, combination therapy offered no clear-cut advantages that could not be attributed to improved blood pressure reduction.

**Vascular and End-Organ Damage**

Wilson and Byrom1 performed a crucial experiment that showed that constriction of one renal artery produced severe hypertension in rats but that no vascular lesions in the clipped kidney occurred. These findings indicated that increased pressure preceded and elicited vascular damage. In their model, Ang II was responsible for pressure elevations and vascular damage. Kincaid-Smith et al22 confirmed these findings and observed that the constricted and dilated areas along the vessel was accompanied by coagulation abnormalities and vascular lesions resembling those of the hemolytic-uremic syndrome. More recently, Ruggenenti and Remuzzi23 have drawn attention to endothelial cell swelling, detachment, proliferation, fibrin deposits, fibrinoid necrosis, and the myointimal rearrangement resulting in irreversible vascular destruction. An example from a 6 week-old, salt-supplemented dTGR exhibiting vascular changes indistinguishable from those of the hemolytic uremic syndrome is shown in Figure 1. A section from the heart of the same animal shows focal areas of myocardial necrosis.

The interplay between hypertension and Ang II appears responsible for these dramatic vascular changes. A direct effect of Ang II on the endothelium has been appreciated for decades. Asscher and Anson24 demonstrated the existence of a vascular permeability factor which was responsible for the development of arterial necroses resembling those found in malignant hypertension. Robertson and Khairallah25 subsequently showed that Ang II increased the permeability of rabbit aortic endothelium to Evans blue, an effect that could be blocked by competitive synthetic peptides. Increased vascular wall permeability undoubtedly is important to the vasculopathy, as Wilson and Byrom1 conjectured. The effects of Ang II on endothelium are more complex than these investigators could imagine. Bech Laursen et al26 showed recently that Ang II-induced hypertension involved vascular O2− production, whereas norepinephrine-induced hypertension did not. Treatment with superoxide dismutase ameliorated the Ang II-induced hypertension but not the norepinephrine-induced hypertension. The authors suggested that the Ang II effect on O2− production occurs via degradation of endothelium-derived NO. Reactive oxygen species were also implicated in Ang II-induced cardiomyocyte hypertrophy by Nakamura et al.27 These investigators found that they could inhibit such hypertrophy by administering antioxidants.

In addition to directly elevating blood pressure, O2− production in the vessel wall, heart, kidney, and elsewhere may...
have been responsible for a host of other consequences. The role of oxidative stress and the mediation of arterial inflammatory responses in hypertension and atherosclerosis have been recently reviewed. Reactive oxygen species may act as signal transduction messengers for several important transcription factors, including NF-κB and activator protein (AP)-1. Binding sites of the redox-regulated transcription factors NF-κB and AP-1 are located in the promoter region of a large variety of genes that are directly involved in the pathogenesis of vascular disease. NF-κB-regulated proteins include proinflammatory cytokines such as tumor necrosis factor, certain interleukins, and granulocyte-macrophage colony-stimulating factor; chemokines such as macrophage chemotactic protein (MCP)-1; lipoxygenases; receptors such as the IL-2 and T-cell receptor; and adhesion molecules such as intercellular adhesion molecule (ICAM)-1, vascular-cell adhesion molecule (VCAM)-1, and E-selectin. Cyclic strain induces an oxidative stress in endothelial cells. Ang II can activate p38 mitogen-activated protein kinase, which is a critical component of the redox-signaling pathway. Further evidence supporting these mechanisms is provided by a model of atherosclerosis, which included amelioration by ACE inhibition. We have accrued evidence of increased \( \cdot \mathrm{O}_2 \) production and NF-κB upregulation in the dTGR model. A mobility shift assay, documenting increased NF-κB expression in kidney tissue from dTGR compared with control kidneys is shown in Figure 2. We are particularly interested in this interconnection because of the considerable MCP-1, ICAM-1, and VCAM-1 expression we were able to detect in our model.

We present evidence that surface adhesion molecules and cell migration into the vessel wall are important to the vasculopathy. With fluorescent antibody cell-sorting analysis, we observed that LFA-1 and VLA-4, the ligands to ICAM-1 and VCAM-1 were expressed on circulating leukocytes and that both ICAM-1 and VCAM-1 were expressed on the endothelial cell surface, cardiac vessels, and elsewhere. Komatsu et al. found that chronic hypertension in spontaneously hypertensive rats also resulted in increased ICAM-1 expression on the endothelium and emphasized the role of ICAM-1 in end-organ damage. Simultaneously, MCP-1 was increased and ED-1-positive cells appeared within the vascular wall in significant numbers. We and others have described surface adhesion molecule expression on the vascular wall in high renin models of hypertension. The appearance is reminiscent of histological findings observed in models of reperfusion injury. Mononuclear cell recruitment via adhesion molecules, GM-CSF, and MCP-1, as in our study, is likely to be important to the vasculopathy on the basis of cytokine release, leading to increased expression of extracellular matrix and vascular smooth muscle cell proliferation. The remarkable MCP-1 expression we observed, to the point that we could measure an increase in this chemokine in urine, is in accord with recent findings reported by Capers et al. We observed increased extracellular matrix production in this and in previous models. Kim and Iwao have recently
reviewed their findings on TGF-β1, fibronectin, and collagen expression in heart and kidney in several rat models of hypertension and the effects of AT1 receptor blockade. Their findings are in accord with those observed in our dTGR model. We previously observed increased expression for the gene encoding the GM-CSF receptor in the hearts of hypertensive rats, concomitant with cardiac macrophage infiltration. It is likely that GM-CSF-related mechanisms also played a role in macrophage proliferation in dTGR. Finally, we have not yet investigated whether or not the infiltrating macrophages contain, or even produce, Ang II. Circulating macrophages and macrophages infiltrating atherosclerotic plaques, have been found to contain generous amounts of Ang II. Whether they take Ang II up from circulating plasma or whether they actually make their own, cannot be answered for certain.

dTGR as a Model for Interventions

Figure 3 (upper panel) shows the effect on blood pressure exerted by the chronic daily gavage-administered ACE-inhibitor cilazapril, the AT1 receptor blocker valsartan, the human renin inhibitor RO 65 to 7219, and the endothelin receptor blocker bosentan in dTGR. The animals were treated for 3 weeks between weeks 4 and 7. The ACE inhibitor and the AT1 receptor blocker effectively lowered blood pressure, the human renin inhibitor lowered blood pressure significantly less, and bosentan lowered blood pressure slightly but not significantly. The ACE inhibitor, AT1 receptor blocker, and the endothelin receptor blocker were effective in ameliorating histological damage. Figure 3 (lower panel) shows the effect of treatment on albuminuria. The albuminuria of dTGR was 1000-fold greater than control rats. All drug treatments markedly reduced albuminuria. Importantly, complete regression of renal and cardiac injury was also observed with human renin inhibition and endothelin receptor blockade, although the blood pressure-lowering effects were modest. These latter two agents therefore appeared to exert positive effects on renal damage independent of blood pressure.

In untreated dTGR, we found severe left ventricular hypertrophy with focal areas of necrosis, probably on the basis of fibrinoid necrosis and vascular occlusion. Rarefaction of capillaries and arteriolar growth have recently been described in Ang II-dependent cardiac hypertrophy. The cardiac changes we observed were as severe as those observed in the kidneys and responded similarly to treatment. Interestingly, although the human angiotensinogen gene was expressed in considerable abundance in the heart, the human renin gene mRNA was barely detectable, even with a quantitative polymerase chain reaction determination. We believe that Ang II is generated locally in the heart and that the renin necessary for this purpose is taken up from the plasma and perhaps processed. Evidence for such uptake has been provided by our group and others in earlier studies. Considerable amounts of the changes we observed may have been primarily related to Ang II and less to blood pressure.

The human renin inhibitor we used had a shorter duration of action compared with the ACE inhibitor and the AT1 receptor blocker, which in part accounts for its lesser effect on blood pressure. Nevertheless, the human renin inhibitor effectively decreased end-organ damage in dTGR. The protection appeared to be greater than we would have predicted from the reduction in blood pressure alone. It is likely that the renin inhibitor acted not only on circulating renin, but also renin incorporated into the vasculature, within the interstitium of the kidney, or even within certain cell types. De Mello has shown that intracellular renin may influence cell-to-cell communications, an effect which could be inhibited with enalaprilat. These observations are particularly interesting, since we have shown that Ang II operates intracellularly in terms of initiating signaling and that this signaling can be spread to adjacent cells, probably through the second messenger IP-3 acting through tight junctions. However, understanding the nature of an intracellular renin-angiotensin system leaves much to be elucidated.

The clinical significance of endothelin in cardiovascular disease has recently been reviewed. Ang II stimulates the expression of endothelin by endothelial cells. Furthermore, Ang II increases tissue endothelin and induces vascular hypertrophy. In vitro studies on vascular smooth muscle cells suggest that endothelin has a stimulating effect on cell proliferation. We were thus not surprised to find that dTGR had increased endothelin concentrations in kidney and heart.
and that bosentan ameliorated the severity of vascular damage, even though blood pressure was scarcely influenced by this intervention. Moreau et al. were able to show that Ang II stimulates endothelin under in vivo conditions and that endothelin thus represents a paracrine local system that interacts with the renin-angiotensin system. The interaction appears more prominent in the vascular wall than in the plasma. Our findings would support that view. Furthermore, the amelioration of vascular damage suggests that endothelin receptor blockade may provide an additional therapeutic avenue. Nephroprotection of an ET\textsubscript{a}-receptor blocker in salt-loaded uninephrectomized stroke-prone spontaneously hypertensive rats has been demonstrated by Orth et al. In spontaneously hypertensive rats, bosentan ameliorated cardiac hypertrophy and fibrosis and improved creatinine clearance, independent of blood pressure-lowering effects.

**dTGR as a Model of Hypertension and Ang II-Related Effects**

We observed that leukocyte infiltration in the vascular wall accompanies PAI-1, MCP-1, and VEGF expression. PAI-1 is a major physiological inhibitor of the plasminogen activator (PA)/plasmin system, a key regulator of fibrinolysis and extracellular matrix turnover. Activation of the renin-angiotensin system can disturb the balance of the fibrinolytic system by stimulating excess production of PAI-1 and thereby increasing the risk of thrombotic events. We believe that the resemblance of the kidneys in our untreated, salt-supplemented dTGR to the hemolytic uremic syndrome, reflects that effect. In our model, we were able to show that ACE inhibition, AT1 receptor blockade, and renin inhibition decreased PAI-1 expression. We observed similar effects on VEGF expression. A stimulatory interaction between VEGF and endothelin-1 on each gene expression has recently been described. This interaction could have an important comitant effect on proliferation of endothelial and smooth muscle cells in the vascular wall.

Ang II is mitogenic for several renal cell types. For instance, Ang II stimulates expression of the chemokine RANTES in rat glomerular endothelial cells; the AT2 receptor may be involved in this response. We are currently investigating this issue in our model. The growth-promoting action of Ang II is largely mediated by autocrine and paracrine factors such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF). ACE inhibition abolishes medial smooth muscle PDGF-AB biosynthesis and attenuates cell proliferation in injured carotid arteries. In the rat carotid artery and in certain mesenteric microvessels, the mitogenic effects of Ang II are mediated by bFGF. The importance of tyrosine phosphorylation in Ang II signaling has been demonstrated by Schieffer et al. as well as by Schmitz et al. Ang II-regulated tyrosine kinases are required for proto-oncogene expression, protein synthesis, and proliferation. Ang II stimulates expression of transforming growth factor-\(\beta\) (TGF-\(\beta\)) in cultured renal cells. However, in vivo, TGF-\(\beta\) expression can also be up-regulated by blood pressure increases, independent of Ang II. Ang II upregulates VEGF expression in cardiac endothelial cells while potentiating VEGF-related effects in microcapillary endothelial cells. In vivo studies to investigate the role of growth factors in this model are planned. Interactions between Ang II, nitric oxide, and endothelin remain to be explored. The role of disturbed carbohydrate metabolism in altering the sensitivity to Ang II in the kidney warrants attention. Finally, apoptosis and its induction appear important in protection from, and regression of, vascular disease. Pollman et al. recently showed that down-regulation of intimal bcl-\(x\)\textsubscript{l} expression with antisense oligodeoxynucleotides induced acute regression of vascular lesions in a rabbit model of balloon-induced vascular injury. Similarly, Yaoita et al showed attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor. The role of apoptosis or its inhibition in our model is yet to be explored. New therapeutic avenues may be introduced by influencing apoptosis.

In summary, we are in the process of investigating a high human renin double transgenic rat model, characterized by severe nephrosclerosis and cardiac injury. We have developed a hypothetical schema shown in Figure 4. We postulate that forces acting on the vascular wall and Ang II stimulate oxidative stress directly or indirectly via endothelin I. We speculate that the transcription factors NF\(\kappa\)B and AP-1 are involved with initiating chemokine and cytokine expression, leading to the above cascade. Adhesion molecule expression, attraction of leukocytes, release of cytokines and chemokines, factors favoring coagulation, cell proliferation, and growth factor-induced matrix production all are likely to promote vascular injury. This rapidly moving area of research will permit novel approaches to test new hypotheses and to develop experimental therapies for hypertension-induced vascular injury.

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