Inhibitors of two zinc metallopeptidases, angiotensin I converting enzyme (ACE) and neutral metalloendopeptidase-24.11 (EP-24.11), are antihypertensive agents. In this issue of Hypertension, Genden and Molineaux report that yet another peptidase inhibitor, metalloendopeptidase-24.15, EC 3.4.24.15 (EP-24.15), lowers blood pressure in normotensive rats. In this editorial we discuss the possible role of kinins as common mediators of part of the vasodepressor action of these peptidase inhibitors. Genden and Molineaux report that the marked fall in blood pressure caused by the EP-24.15 inhibitor is almost abolished by a kinin receptor antagonist, supporting the hypothesis that kinins play a role in the regulation of normal blood pressure. We have confirmed that the EP-24.15 inhibitor used by these investigators lowers blood pressure. Up to now, EP-24.15 has not been implicated in in vivo metabolism of kinins. Although a number of kininases have been identified, our own previous work indicated that the metabolic pathway responsible for clearing kinins from the circulation involves the action of kininase II (angiotensin I converting enzyme) and renal peptidases. Nevertheless, the main metabolic pathway involved some other unidentified enzyme, since in these experiments disappearance of kinins from the circulation was only marginally reduced by a “cocktail” of inhibitors of ACE, EP-24.11, and carboxypeptidase N. It could be that EP-24.15 is involved in kinin metabolism. However, a number of questions need to be answered with regard to the mechanism by which the EP-24.15 inhibitor lowers blood pressure. The data obtained with the EP-24.15, NE-24.11, and ACE inhibitors suggest that potentiation of vasodilator peptides is yet another possible relevant therapeutic approach to hypertension and perhaps heart failure. (Hypertension 1991;68:366–371)
These investigators used a dose of the kinin antagonist to select the dose of the kinin antagonist operating phenomenon. However, we have reported that part of the acute antihypertensive effect of ACE inhibitors in an experimental hypertensive model was blocked by a kinin antagonist even though the ACE inhibitor did not significantly alter plasma kinins. Thus, it is possible that the EP-24.15 inhibitor decreased degradation of tissue kinins, leading to buildup of the peptide in tissues (presumably the vascular wall) but not in plasma. This would agree with our current view that the kallikrein-kinin system acts as a paracrine system. Local increase of kinins in various tissues would support this hypothesis; however, at present reliable methods of measuring kinins in tissues are not available.

2) Which enzymes are responsible for endogenous kinin generation? Increasing kinins by inhibiting their degradation presupposes that kinin generation is continuous. Since acute administration of kinin antagonist does not alter blood pressure in normotensive animals, it follows that kinins should normally be immediately destroyed, keeping the final concentration low. There is little evidence of the existence of such a continuous kinin-generating pathway. However, the study of Genden and Molineaux and our studies showing that the blood pressure effects of ACE inhibitors are blocked by kinin antibodies or antagonists suggest that kinins may be formed and hydrolyzed continuously. We do not know which enzymes release tissue kinins. We have reported that vascular tissue contains a kininogenase that has the same properties as glandular kallikrein and that messenger RNA for kallikrein is present in vascular tissue and smooth muscle cells in culture. Oza et al reported that vascular smooth muscle cells in culture release both kallikrein and kininogen (kallikrein substrate). Thus, it may be that vascular kininogenase generates kinins continuously and that they are rapidly destroyed by peptidases such as EP-24.15 and ACE. Plasma kallikrein is a different enzyme from glandular kallikrein, and its concentration in plasma is quite high. Although it normally circulates as prekallikrein, the inactive precursor, it is possible that kinins are generated via activation of the plasma kallikrein-kinin system. However, there is no evidence that this is a normally operating phenomenon.

3) What criteria were used by Genden and Molineaux to select the dose of the kinin antagonist? These investigators used a dose of the kinin antagonist at least fivefold higher than that needed to block 70% or more of the hypotensive effect of a bolus dose of kinins. It is not known whether lower doses also blunt the hypotensive response to the EP-24.15 inhibitor or whether high doses are necessary. Although these authors report that the dose they used has no effect on blood pressure, we and others have demonstrated that high doses of a kinin antagonist increase blood pressure in normal rats. We could not block this increase with sympathetic and parasympathetic blockers, indomethacin, adrenalectomy, or nephrectomy. These data are consistent with the notion that high concentrations of the kinin antagonist are needed to displace kinins from vascular tissue receptors. If high doses are also needed to blunt the hypotensive effect of the EP-24.15 inhibitor, this would support the contention that tissue kinins are responsible for the changes in blood pressure. However, these results should be interpreted with caution, since at high doses the antagonist may stimulate the release of catecholamines or have other unforeseen effects such as causing pain due to residual agonistic activity. Furthermore, when the antagonist is administered at lower doses that block the hypotensive effect of exogenous kinins by 70% or more, they do not change blood pressure; in addition, kinin antibodies that block part of the acute antihypertensive effect of ACE inhibitors do not increase blood pressure in rats not treated with the ACE inhibitor. It would be of interest to determine whether lower doses of the kinin antagonist block the hypotensive effects of the EP-24.15 inhibitor. A positive finding would support the hypothesis that kinins mediate part of the hypotensive effect of the EP-24.15 inhibitor.

4) Why is the vasodepressor response to EP-24.15 only transient? The hypotensive effect of the EP-24.15 inhibitor was observed only after the initial administration but was greatly diminished or absent when the inhibitor was given a second time. These investigators postulate some undefined compensatory mechanism to explain the decreased response on successive administrations of the peptidase inhibitor. This is possible, but it also raises the question of an exhaustible substance linked to the initial hypotensive response. It is also possible that the hypotensive effects of the inhibitor are due not to blockade of EP-24.15 but to some other effects of the peptide. We have confirmed that the inhibitor lowers blood pressure by as much as 40 mm Hg with little change in heart rate. However, it would be convenient to demonstrate that other inhibitors of EP-24.15 have similar hypotensive effects. This would strengthen the contention that inhibition of EP-24.15 is the mechanism whereby blood pressure is decreased. The lack of tachycardia is surprising considering the magnitude of the decrease in blood pressure produced by EP-24.15. It could be that EP-24.15 inhibitors directly or indirectly alter the sympathetic-mediated baroreceptor response to hypotension.
5) Does the EP-24.15 inhibitor lower blood pressure by decreasing cardiac output or total peripheral resistance? It could be that EP-24.15 inhibitors alter heart inotropic and chronotropic activity. These possibilities need to be studied further.

We now know of inhibitors of three different zinc metallopeptidases that have either a hypotensive or antihypertensive effect, namely ACE, EP-24.11, and EP-24.15. These are three distinct enzymes that only cleave small peptides; their inhibitors reduce blood pressure by either blocking formation of vasopressor peptides such as angiotensin II or inhibiting degradation by vasodepressor peptides such as kinins and atrial natriuretic factor (ANF). However, in vitro neither ACE, EP-24.11, nor EP-24.15 is specific for a particular substrate but rather cleaves a number of peptides. In vivo, hydrolysis of a particular substrate by these peptidases may depend on whether enzyme and substrate have the opportunity to interact, as well as on factors that alter the kinetics of the reaction. Kinins, which are potent vasodepressor peptides, are common substrates for these three peptidases (Figure 1).

**Metalloendopeptidase EC-3.4.24.15**

EP-24.15 was first identified by Orlowski et al in 1983 as a metalloendopeptidase cleaving several bioactive peptides (including bradykinin). Work on the specificity of the enzyme with model synthetic substrates led to the discovery of potential, active site-directed inhibitors that could be used as probes in studies of the role of the enzyme in in vivo metabolism of bioactive peptides. EP-24.15 is highly concentrated in the testes, with lower levels in the brain, pituitary, kidney, heart, adrenal, lung, and liver. In the brain, part of the enzyme is soluble in form and part is bound to membranes. The enzyme preferentially cleaves bonds on the carboxyl side of hydrophobic amino acid residues in the P1 position. A bulky or hydrophobic residue in the P1' position greatly increases the affinity of the substrate for the active site of the enzyme. EP-24.15 converts large opioid peptides to Leu- and Met-enkephalin and degrades neurotensin, lutetating hormone-releasing hormone and kinins. Like ACE or EP-24.11, EP-24.15 is not specific for a single peptide substrate such as kinins and probably metabolizes other peptides containing hydrophobic amino acids. Thus, the effects of EP-24.15 inhibitors may be more complex than Genden and Molineaux proposed. Nevertheless, this is an important study, since EP-24.15 inhibitors have the potential to become a new class of antihypertensive drug.

**Neutral Metalloendopeptidase EC-3.4.24.11**

The effects of EP-24.11 inhibitors, like those of ACE inhibitors, are probably due to blockade of the hydrolysis of a variety of peptides; some lower blood pressure, such as ANF, kinins, neurotensin, enkephalins, and endorphins, whereas others may increase blood pressure, among them angiotensin, endothelin, and perhaps vasopressin. However, the final result is a decrease in blood pressure. The prevailing hypothesis is that they lower blood pressure by inhibiting the hydrolysis of ANF. However, EP-24.11 is an important kininase, both in the kidney and the brain. In our laboratory, Ura et al have shown that EP-24.11 is the main renal kininase in the rat and that acute inhibition of this enzyme by phosphoramidon resulted in an increase in urinary kinins and sodium and water excretion. However, phosphoramidon caused no changes in plasma kinins.

We hypothesized that the saluretic effect of phosphoramidon is mediated by an increase in both renal kinins and ANF. The work of Smits et al further supports this hypothesis; they reported that thiorphan, which is also an inhibitor of EP-24.11, potentiated the acute effects of ANF on water and sodium excretion and that these effects were blocked completely by a kinin antagonist. At the dose infused, ANF had no effect on blood pressure. However, it has also been reported that the acute natriuretic effect of EP-24.11 inhibitors is blocked by ANF antisera. These studies support the hypothesis that the natriuretic and diuretic effects of EP-24.11 inhibitors are mediated by an increase in both ANF and renal kinins, which may act synergistically. It is not clear whether an increase in plasma or renal ANF is needed for these effects.

In normotensive humans, UK 79300, also an inhibitor of NE-24.11, increased urinary sodium, water, ANF, and cyclic GMP excretion. At the dose used, the inhibitor caused no changes in blood pressure; however, plasma renin activity and aldosterone were significantly decreased, and a small increase in plasma ANF was observed. (Kinins were not measured.) Sybertz et al and Seymour et al reported that inhibition of EP-24.11 in deoxycorticosterone acetate (DOCA)–salt hypertension has an acute antihypertensive effect. In this model, plasma ANF is increased; however, the EP-24.11 inhibitor did not cause a further increase in ANF, and yet polyclonal antiserum to ANF blocked most of the antihypertensive effect of EP-24.11 inhibitor while the kinin antagonist had no effect. In SHR, EP-24.11 inhibitors also had an antihypertensive effect that was smaller than in DOCA-salt rats, and in some cases...
the inhibitor took 3 days to lower blood pressure. In humans, it has been reported that EP-24.11 inhibitors have an antihypertensive effect that was more pronounced in black patients.34

In conclusion, EP-24.11 inhibitors may be useful drugs in the treatment of hypertension and perhaps heart failure. Their antihypertensive effect is probably due to a direct vasodilator action of ANF and the combined natriuretic effect of both ANF and kinins. However, the roles of other vasoactive peptides that may be cleaved by EP-24.11 cannot be completely excluded.

**Angiotensin Converting Enzyme EC-3.4.15.1**

Inhibition of ACE has resulted in effective treatment of hypertension and congestive heart failure.35 ACE inhibitors act by blocking conversion of angiotensin I to II; however, it is difficult to explain their effect by this mechanism alone, especially in low-renin hypertension.36-44 In addition to blocking conversion of plasma angiotensin I to II, two other major hypotheses have been postulated to explain the effect of ACE inhibitors in low-renin hypertension: 1) their effect is mediated by blockade of the extra-renal tissue renin-angiotensin system45 and 2) their effect is partially due to inhibition of kinin degradation in tissue, which lowers vascular resistance directly or via prostaglandins and endothelium-derived relaxing factor.46 Due to limitations of space, the first hypothesis will not be discussed here.

The hypothesis that kinins participate in the effect of ACE inhibitors is supported by the following data: 1) ACE has higher affinity for kinins than for angiotensin I.19,47 2) Although plasma kinins are unchanged or moderately increased after ACE inhibitors,48 urinary kinins reportedly increase, indicating that their renal concentration likewise increases.28,49,50 This in turn may contribute to the antihypertensive effect of ACE inhibitors by altering blood flow and increasing sodium and water excretion. Kinins also increase in the venous effluent of organs rich in tissue kallikrein.51 3) Inhibition of the kallikrein-kinin system with aprotinin,52 kinin antagonist7-55-57 partially blocks the acute effects of ACE inhibitors on renal function, blood pressure, and blood flow.4 In kininogen- and kinin-deficient Brown-Norway rats with two-kidney, one clip renovascular hypertension, the drop in blood pressure caused by intravenous injection of ACE inhibitors is significantly reduced in both magnitude and duration.58 4) An ACE inhibitor increases water and sodium excretion and renal papillary blood flow in rats pretreated with the angiotensin antagonist DuP 753; these effects were blocked by the kinin antagonist.59 These studies indicate that some of the acute effects of ACE inhibitors on renal function, blood flow, and blood pressure are mediated by kinins; however, whether they play a role in the chronic effects of ACE inhibitors is not known.

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O A Carretero and A G Scicli

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