The circulating renin-angiotensin system plays an important role in blood pressure regulation as well as in fluid and electrolyte balance. Its contribution to cardiovascular homeostasis has been well documented in studies that employ pharmacological or immunological inhibitors. Blockade of the renin-angiotensin system using peptide inhibitors or specific antibodies to renin, angiotensin converting enzyme (ACE) inhibitors, or angiotensin antagonists results in acute hypotensive responses in sodium-depleted animals or humans. In conditions associated with elevated plasma angiotensin II levels, such as experimental or clinical renovascular hypertension and congestive heart failure, these agents also bring about marked depressor responses. In all studies, the magnitude of acute depressor response can be predicted by the pretreatment plasma renin-angiotensin activity, indicating a causal relationship. In experimental as well as clinical studies, chronic administration of renin-angiotensin inhibitors has proven efficacious in lowering blood pressure in hypertension and in ameliorating edema formation in heart failure. As a result, specific inhibition of the renin-angiotensin system has become an important strategy in cardiovascular drug development. As drug development technology becomes more sophisticated, novel pharmacological agents with unique properties become available for experimental and clinical trials. A frequent outcome of these trials is an improved understanding of the biology and pharmacology of the particular system under study. For example, the development of pharmacological ligands has improved our understanding of the properties and roles of adrenergic, dopaminergic, serotoninergic, and histaminergic receptor subtypes. The development of various opioid agonists and antagonists has provided new insights into the biology of this peptidergic system.

What have we learned from experiments of renin-angiotensin inhibition in the last decade? As stated earlier, studies of acute inhibition of this system have provided evidence that the renin-angiotensin system is important in acute blood pressure regulation during sodium depletion, anesthesia, and hemorrhage, as well as in the initiation of renovascular hypertension and in the development of the syndrome of congestive heart failure. These findings could have been predicted by the earlier observations that plasma renin activity (PRA) was elevated in the early phases of these conditions. However, during the chronic phase of these conditions, PRA was frequently normal and yet response to renin-angiotensin inhibition persisted, albeit attenuated. Therefore, invaluable and somewhat surprising lessons can be learned from the chronic administration of renin-angiotensin inhibitors. Although the acute blood pressure lowering effect of ACE inhibitors in humans correlates with the initial PRA, the
chronic response appears to bear little relationship to pretreatment plasma hormonal levels. Clearly, ACE inhibitors can lower blood pressure in many hypertensive patients whose plasma renin levels are normal or even low. This observation is supported further by animal studies that demonstrated that chronic administration of ACE inhibitors or angiotensin II antagonists lowered the blood pressure in various hypertensive animal models whose plasma renin activities were not elevated.

Several postulates have been proposed to explain the “non-renin-mediated” antihypertensive effect of these antagonists, especially captopril. Since ACE (kininase II) also degrades bradykinin, it has been suggested that an accumulation of tissue bradykinin resulting from the inhibition of this enzyme might be responsible for the additional vasodilating effect. Carretero et al. reported that the antihypertensive effect of captopril could be attenuated by the administration of antikinin antiserum to the rabbit. Another hypothesis proposed that inhibition of the renin-angiotensin system resulted in a reduction in sympathetic nervous activity through a reduction in catecholamine release at noradrenergic nerve endings. Alteration in baroreceptor reflex activity has been suggested as another possible mechanism. Current evidence also suggests a prostaglandin-mediated effect of captopril. Plasma prostaglandin E₂ metabolite levels have been observed to increase after the administration of captopril in humans. Furthermore, the antihypertensive action of captopril is attenuated by indomethacin, especially in patients on a high sodium diet. The mechanism by which captopril stimulates prostaglandin release is not clear. Zusman demonstrated that captopril stimulated prostaglandin E₂ biosynthesis in cultured renomedullary interstitial cells in vitro. This effect appears to be unique to captopril, since enalapril (a structurally different ACE inhibitor) failed to stimulate prostaglandin release from these cells. This observation is supported by the clinical data of Given et al., who failed to observe an increase in plasma prostaglandin E₂ metabolites after enalapril administration.

The prostaglandin hypothesis would not explain the “nonrenin” antihypertensive response to chronic enalapril therapy, saralasin, or antirenin monoclonal antibodies, since none of these can activate prostaglandins. In addition, peptide inhibitors of renin have recently been shown to induce an antihypertensive response that cannot be explained solely by the inhibition of plasma renin. For example, the renin inhibitory peptide resulted in a large reduction of blood pressure in hypertensive humans with normal PRA. The statine-containing renin inhibitory peptide reduced blood pressure in dogs in a dose-dependent fashion; however, its effect on blood pressure appears to be dissociated from PRA inhibition. At low infusion rates (up to 20 μg/kg/min), the slope of inhibition of PRA was steeper than that for the decline of blood pressure. At doses greater than 20 μg/kg/min, when PRA was completely suppressed, blood pressure continued to fall in a dose-dependent fashion independent of PRA. The data of Okamura et al. published in this issue of Hypertension, provide a possible explanation of these findings. These investigators demonstrated that vascular renin-angiotensin activity is increased during the chronic phase of rat two-kidney, one clip hypertension, when PRA is almost normal. This increase was documented by increases in ACE activity of vascular tissues and in the constrictor response of isolated arteries to angiotensin I. Their data suggest that increased angiotensin II concentration is present in the vascular tissues and may be responsible for chronic hypertension. The dissociation of vascular and circulating renin-angiotensin activities supports the postulate that a local system exists in blood vessels that contributes to the control of vascular tone. Indeed, Okamura et al. reported that the ACE inhibitor enalapril and the angiotensin II antagonist [Sar¹, Iso³]-angiotensin II lowered blood pressure in rats with chronic two-kidney, one clip hypertension despite near-normal plasma renin level.

The importance of tissue ACE, rather than its serum counterpart, in determining long-term response to ACE inhibitors has been demonstrated by several investigators. In these studies, the magnitude and duration of blood pressure reduction appeared to correlate better with the inhibition of ACE activity in certain critical tissues than with the inhibition of serum enzyme activity. For example, hours after a single dose of ACE inhibitor (when serum ACE activity as well as the pressor response to exogenous angiotensin I had returned to normal) blood pressure remained reduced. It is important to note that the duration of the antihypertensive response paralleled the suppression of aortic and kidney ACE activities and, to a lesser extent, lung ACE activity, and did not follow serum ACE activity at all. This result suggests that certain tissue ACE (e.g., vascular and renal) may be more important in determining blood
pressur response to ACE inhibition. In addition, these studies also demonstrate that individual ACE inhibitors may differ in their tissue distribution, pattern, and duration of inhibition.

The concept that the major target organs of angiotensin II may have independent endogenous renin-angiotensin systems is gaining increasing support. Many studies have demonstrated biochemically the presence of renin-angiotensin components in many tissues, including those of brain, heart, kidney, adrenals, and especially blood vessels. Immunoreactive renin has been isolated using immunoaffinity chromatography from rat brain, rabbit uterus, canine aorta, and cultured vascular smooth muscle and endothelial cells. In addition, with the use of monoclonal and polyclonal antibodies, immunostaining for renin could be observed in these tissues.

Although these data support the existence of immunoreactive renin as well as other components of the system in various extrarenal tissues, they do not distinguish between local synthesis, selective uptake, or contamination. To study further the issue of local synthesis, we and others have examined for the presence of renin or angiotensinogen messenger (m) mRNA, or both, in various tissues using Northern blot hybridization analyses. Nick-translated 32P-labeled renin and angiotensinogen complementary (c) DNAs were used to hybridize to total mRNA from various tissues of the rat and mouse. Renin mRNA could be demonstrated in the brain, adrenal glands, kidney, testes, and heart of both species. In all these tissues, angiotensinogen mRNA sequences were also detected. These data provide evidence of renin and angiotensinogen gene expressions in various tissues allowing for the potential for local angiotensin production.

We have been unable to address the above question in vascular tissues using these techniques since the quantity of RNA recovered in these tissues thus far has been too low for reliable hybridization studies. Instead, we have employed cultured vascular cells for further studies of the cell biology of the vascular renin-angiotensin system. Cultured vascular smooth muscle cells from bovine and canine aorta as well as rat mesenteric artery and cultured bovine vascular endothelial cells contain renin-like activities that are inhibited by renin antibodies at 50% inhibitory concentrations similar to those of renal renin. Pulse-labeling studies have demonstrated that vascular renin is synthesized in situ in cultured endothelial and smooth muscle cells. Immunoreactive angiotensinogen as well as angiotensins I, II, and III can be measured intracellularly. In addition, angiotensins appear in the culture media in a time-dependent fashion. Our data suggest that renin-angiotensinogen reaction occurs intracellularly, leading to the synthesis and subsequent secretion of angiotensin II. The complete renin-angiotensin system has also been demonstrated in several other cell cultures, including neuroblastoma, glomerular, and adrenal cells. We have proposed that this intracellular process may be operative in many cells that release angiotensin and may serve as a regulator or amplifier of local function. Thus, angiotensin release by vascular cells may have an important autocrine or paracrine influence on blood vessel function. As far as autocrine functions are concerned, endothelial angiotensins may have a number of cellular actions, such as stimulation of prostaglandin biosynthesis.

Recent experiments provide suggestive but preliminary data that angiotensin may bind to acceptors on nuclear chromatin and initiate nuclear events that may result in protein synthesis and cell proliferation. In the case of paracrine function, endothelial angiotensin may activate vascular smooth muscle angiotensin receptors and affect vascular tone. Vascular angiotensin may also influence catecholamine release and reuptake by noradrenergic nerve endings. Angiotensin has been shown to increase norepinephrine release and block its reuptake by noradrenergic nerve endings. Indeed, animal studies have suggested that locally generated angiotensins may regulate vascular tone by potentiating sympathetic activity. Recently, Kawasaki et al. and Nakamura et al. suggested that the isoproterenol-induced facilitation of vascular noradrenergic neurotransmission of rat mesentery is due to the activation of the vascular renin-angiotensin system, which in turn is mediated by the β-adrenergic receptor. In support of the potential physiological relevance of this influence is the observation of Antonaccio and Kerwin. These investigators demonstrated that captopril resulted in prejunctional and postjunctiional inhibition of vascular sympathetic function in spontaneously hypertensive rats (SHR).

An important issue that has not been adequately addressed is whether renin is the only angiotensinogen-processing enzyme in the extrarenal and extrahepatic tissues.
Although our data, as well as those of other investigators, demonstrate that immunoreactive renin is present in many extrarenal tissues, a significant fraction of the reninlike activity in many tissues is not inhibited by renin-specific antibodies. Some investigators have reported that brain reninlike activity is due to tissue cathepsin D activity. As a result, recent experiments have employed neutral pH in assaying for tissue reninlike activity to avoid the contribution of cathepsin D. These studies have demonstrated the existence of neutral pH, nonrenin angiotensin-forming enzymes in addition to renin. Husain et al. reported that a neutral aspartyl protease, which they called isorenin, was the major angiotensin-forming enzyme in the dog brain and other tissues in vitro. Whether this enzyme plays a physiological role in brain angiotensin formation remains to be determined. Boucher et al. isolated a neutral serine protease named tonin, which is capable of generating angiotensin II directly from angiotensinogen. Recently, human neutrophils have been shown to release a serine protease, cathepsin G, that can act at multiple steps of the renin-angiotensin pathway. Cathepsin G activates human prorenin, hydrolyzes angiotensinogen directly to angiotensin II, and cleaves angiotensin II from angiotensin I. We have speculated that this enzyme may be released by neutrophils at sites of inflammation, allowing for activation and amplification of local renin angiotensin that may modulate vascular permeability and edema formation. The exact physiological role of this enzyme, however, has not been defined. Recently, Okunishi et al. observed that a nonrenin protease was responsible for generating angiotensin II in isolated arterial strips. These data are in contradistinction to that of Oliver and Sciacca, who demonstrated that the angiotensin I generated in an isolated perfused artery was completely inhibited by renin inhibitory peptide. Our recent data indicate that renin mRNA sequences are present in low quantities in mouse and rat brains, in contrast to angiotensinogen mRNA, which exists in abundance. Taken together, these findings suggest that in certain tissues, nonrenin proteases rather than renin may play an important role in the processing of angiotensinogen to angiotensin. This will be an important area of future investigation.

Based on the above data, we have hypothesized that the renin-angiotensin system consists of two compartments, one in circulation and the other in local tissues. The tonic control of vascular resistance and local tissue function (e.g., adrenal, kidney) is influenced by the intrinsic tissue renin-angiotensin system. This autocrine or paracrine system contributes little to the circulating renin-angiotensin levels. The traditional circulating renin-angiotensin system is an endocrine system whose principal function is short-term cardiorenal homeostasis. To a limited extent, an analogy can be drawn between the renin-angiotensin system and the sympathetic nervous system. For example, tonic control of sympathetic function is provided by local noradrenergic nerve activity. During flight or fight response, additional support is provided by the acute release of catecholamines from the endocrine adrenal medulla. The local renin-angiotensin system may provide tonic influence of vascular function. During cardiovascular decompensation, the endocrine renin-angiotensin system is activated for acute homeostasis. The proposal that the circulating renin-angiotensin system is involved with only short-term control of cardiovascular function is supported by the observation that plasma renin-angiotensin activity follows the principle of a closed-loop hormonal system. Except for states of severe cardiovascular decompensation, after acute activation plasma renin-angiotensin activity almost always returns to normal if homeostasis is achieved. Indeed, in both congestive heart failure and renovascular hypertension, plasma renin-angiotensin levels are no longer elevated in the chronic compensated phase. We hypothesize that the "non-renin-dependent" phases of heart failure or renovascular hypertension are conditions in which acute circulatory support afforded by the activation of circulating renin-angiotensin system is no longer necessary. During this phase, the tissue (vascular) renin-angiotensin system maintains vascular tone. This mechanism is well demonstrated by the data of Okamura et al. published in this issue of Hypertension. Thus, non-renin-dependent is really a misnomer, since the local vascular renin-angiotensin system may be playing an important role in maintaining hypertension.

Based on this reasoning, abnormal tissue renin-angiotensin activities may be postulated to lead to hypertension. Enhanced local angiotensin II production can result in increased angiotensin II-mediated responses such as increased vascular tone (due to direct angiotensin II vasoconstriction or to angiotensin II--induced increased sympa-
thetic activity) or adrenal aldosterone overproduction (or both), independent of the influence of circulating angiotensin II. This possibility is supported by the study of Okamura et al.,

25 which showed that increased vascular renin-angiotensin activity may be responsible for the maintenance of chronic two-kidney, one clip hypertension. This reasoning may be extended to question whether abnormalities in tissue renin-angiotensin activity are responsible for certain forms of genetic hypertension. Indeed, several investigators have reported that the Okamoto-Aoki strain of SHR has elevated adrenal and vascular renin levels.

12 Schelling et al. 66 reported increased renin-angiotensin levels in certain regions of the brain of SHR. The contribution of the tissue renin-angiotensin system to hypertension in SHR is elucidated by the depressor response to renin-angiotensin inhibition despite normal plasma renin levels. Although data are still too premature to allow a definitive conclusion of causal relationship, the data can be interpreted to support the notion that increased local tissue angiotensin II concentration in genetically hypertensive rats is at least one mechanism that may be responsible for sustained hypertension.

What about human essential hypertension? Little or no data exist on tissue renin-angiotensinogen expression in humans; however, recent reports have suggested that as many as 40% of patients with essential hypertension show an alteration in renal, vascular, and adrenal responses to alterations in plasma angiotensin II levels and to exogenous angiotensin II infusion.

67 These patients have normal plasma renin, angiotensin, and aldosterone levels on a high sodium diet. On a low sodium diet, however, their plasma renin and angiotensin levels are higher, and plasma aldosterone levels lower, than normal. In addition, the renal blood flow and adrenal responses to changing sodium diets (and thus plasma angiotensin II levels) and exogenous angiotensin II infusion are significantly attenuated. These patients have been termed the nonmodulators. 61 Interestingly, the abnormal tissue responses to exogenous angiotensin in these nonmodulating hypertensive patients are similar to those reported for SHR. In addition, treatment of these patients with ACE inhibitors appears to normalize their response to exogenous angiotensin II.

68 Taken together, these findings suggest that excessive tissue renin-angiotensin activity (as in the SHR) might have resulted in increased vascular tone and other angiotensin-mediated functions in local tissues. The elevated local angiotensin II concentration may simultaneously down-regulate the tissue angiotensin II receptors, rendering them less responsive to exogenous angiotensin II. The response to ACE inhibitors in these patients can be explained by the inhibition of local angiotensin II production, which may result in up-regulation of angiotensin II receptors, thus allowing the tissue to be responsive to exogenous angiotensin II again.

In summary, important lessons have been learned from the experiments employing inhibitors of the renin-angiotensin system in experimental animals and humans. The application of biochemical, cellular, and molecular biological techniques to these studies has allowed us to explore these questions in greater depth. These investigations have provided evidence that renin and angiotensinogen genes are expressed at multiple tissue sites and the idea that locally produced angiotensin may influence tissue function. The data of Okamura et al. 25 published in this issue of Hypertension demonstrate further that activation of the tissue (vascular) renin-angiotensin system may contribute to chronic two-kidney, one clip hypertension. Taking Page's 69 mosaic theory of hypertension into due consideration, we cautiously speculate that aberrant tissue renin-angiotensinogen gene expressions may be another mechanism by which certain forms of genetic hypertension can develop. If correct, this hypothesis may have important implications in understanding the pathophysiology of certain forms of essential hypertension and in the strategy of new drug development.

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