New Insights into Target Organ Involvement in Hypertension

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The therapy of hypertension is in large measure directed to the prevention of target organ disease in the brain, kidney, heart, and vasculature. It is noteworthy that some of the factors that participate in the raising of arterial pressure also participate in the pathogenesis of hypertension-related target organ disease. Foremost among these factors is the renin-angiotensin system (RAS), which is both a powerful regulator of intravascular volume and arterial pressure as well as a mediator of tissue damage. Over recent years new knowledge has been developed regarding the participation of the RAS in the development of target organ damage and these insights in turn suggest improved methods of therapy.1–8

THE RENIN-ANGIOTENSIN SYSTEM AND VASCULAR DISEASE

It has been shown that infusion of angiotensin II, the major effector peptide of the RAS, can lead to hypertension accompanied by cardiac fibrosis, vascular remodeling, vascular hyalinization, and fibrinoid necrosis in various animal models.4–9 Thus, many of the major findings in acute severe hypertension can be reproduced by angiotensin-induced hypertension. At the same time, atherosclerosis, a longer term potential sequela of high blood pressure, has come to be seen as a complex process involving the genesis of fatty streaks, vascular inflammation, plaque formation, and at times plaque rupture. Angiotensin II participates in many phases of this process. Recent work in a primate model of atherogenesis demonstrates that blockade of the AT-1 angiotensin II receptor results in marked diminution in fatty streak formation in large arteries. Monocyte adherence to the vessel walls of these animals is reduced and in fact remains reduced for some weeks after cessation of AT-1 blocker therapy suggesting an effect of angiotensin II blockade on the differentiation of monocyte precursors.10,11 Similarly, angiotensin has been shown to be proinflammatory, through
the production of oxidative stress as well as the up-regulation of inflammatory mediators such as transforming growth factor (TGF)-beta.\textsuperscript{4,12} In cell culture and other models, angiotensin can either stimulate cellular proliferation or hypertrophy depending on cell type and culture conditions.\textsuperscript{13-16} In this regard it is interesting to note that converting enzyme inhibition or AT-1 blockade when administered to hypertensive subjects can be shown to improve hypertension-induced vascular remodeling in arterioles studied at biopsy. Beta blockers do not produce a similar effect even when blood pressure is equally controlled.\textsuperscript{17} Angiotensin-converting enzyme is up-regulated in the shoulders of arterial plaques and this makes it reasonable to suppose that increased angiotensin II production at these sites could lead to a proinflammatory environment, an up-regulation of matrix metalloproteinases, and ultimately to plaque rupture.\textsuperscript{18,19}

Thus considerable evidence points to the participation of the RAS in the development of the sequelae of hypertension. Recent findings further extend this participation.

THE (PRO)RENIN RECEPTOR

One of the most exciting findings of recent years has been the discovery and initial characterization of the (pro)renin receptor.\textsuperscript{20,21} It has for some time been known that glycosylated renin could be taken into cells via the mannose/insulin-like growth factor II cell surface receptor. Renin binding to this receptor leads to receptor signaling but the internalized renin does not act within the cell and appears to be destroyed.\textsuperscript{22,23} That is, this appears to be a clearance receptor without significant implications for local angiotensin II generation. More recently, a second receptor has been characterized. Termed the (pro)renin receptor, this molecule is a portion of the vacuolar ATPase-associated protein, a near ubiquitously expressed intracellular protein. On the cell surface, however, this receptor binds both prorenin and renin with the generation of second messengers and the up-regulation of among other factors, plasminogen activator inhibitor-1. These cell surface receptors have been reported on vascular smooth muscle cells, mesangial cells of the kidney, and other cell types. Also, upon prorenin binding to the receptor, the pro segment of prorenin is moved away from the enzymatic active site and surface-bound prorenin becomes non-enzymatically activated. That is to say, the pro segment is not cleaved from the protein but, nonetheless, the enzyme on the cell surface can generate angiotensin I from angiotensinogen. The availability of angiotensin-converting enzyme on cell surface in proximity to angiotensin AT-1 receptors on targets cells makes local generation of angiotensin II by cell surface bound prorenin of considerable physiologic importance. Moreover, as already noted, the receptor binds renin whereupon bound renin becomes more enzymatically active than when in an unbound state, a situation that again leads to the generation of large amounts of angiotensin II in close proximity to the cell’s AT-1 receptors.\textsuperscript{20,21,24} Recently, small decoy peptides designed to bind to the so-called handle region of the (pro)renin receptor have been synthesized. Because the handle region is responsible for displacing the pro segment from the prorenin active site and thereby activating the enzyme, it was assumed that these small peptides would interfere with the activation of prorenin at the receptor. When these peptides were administered to diabetic rodents, diabetic nephropathy was almost entirely prevented—an effect more robust than was seen with traditional AT-1 blockers. Moreover, this beneficial effect was seen in AT-1 knockout mice suggesting a novel mode of producing pharmacologic benefit. These results are controversial in part because they have not yet been reproduced and in part because it is unclear why the decoy peptides provided benefit when renin was presumably left free to bind to
the receptor. Nonetheless, these are promising observations suggesting the existence of previously unsuspected ways of mitigating vascular disease in the glomerulus. Irrespective of the efficacy of decoy peptides, the (pro)renin receptor has important implications for the pathogenesis of disease. It has long been known that circulating prorenin concentration can be very high in diabetic patients. Prorenin concentrations are also high in eyes of diabetic patients and, in fact, prorenin can be synthesized in situ in the retina. In the 1970s it was reported that elevations of circulating prorenin concentration in type I diabetic patients presage microvascular complications such as retinopathy and nephropathy. Until the discovery of the prorenin receptor these findings were inexplicable. The only known site of prorenin activation was the kidney. Thus, any circulating prorenin would be expected to be inert. However, the existence of the (pro)renin receptor means that high concentrations of prorenin can be activated at local sites and lead to local generation of angiotensin II. In this way, the (pro)renin receptor greatly expands the complexity of the local renin angiotensin system. Moreover, direct signaling by high concentrations of prorenin at the (pro)renin receptor could also have physiologic implications. Aliskiren is a recently available renin inhibitor. It can block the enzymatic activity of prorenin and renin bound to the (pro)renin receptor and therefore should be beneficial in cases of high local angiotensin generation. An interesting question is whether aliskiren can prevent the direct activation of receptor signaling by either prorenin or renin; cell culture studies suggest that it cannot. Receptor signaling is not impaired by aliskiren but nonetheless the drug likely will produce considerable benefit through the inhibition of local angiotensin II formation. Another facet of aliskiren action should be touched on. Aliskiren is associated with marked reactive up-regulation of renin. Circulating renin concentrations are much higher after aliskiren therapy than after AT-1 blocker therapy. Although this was initially felt to be the result of more complete blockade of the renin angiotensin system, it seems more likely a result of the measurement of renin concentration in the blood being artifactually elevated because of bound inhibitor. Also, the clearance of renin may be reduced once it is bound to aliskiren, another possible cause for artifactually high concentrations of renin. Nonetheless, renin concentration rises after aliskiren as it does after AT-1 blocker therapy or after the administration of converting enzyme inhibition. Even though circulating renin in the case of aliskiren is not enzymatically active, could it be detrimental by virtue of direct stimulation of the (pro)renin receptor? A recent cell culture study suggests that there is a mitigating process that would lessen any such deleterious effects. The binding of renin to the (pro)renin receptor activates a rapid down-regulation of the receptor gene, thereby presumably down-regulating receptor number at the cell surface and dampening any effects of high circulating renin. The lack of any obvious deleterious effects of the hyperreninemia associated with AT-1 blocker or converting enzyme inhibitor therapy is consistent with this view.

THE AT-1 RECEPTOR

The effects of angiotensin II at the cell surface are mediated by two G-protein coupled receptors termed the AT-1 and AT-2 receptors. Although the AT-2 receptors are abundantly expressed in the fetus, their number declines in adults with the result that the AT-1 receptor becomes the predominant receptor in most tissues. The most well-known actions of angiotensin II, such as vasoconstriction, as well as stimulation of hypertrophy, hyperplasia and fibrosis, stimulation of aldosterone secretion, and augmentation of renal tubular sodium re-absorption, are mediated by the AT-1 receptor. Angiotensin II binding to the AT-2 receptor for the most part appears to
have actions opposite to angiotensin II binding at the AT-1 receptor so that the AT-2 receptor in part mitigates the stimulation of the AT-1 receptor.\textsuperscript{4,5,32} AT-2 receptor stimulation up-regulates bradykinin and eventually nitric oxide synthesis thereby producing a vasodilating effect. AT-2 stimulation also plays a role in enhancing natriuresis and in the blunting of cell proliferation.

It has recently become apparent that the AT-1 receptor participates in cross-talk with the epidermal growth factor (EGF) receptor such that activation of the AT-1 by angiotensin II leads to the cleavage of bound EGF from the cell surface with subsequent stimulation of cell-surface EGF receptors.\textsuperscript{33} This provides a link between the proliferative effects of angiotensin II and EGF such that stimulation of target cells can produce enhanced actions via the stimulation of EGF receptors and therefore the actions of both hormones become involved in generating tissue pathology. Also of note, it is now clear that the AT-1 receptor can physically interact at the cell surface with the bradykinin B2 receptor with the result that AT-1 signaling in the presence of angiotensin II is enhanced.\textsuperscript{34,35} This receptor interaction has the effect of sensitizing target cells to angiotensin II with likely pathologic results. Indeed, there is evidence to indicate that AT-1 B 2 interaction plays a role in the genesis of the hypertension and vascular pathology seen in preeclampsia.

Yet other recent findings point to an interaction between the pathogenic effects of hyperlipidemia and hypertension—two potent risk factors for atherosclerosis. Oxidized low-density lipoprotein—an established risk factor for atherogenesis—can be oxidized whereupon it can bind to the so-called lectin-like oxidized low density lipo-protein (LDL) receptor, LOX-1. Angiotensin II increases the synthesis of oxidized LDL in the arterial wall through the production of oxidative stress with the result that increased amounts of oxidized LDL are taken up by macrophages through a scavenger pathway leading to enhanced macrophage synthesis of cholesterol. However, angiotensin II also produces pro-atherogenic effects on endothelial cells including the stimulation of apoptosis and these effects are mediated by LOX-1. It has recently been shown that angiotensin II up-regulates LOX-1 expression, whereas oxidized LDL up-regulates AT-1 expression.\textsuperscript{36,37} This suggests that AT-1 and LOX-1 act synergistically in the production of vascular inflammation and pathology.

A homolog of angiotensin-converting enzyme (ACE) has been identified, so-called ACE2. This enzyme does not generate angiotensin II from angiotensin I but rather cleaves angiotensin I to angiotensin, (1-9) which in some circumstances may be cleaved to angiotensin (1-7) by ACE. But more important, it also cleaves angiotensin (1-7) directly from angiotensin II.\textsuperscript{38–40} Angiotensin (1-7) is an important mediator of angiotensin action, likely operating through the Mas receptor.\textsuperscript{41} Angiotensin (1-7) is antiproliferative and vasodilating. Thus, it tends to offset the pathogenic actions of angiotensin II. The balance between angiotensin II and angiotensin (1-7) is an important determinant of vascular health and the ratio of these factors in large part determined by the availability and regulation of ACE2.\textsuperscript{38–41}

**TRANSFORMING GROWTH FACTOR**

An exciting, although still preliminary, new field of investigation involves the role of TGF in the vascular pathology of Marfan’s syndrome. Patients with Marfan’s syndrome suffer from a variety of abnormalities, most notably from aortic aneurysm and dissection. The defect in Marfan’s syndrome involves the gene for fibrillin, a structural protein in the vascular wall. Conventional thinking assumed that abnormalities of a connective tissue protein such as fibrillin resulted in abnormal connective tissue strength in large vessels leading to lessened tissue integrity and eventual aneurysm formation.
Treatment consisted in lowering blood pressure and pulse pressure so as to reduce stress on the aortic wall and this therapy has provided significant benefit by slowing the progression of aortic distension. However, recent studies have revealed that the fibrillin molecule contains multiple binding sites for TGF and that the protein serves as a reservoir for this protein, binding it in tissue in an inactive state. Defects in the fibrillin gene are associated with a reduction in TGF binding sites. These observations and studies in transgenic animal models of the disease led to the hypothesis that a major lesion in Marfan’s syndrome was the absence of a tissue sink for TGF leading to enhanced TGF activity in the tissue, the stimulation thereby of metalloproteinases, and the degradation of vascular connective tissue. Because angiotensin II up-regulates TGF, it was hypothesized that AT-1 blockade could blunt the progression of vascular pathology in Marfan’s syndrome. Studies in the transgenic model of the disease, and more recently a preliminary study in humans, bear this out. AT-1 blockade results in a slowing of the progression of aortic root dilatation. This suggests that the possibility that similar benefit can be obtained in patients with non-Marfan’s vascular dilatation should be explored.

**INTRACRINE RENIN-ANGIOTENSIN**

Considerable evidence has been developed over recent years to indicate that a variety of peptide factors, including hormones, growth factors, DNA binding proteins, enzymes, and others, can serve both as extracellular signaling molecules and also function in the intracellular space either after retention in the cells that synthesized them or after internalization by target cells. Early on we termed these factors *intracrines* and developed hypotheses regarding their biologic origins and the principles that regulate their function. These ideas are reviewed in detail elsewhere. Important for the present discussion is the realization that angiotensin II was one of the earliest identified intracrine hormones and recent evidence strongly suggests that renin, angiotensinogen, and even angiotensin-converting enzyme can act as intracrines in their own right.

Recent studies using either an angiotensinogen construct lacking the signal sequence for secretion and therefore destined to be retained in the cell, or using a construct expressing an angiotensin II molecule that must be retained in the intracellular space demonstrated that up-regulation of intracellular angiotensin II is associated with proliferation in several cell types. These observations were followed by the demonstration that the transfection of cultured cardiac myocytes with a construct expressing a nonsecreted angiotensin II moiety resulted in marked cellular hypertrophy within 48 to 96 hours. Moreover, when the intracellular angiotensin construct was incorporated in a plasmid under the regulation of the alpha myosin heavy chain promoter and the plasmid injected into the left ventricles of rodents, marked cardiac hypertrophy again occurred within 96 hours. Collectively, these results point to the possibility that the intracrine angiotensin system could play an important role in cardiac hypertrophy. Still more recent studies indicate that both in cell culture and in vivo hyperglycemia up-regulates intracellular cardiac myocyte angiotensinogen and renin, leading to apoptosis. These findings support earlier reports of increased angiotensin II and apoptosis in human diabetic heart disease.

Other studies have demonstrated that intracellular angiotensin II can have important effects on intracellular calcium currents and on junctional conductance. Moreover, intracellular angiotensin as opposed to extracellular hormone causes cell volume to decrease. All these effects suggest a role for the intracellular renin angiotensin system in the regulation of cardiac rhythm and in the genesis of pathologic arrhythmia.
SUMMARY

Hypertension and its sequelae are complex processes. Optimization of the care of the hypertensive patient requires not only attention to the regulation of arterial pressure but also attention to blunting the hypertension-related processes that lead to vascular disease. It is clear that the regulation of these processes is much more complex than previously understood. Here several new insights into the pathogenesis of hypertension-related vascular disease have been explored. While this review is not exhaustive, it does serve to point out the varied nature of the biologic processes that must be taken into account and it points to new avenues for the development of therapeutic agents.

REFERENCES


