Role of Kinins and Nitric Oxide in the Effects of Angiotensin Converting Enzyme Inhibitors on Neointima Formation

Rodolfo F. Farhy, Oscar A. Carretero, Khang-Loon Ho, and A. Guillermo Scicli

Marked neointima formation occurs after balloon injury to the intima of rat arteries. Angiotensin II has been implicated as a growth factor in this process, since angiotensin converting enzyme (ACE) inhibitors block neointima formation after injury. However, ACE is an important kininase, and its inhibitors may act in part by a kinin-mediated mechanism. Kinins are also known to stimulate synthesis of endothelium-derived relaxing factor/nitric oxide (EDRF/NO) and prostacyclin, both of which have antiproliferation effects. To determine whether the effect of ACE inhibitors on neointima formation is due to blockade of angiotensin II synthesis alone and/or inhibition of kinin inactivation, we followed two approaches. First, we compared the inhibition of neointima formation induced by the AT,-type angiotensin II receptor antagonist losartan with that caused by the ACE inhibitor ramipril. We also studied whether a kinin receptor antagonist, Hoe 140, blocks the effect of two different ACE inhibitors, ramipril and enalapril, on neointima formation. In addition, we studied whether the effect of ramipril is blocked by an NO synthesis inhibitor, Nω-nitro-L-arginine-methyl ester (L-NAME). Although both ramipril and losartan significantly reduced neointima formation, ramipril had a more marked effect (p<0.05 for ramipril versus losartan). The kinin antagonist Hoe 140 reduced the inhibitory effect of ramipril and enalapril by 73% and 62%, respectively. The remaining effect of the ACE inhibitors was now similar to that of losartan. Inhibition of neointima formation by ramipril was also blocked by the NO synthesis inhibitor L-NAME. Therefore, we suggest that the protective effect of ACE inhibitors is due to both blockade of angiotensin II formation and kinin degradation. We also suggest that NO may mediate the effect of kinins. The fact that L-NAME blocked the effect of ramipril on neointima formation suggests that NO may play a major role in the inhibitory effect of ACE inhibitors. (Circulation Research 1993;72:1202–1210)

KEY WORDS • kinins • angiotensin converting enzyme inhibitors • endothelium-derived relaxing factor/nitric oxide • neointima

Marked neointima formation occurs after balloon injury to the intima of rat arteries. The sequence of events involved in neointima formation is proliferation of vascular smooth muscle cells (VSMCs) in the media, followed by migration and further proliferation of medial VSMCs in the neointima. The mechanisms that produce migration and proliferation of VSMCs are not fully understood, and several factors (proto-oncogenes, growth factors, and angiotensin [Ang] II) have been implicated in this process. The use of treatments that affect some of these factors may provide information about the pathophysiological processes involved and could also facilitate therapeutic intervention in clinical situations characterized by neointima formation, such as arteriosclerosis, or after balloon dilatation of arterial stenosis.

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Powell et al. have shown that angiotensin converting enzyme (ACE) inhibitors such as cilazapril and captopril reduce neointima formation after balloon injury to the rat carotid. They concluded that inhibition of conversion of Ang I to Ang II is the mechanism whereby ACE inhibitors block neointima formation. However, ACE (EC 3.4.15.1) is a dipeptidyl carboxypeptidase that not only converts Ang I to Ang II but is also an important kininase, kininase II. There is evidence that vascular tissue contains an intrinsic kallikrein–kinin system and that some of the pharmacological actions of ACE inhibitors are mediated in part by endogenous kinins, which act as paracrine hormones. Kinins are potent vasodilators and act in part via release of endothelium-derived relaxing factor/nitric oxide (EDRF/NO) and prostaglandins. It has been shown that NO has an antiproliferative influence on VSMCs. Therefore, we hypothesized that the effect of ACE inhibitors on neointima formation is mediated in part by inhibition of kinin hydrolysis and that the effect of kinins may be due to stimulation of NO synthesis. Our objective was to study the role of kinins and NO in the inhibition of neointima formation induced by ACE inhibitors.

In the present study, we sought to determine whether the effect of ACE inhibitors on neointima formation is due to inhibition of Ang II formation alone or attribut-
able at least in part to the inhibition of kinin degradation. Therefore, we compared the effect of the Ang II receptor antagonist losartan (a nonpeptidic antagonist of AT1-type Ang II receptors) with that of the ACE inhibitor ramipril. In addition, we studied whether a kinase receptor antagonist, Hoe 140, blocks part of the effect of two different ACE inhibitors (ramipril and enalapril) on neointima formation. We also studied whether the effect of ramipril is blocked by an NO synthesis inhibitor, Nω-nitro-L-arginine-methyl ester (L-NAME). Our data show that part of the inhibitory effect of ACE inhibitors on neointima formation is mediated by kinins and NO.

Materials and Methods

Experimental Protocols

Protocol 1. In this protocol, we tested 1) whether there are quantitative differences between the reduction in neointima formation induced by ramipril and losartan and 2) whether administration of Hoe 140 would prevent ramipril from exerting its protective effect on neointima formation.

Male Sprague-Dawley rats weighing 450–500 g (Charles River Laboratories, Portage, Mich.) were divided into five groups. The first group was given vehicle (saline) and used as a control, the second group received losartan (10 mg/kg per day), the third group received ramipril (5 mg/kg per day), the fourth group received Hoe 140 (70 μg/kg per day), and the fifth group received both ramipril (5 mg/kg per day) and Hoe 140 (70 μg/kg per day). These doses have been used by other investigators,11,12 and we found them to be effective as described below.

Protocol 2. In this protocol, we tested the effect of a second ACE inhibitor, enalapril, and questioned whether that effect is blocked by the kinase antagonist Hoe 140. Rats were divided into three groups. One group was given vehicle (control), the second group was given enalapril (20 mg/kg per day), and the third group was given both enalapril and Hoe 140 (70 μg/kg per day).

Protocol 3. The objective of this protocol was to determine whether suppression of neointima formation by ramipril is blocked when NO synthesis is inhibited. Rats were divided into four groups. The first group was given vehicle (saline), the second group was given ramipril (5 mg/kg per day), the third group was given both ramipril and the NO synthase inhibitor L-NAME (1.44 mg/kg per day), and the fourth group was given L-NAME alone.

In all protocols, drugs were dissolved in saline and given intraperitoneally as a continuous infusion by use of an osmotic pump (Alzet 2ML2, Alza Corp., Palo Alto, Calif.). The pump was implanted in the peritoneal cavity under ether anesthesia 2 days before balloon deendothelialization.

Drugs

Ramipril and Hoe 140 were obtained from Hoechst-Roussel, Somerville, N.J.; losartan was from Du Pont–Merck, Wilmington, Del.; enalapril was from Merck Sharp & Dohme, Rahway, N.J.; and L-NAME was from Sigma Chemical Co., St. Louis, Mo.

Compound Dose Selection

Initially, we defined the kinase antagonist dose needed to block 70% or more of the blood pressure response to 100 ng bradykinin. Five rats were anesthetized, and catheters were placed in the femoral artery and right jugular vein for blood pressure measurements and intravenous drug administration, respectively. Blood pressure was measured with a Statham transducer (Viggo-Spectramed, Oxnard, Calif.) and monitored on a recorder (Gould, Valley View, Ohio). Rats received the ACE inhibitor enalapril (200 μg/kg i.v.) to potentiate the response to kinins. After blood pressure had stabilized, they were challenged with intravenous bradykinin (100 ng in 0.05 ml saline), and the fall in blood pressure was monitored. Alzet osmotic pumps (model 2001D, which delivers 8 μl/hr) were implanted intraperitoneally. The pumps contained Hoe 140, a potent B2 kinase receptor antagonist,13 with the concentration adjusted to deliver 35 μg/kg per day. After 24 hours, the rats were anesthetized and given enalapril, and the blood pressure response to 100 ng bradykinin was measured again. Before the administration of Hoe 140, bradykinin decreased blood pressure in these five rats by 22, 12, 14, 22, and 22 mm Hg, whereas after 24 hours of Hoe 140 the response had decreased to 0, 0, 0, 8, and 2 mm Hg, respectively, or 89% blockade. In subsequent chronic experiments with Hoe 140, the dose was doubled to 70 μg/kg per day.

Blood Pressure Measurements

Systolic blood pressure was measured twice a week by the tail-cuff method. To assess the effect of the drugs and the degree of blockade of ACE and/or the different receptors, the animals were anesthetized with sodium pentobarbital 14 days after deendothelialization (50 mg/kg i.p.), and a PE-50 polyethylene catheter was introduced into the abdominal aorta via the femoral artery to measure mean blood pressure. The rats were then randomly challenged with 100 ng i.v. Ang I, Ang II, or bradykinin, and the change in mean blood pressure was monitored.

We found that the tail-cuff method is not reliable when rats are treated with L-NAME because of significant constriction of the tail artery. Thus, in protocol 3, the rats were anesthetized with ether, and a PE-50 catheter was introduced into the abdominal aorta via the femoral artery to measure mean blood pressure. After they had completely recovered from the anesthesia (approximately 4 hours), mean blood pressure was measured in awake, restrained rats for 2 hours with a pressure transducer connected to a Zenith Z100 computer via an analog-to-digital board. Four readings were taken per minute and were averaged to give a single value for each rat.

Arterial Injury Model

A balloon catheter was constructed by attaching a 10–15-mm Silastic tube to the end of a PE-10 catheter by means of cyanoacrylate and then closing the other end with Silastic glue. The diameter of the balloon when inflated with saline (approximately 30 μl) was 2.5 mm, enough to produce considerable distension of the common carotid artery. The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and the left
common and external carotid arteries were exposed. The balloon catheter was passed through the external carotid artery and advanced to the aorta; the balloon was inflated with saline to distend the common carotid artery and resist withdrawal and then pulled back to the external carotid artery. This procedure was repeated three times, after which the catheter was removed, the external carotid was ligated, and the incision was sutured. In pilot studies, we confirmed that the endothelium was completely removed, using silver nitrate and Evans blue. We also observed that the balloon injury produced mild damage to the arterial media.

**Morphology/Morphometry**

Fourteen days after balloon injury, the rats were anesthetized, a catheter was placed in the ascending portion of the thoracic aorta via the left ventricle, and the right atrium was cut open. The vascular system was first rinsed with phosphate-buffered saline (approximately 100 ml) and then perfusion-fixed with 2.5% glutaraldehyde (approximately 250 ml) at a perfusion pressure of 100 mm Hg. The left common carotid was removed, and adipose connective tissue in the adventitia was excised and placed in 2.5% glutaraldehyde for further fixation. Each artery was divided into three equal segments that were separately embedded in paraffin. Cross-sectional rings (1 μm) were cut from each segment and stained with hematoxylin and eosin.

In the first protocol, the slices were displayed with a Prado-Leitz projecting microscope at a magnification of ×100. The lumen, neointima, media, and total arterial area (defined as the entire area within the external lamina elastica minus the lumen) were traced on paper and measured by use of a morphometric computer system (Sigma-Scan, Jandel Scientific, Corte Madera, Calif.). In subsequent protocols, the same areas were measured directly from the slices by use of a microscope with an attached camera lucida (Leitz Wetzlar, FRG) and a Summasketch digitizer tablet (Summographics Corp., Seymour, Conn.). Both systems were calibrated by the same standard. Measurements by the two methods did not differ from each other by more than 0.1%. Neointima formation was expressed as the ratio of neointimal area to total arterial area.

**Statistics**

A single slice was chosen at random from each segment, giving three slices per artery. When areas of each segment were compared (one-way analysis of variance [ANOVA]), no differences between segments were found; therefore, the three slices were averaged to give a single measurement for each carotid artery. The results were expressed as a percentage of the neointimal area/total arterial area ratio.

For each protocol, ANOVA was used to compare the neointimal area/total arterial area ratio. Tukey's studentized range test was used to perform all pairwise comparisons in the presence of an overall statistically significant group difference. The type I experimental error rate was maintained at 0.05.

The neointimal area/total arterial area ratio was considered the most appropriate choice for analysis because the area of the neointima without adjustment was found to correlate highly with arterial size. This, coupled with the unequal arterial size across comparision groups, rules out using neointimal area alone. A significant effect in this setting could be due to the treatment or to differing arterial size among groups.

We did not use analysis of covariance (ANCOVA) to examine the data. This approach was considered, but the data for all three protocols did not satisfy the assumptions of equality of slopes across treatment groups. Without this assumption proving to be correct, ANCOVA is inappropriate.

Finally, the neointimal area/total arterial area ratio did not show a strong correlation with arterial size and satisfied the assumptions of normality and homogeneity of variance. For these reasons, we chose this end point to express our results.

The change in systolic blood pressure in protocols 1 and 2 was analyzed by ANCOVA, with the covariate being baseline blood pressure. Pairwise comparisons were made using Bonferroni’s multiple-comparison adjustment.

The differences in mean blood pressure in protocol 3 and the blood pressure response to either Ang I or II in protocols 1 and 2 were analyzed by ANOVA. Tukey’s studentized range test was used to adjust for multiple comparisons.

**Results**

**Protocol I**

There was no difference in body weight among the five groups, either before surgery or 2 weeks afterward. After 2 weeks of treatment, systolic blood pressure did not change in the vehicle-treated group (−2.8 mm Hg) but dropped significantly in the groups treated with losartan (−18.6 mm Hg), ramipril (−18.9 mm Hg), and ramipril+Hoe 140 (−13.6 mm Hg) (by ANCOVA, p<0.002; by Bonferroni’s adjustment, p<0.05 versus control group) (Table 1).

At the end of the experiment, the blood pressure response to Ang I (100 ng i.v.) was similar in the losartan- and ramipril-treated groups (by Tukey’s test, p=NS); however, compared with the untreated control group, it was blocked by 80.1±1.9% and 80.8±1.1%, respectively (by ANOVA, p<0.001; by Tukey’s test, p<0.05). The response to Ang II was not altered by ramipril, either alone or with Hoe 140; however, it was blocked by 78.9±2.5% in the losartan-treated group (by ANOVA, p<0.001; by Tukey’s test, p<0.05 for control group versus losartan-treated group).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Basal (mm Hg)</th>
<th>2 Weeks (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n=11)</td>
<td>129.5±2.9</td>
<td>126.7±4.9</td>
</tr>
<tr>
<td>Losartan (n=11)</td>
<td>130.4±3.2</td>
<td>111.8±1.8*</td>
</tr>
<tr>
<td>Ramipril (n=11)</td>
<td>130.3±2.0</td>
<td>111.4±1.2*</td>
</tr>
<tr>
<td>Ramipril+Hoe 140 (n=9)</td>
<td>126.7±4.1</td>
<td>113.1±2.5*</td>
</tr>
<tr>
<td>Hoe 140 (n=8)</td>
<td>122.4±2.8</td>
<td>130.0±4.5</td>
</tr>
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Values are mean±SEM. *p<0.002 by analysis of covariance and p<0.05 by Bonferroni’s adjustment vs. vehicle-treated group.
The vehicle-treated group showed marked neointima formation in the left carotid artery after balloon injury. In both ramipril- and losartan-treated groups, the neointimal area/total arterial area ratio was significantly decreased compared with the vehicle-treated group (by ANOVA, p < 0.001; by Tukey’s test, p < 0.05); the neointimal area of the ramipril-treated group (0.021 ± 0.003 mm²) was less than half that of the losartan-treated group (0.054 ± 0.009 mm²). The neointimal area/total arterial area ratio revealed that in the rats treated with ramipril, neointima formation was significantly decreased compared with both vehicle- and losartan-treated rats (by ANOVA, p < 0.001; by Tukey’s test, p < 0.05). This effect of ramipril was blunted 73% by Hoe 140 (by Tukey’s test, p < 0.05 for ramipril+Hoe 140–treated rats versus ramipril–treated rats), with neointima formation becoming similar to that of the losartan-treated rats (by Tukey’s test, p = NS for ramipril+Hoe 140–treated rats versus losartan–treated rats) (Figures 1 and 2). In addition, when Hoe 140 was given alone, it induced a 24% decrease in neointima formation compared with the control rats (by Tukey’s test, p < 0.05 for Hoe 140–treated rats versus vehicle–treated rats) (Figure 2).

No differences in medial area were found after the various treatments.

Protocol 2

Two weeks after surgery, systolic blood pressure did not change in the vehicle-treated rats (1.6 mm Hg). It dropped significantly (by ANCOVA, p < 0.001; by Bonferroni’s adjustment, p < 0.05) in the rats treated with enalapril (−22.8 mm Hg), and similar changes were observed in the rats treated with enalapril+Hoe 140 (−23 mm Hg; by Bonferroni’s adjustment, p < 0.05) (Table 2). The hypertensive response to 100 ng Ang I was blocked by 89.8 ± 3.9% in the rats treated with enalapril (by ANOVA, p < 0.001; by Tukey’s test, p < 0.05 for enalapril–treated rats versus control rats) and by 80.6 ± 5.1% in the rats treated with ACE inhibitor and the kinin receptor antagonist (by Tukey’s test, p = NS for enalapril–treated rats versus enalapril+Hoe 140–treat-
ed rats; by Tukey's test, \( p<0.05 \) for enalapril+Hoe 140–treated rats versus control rats).

Enalapril significantly decreased the neointimal area-total arterial area ratio compared with the control condition (by ANOVA, \( p<0.001 \); by Tukey's test, \( p<0.05 \)). This effect was markedly blunted when enalapril was given together with Hoe 140 (by Tukey's test, \( p<0.05 \) for enalapril-treated rats versus enalapril+Hoe 140–treated rats) (Figure 3).

**Protocol 3**

The mean arterial pressure in awake restrained rats was significantly lower in the group treated with ramipril alone compared with the control group (by ANOVA, \( p<0.001 \); by Tukey’s test, \( p<0.05 \)). There were no differences between rats treated with saline and L-NAME alone, even though blood pressure tended to be higher in the L-NAME group (by Tukey's test, \( p>0.05 \)). The rats treated with both ramipril and L-NAME tended to have higher blood pressure than those receiving ramipril alone, but the difference was not statistically significant (by Tukey’s test, \( p>0.05 \)) (Table 3).

**Table 3.** Mean Blood Pressure After 2 Weeks of Treatment With Vehicle, Ramipril, or Ramipril+L-NAME

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean blood pressure (mm Hg)</th>
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<tbody>
<tr>
<td>Vehicle (n=12)</td>
<td>125.0±2.1</td>
</tr>
<tr>
<td>Ramipril (n=7)</td>
<td>97.8±3.7†</td>
</tr>
<tr>
<td>Ramipril+L-NAME (n=6)</td>
<td>106.8±3.2†</td>
</tr>
<tr>
<td>L-NAME (n=7)</td>
<td>129.7±2.8</td>
</tr>
</tbody>
</table>

*\( p<0.001 \) by analysis of variance; \( p<0.05 \) vs. vehicle group by Tukey's test.

There were no differences in the neointimal area-total arterial area ratio between rats treated with saline (control rats) and those given L-NAME alone (by Tukey’s test, \( p>0.05 \)). In the rats treated with ramipril alone, there was a significant decrease in the neointimal area-total arterial area ratio (by ANOVA, \( p<0.001 \); by Tukey’s test, \( p<0.05 \) for ramipril-treated rats versus control rats). Given together, L-NAME and ramipril resulted in reversal of the inhibitory effect of the ACE inhibitor on neointima formation, since there was no statistical difference between these rats and rats treated with saline (by Tukey’s test, \( p>0.05 \) for ramipril+L-NAME–treated rats versus control rats) (Figure 4). However, there was a significant difference between rats treated with L-NAME alone and those treated with ramipril+L-NAME (by Tukey’s test, \( p<0.05 \)).

**Discussion**

We have shown that two different ACE inhibitors, ramipril and enalapril, prevent neointima formation after balloon injury to the intima of the rat carotid artery. We found that the effect of both agents on neointima formation was reduced by more than 50% by the kinin receptor antagonist Hoe 140 and that the effect of ramipril was...
blocked by the NO synthesis inhibitor L-NAME. These results confirm our previous findings, which in turn were recently reproduced by deBlois et al. These data support our hypothesis that the effect of ACE inhibitors on neointima formation is mediated in part by inhibition of kinin hydrolysis and that the effect of kinins may be due to stimulation of NO synthesis. We also found that the AT1 Ang II receptor antagonist losartan inhibits neointima formation but is less effective than ACE inhibitors. These results are consistent with the hypothesis that Ang II is a component of the process leading to neointimal thickening.

Powell et al. have shown that cilazapril and captopril inhibit neointima formation. Similar results were obtained by Prescott et al. using benazepril and by us using ramipril and enalapril. Therefore, since five different ACE inhibitors of the carboxyalkyl and sulphydryl types have now been shown to prevent neointima formation, we conclude that their effect is class specific and that it is due to inhibition of ACE rather than related to the chemical characteristics of the various ACE inhibitors. ACE is a dipeptidyl carboxypeptidase found in high concentrations in vascular tissue, especially the endothelial cells, although it is also contained in VSMCs. Dzau et al. found that the cells of the neointima express substantial amounts of ACE. ACE not only converts Ang I to Ang II but also hydrolyzes kinins and other peptides. In the present study, we have shown that kinins contribute to the effects of ACE inhibitors on neointima formation. This is not a unique occurrence, since we and others have found that the effects of ACE inhibitors on blood flow, renal function, blood pressure, and cardiac hypertrophy are partially blocked by kinin antibodies and kinin antagonists. Thus, there is clear evidence supporting the hypothesis that kinins do participate in the physiological actions of ACE inhibitors in some experimental situations. On the other hand, kinins may not contribute to the effects of ACE inhibitors in some other experimental situations. Kinin antagonists and kinin antibodies have been reported to partially block the antihypertensive effects of ACE inhibitors. However, in the present work, the significant decrease in blood pressure induced by ACE inhibitors was not altered by blocking kinin receptors, suggesting that kinins are not involved. Previously, we found that the effects of ACE inhibitors on sodium-depleted rats were likewise not affected by blocking kinins. These data suggest that under normal conditions, or in sodium depletion, most (if not all) of the blood pressure-lowering effect of ACE inhibitors is due to blockade of the renin-angiotensin system.

Although kinin antibodies and kinin antagonists block some of the effects of ACE inhibitors, ACE inhibitors do not significantly alter plasma kinin concentrations, suggesting that kinins act mainly as local (paracrine) hormones before they are rapidly hydrolyzed by various peptidases including ACE. Our study suggests that in the absence of ACE inhibitors, kinins appear to have little influence on the regulation of neointima formation, since in our experiment the kinin antagonist alone tended to decrease rather than increase neointima formation after vascular injury. However, deBlois et al. reported that the same antagonist appears to increase neointimal thickness. We have no explanation for these results, although they may be related to the quite different doses used in these studies. We have interpreted these findings as suggesting that ACE inhibitors inhibit neointima formation by increasing kinin concentrations within the vascular wall from subthreshold to biologically active levels. There is evidence indicating the presence of a vascular kallikrein-kinin system. We have reported that both glandular kallikrein and its mRNA are present in vascular tissue and that vascular tissue releases kallikrein. Recently, we found that the isolated perfused rat hindquarter releases not only kallikrein but also kinins and that endogenous kinin release is augmented by ACE inhibitors (authors’ unpublished observations). We also found that VSMCs in culture contain mRNA for glandular kallikrein, whereas Oza et al. reported that these cells contain and release not only glandular kallikrein but also the kinin precursor kininogen. VSMCs have also been reported to contain ACE. Thus, VSMCs contain a local (paracrine) kallikrein-kinin system that is likely responsible for the kinin-mediated effects of ACE inhibitors; however, arterial injury may induce activation of the extrinsic coagulation pathway and activation of plasma prekallikrein to kallikrein. Thus, we cannot exclude the possibility that the kinins involved are those released by plasma kallikrein from high molecular weight kininogen.

The vasodilator effects of kinins are mediated in part by stimulation of prostaglandin and EDRF/NO synthesis; in addition, part of the antihypertensive effect of ACE inhibitors is mediated by kinins via the release of EDRF/NO. In the present study, we have shown that the effect of ACE inhibitors on neointima formation is blocked by an NO synthesis inhibitor. One explanation for the effect we observed is that kinins act via NO. It was reported that there is little reendothelialization 14 days after balloon injury; using Evans blue stain, we also confirmed that it was confined to the borders of the injured carotid artery (authors’ unpublished data). We cannot exclude the possibility that kinins stimulate EDRF/NO from the aortic endothelium or the rest of the vascular tree. This mechanism requires that some form of EDRF act more as an endocrine than as a paracrine mediator. Stamler et al. have shown that NO can circulate in the plasma with albumin as an adduct in the form of NO or nitroso compounds that can liberate NO. An alternative source of NO may be VSMCs, which have been reported to contain NO synthase (the enzyme responsible for the release of NO from the precursor arginine). Joly et al. have presented data suggesting that 24 hours after balloon injury there is an increase in cGMP in the injured carotid artery and that this increase can be stimulated by interleukin 1 and blocked by L-NAME, suggesting that the inducible form of NO synthase is present. If the NO synthase involved is indeed the inducible form, it is not clear how kinins participate in this mechanism, since it is not known whether these peptides stimulate NO formation by the inducible NO synthase. However, kinins exert complex and multiple actions on a number of systems such as interleukins; thus, it is possible that their effect on neointima formation is not necessarily due to direct release of NO but may be indirect through the release of
interleukins followed by stimulation of inducible NO synthase.48,49,51,52

One puzzling aspect of our results is the fact that the NO synthase inhibitor reversed the protective effects of ACE inhibitors to levels not significantly different from those in saline-treated rats. However, if we consider the group treated with L-NAME alone an appropriate control, they were different from the ramipril+L-NAME–treated group. It may be that L-NAME reverses the kinin component of the inhibitory effect of ramipril on neointima formation. Our results suggest that kinins mediate part of the effect of ACE inhibitors via NO but do not prove that such a linkage exists. It is still possible that kinins and NO act at different steps of the cascade of events involving ACE inhibitors.

It is difficult to relate changes in blood pressure to the effect of ACE inhibitors on neointima formation.53 Powell et al.3 reported that compounds such as hydralazine and verapamil, which lowered blood pressure to levels similar to those found with ACE inhibitors, were less effective in suppressing neointimal thickness after balloon injury. In the present study, both ramipril and enalapril lowered blood pressure by approximately 15 mm Hg. Although the kinin receptor antagonist did not affect this decrease, it reversed the effect of the ACE inhibitors on neointima growth by more than 50%. Losartan had the same blood pressure–lowering effect as ACE inhibitors but exerted a less suppressive effect on neointima formation. These results show that there is little relation between lowering blood pressure and protection from response to vascular injury.

We based our dose of ramipril on published data indicating that this dose (5 mg/kg per day) was effective in blocking vascular ACE12 and not on the smaller dose needed to lower blood pressure during experimental hypertension.54 These data are consistent with Powell et al.3 who postulate that the doses of ACE inhibitors required to inhibit neointima formation may be higher than those needed to decrease blood pressure. Also, it has been proposed that the failure of an ACE inhibitor to decrease restenosis after angioplasty in the MERCATOR study55 may be due to the fact that the dose used was based on antihypertensive activity.55,56 However, Linz et al.54 using an aortic coarctation model, reported that doses of ramipril that are not antihypertensive nevertheless decrease left ventricular hypertrophy and that this effect is blocked by the kinin antagonist Hoe 140. It is not clear whether the same mechanisms by which kinins mediate ACE inhibition of neointima formation after vascular injury are also involved in inhibition of cardiac hypertrophy secondary to hypertension.

Although we found that losartan was less effective than ramipril, it still resulted in a significant decrease in neointima formation, suggesting that Ang II acting via AT1 receptors plays an important role in the process leading to neointima formation after vascular injury. Other investigators have found no difference between ACE inhibitors and losartan with regard to the suppression of neointima formation.18,57,58 These differences may be related to the dose and mode of administration. However, compared with the control rats, the effect of losartan in our study was similar in magnitude to those effects reported previously.

The greater effect of ACE inhibitors in our study (compared with the Ang II antagonist, Figure 2) was not due to a greater bioactive dose of ACE inhibitors, since the hypertensive response to Ang I was blocked to a similar degree by both inhibitors (approximately 80%); moreover, losartan also blocked the hypertensive response to Ang II to the same extent. In addition, the dose of losartan we used has repeatedly been shown to be effective in blocking Ang II receptors in chronic in vivo studies.11 The fact that we used a relatively high dose of ramipril may also affect the comparisons. Variability in the magnitude of the induced injury and repair process as well as in the experimental conditions may explain these discrepancies.

Thickening of the intima in balloon-induced vascular injury has three phases or waves. The first is activation and replication of VSMCs in the media, which occurs during the first 3 days after injury. This first wave appears to be driven by basic fibroblast growth factor, which is released by injured medial VSMCs.59 This is followed by migration of VSMCs across the internal lamina and into the intima, which occurs from approximately day 3 to day 5. It is likely that the initial migration response is linked to production of platelet-derived growth factor, a known stimulator of cell migration, which appears to have largely more chemotactic than mitogenic properties in this model.1 In the intima, VSMCs continue to proliferate, reaching a maximum at approximately 2 weeks.14 Other known and unknown growth factors are involved, but the mechanisms are not yet well understood. It is not clear which of these sequences of events are influenced by kinins and NO.

The mechanism by which Ang II stimulates neointima formation after vascular injury is not known. Data obtained in vitro by cell culture techniques indicate that, when Ang II is added to VSMCs cultured in serum-free medium, it induces hypertrophy but not hyperplasia60; however, when administered in vivo, it increases cell proliferation not only in the injured intima but also in the media of normal vessels.61

Changes in the expression of platelet-derived growth factor A chain, c-myc, c-jun, and c-fos20,62–65 may be the basis of the growth-promoting activity of Ang II, since these genes are known to be mitogenic. Stouffer and Owens66 showed that Ang II potentiates the effects of platelet-derived growth factor-BB and epidermal growth factor on VSMCs in culture and that this effect is mediated by the autocrine release of transforming growth factor-β. Dzau et al.67 postulate that the endothelium regulates the VSMC response to Ang II and other growth factors and that in the absence of the endothelium the balance is shifted in favor of mitogenesis. In balloon-induced arterial injury, it is possible that the increased endogenous kinins produced after ACE inhibition counteract the mitogenic influence induced by removal of the endothelium. Thus, the action of ACE inhibitors may involve removal of a progrowth influence (Ang II) and increase of an antigrowth influence (kinins/NO).

In conclusion, these data represent the first known evidence that endogenous kinins mediate part of the inhibitory effect of ACE inhibitors in neointima formation after injury to the rat carotid artery. These data also suggest that NO is involved in the protective effects of ACE inhibitors.

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Role of kinins and nitric oxide in the effects of angiotensin converting enzyme inhibitors on neointima formation.

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