Chronic Captopril Administration Decreases Vasodilator Responses in Skeletal Muscle Arterioles

Jefferson C. Frisbee, David S. Weber, and Julian H. Lombard

Changes in arteriolar reactivity to dilator agonists were assessed in the cremaster muscle of Sprague-Dawley rats fed normal rat chow with captopril (100 mg/kg/day) in the drinking water for 8 weeks and in nontreated controls. The in situ cremaster muscle was prepared, superfused with physiologic salt solution, and arteriolar diameter was measured using television microscopy. Changes in the diameter of distal arterioles in response to topical application of iloprost, forskolin, cholera toxin, acetylcholine, and nitroprusside were measured with a video micrometer. Arteriolar responses to each of the vasodilator agonists used in this study were significantly reduced in the captopril-treated rats, relative to the untreated controls. The maximum dilation of the arterioles, determined during superfusion with Ca$^{2+}$-free physiologic salt solution containing $10^{-4}$ mol/L adenosine, was also reduced in the captopril-treated rats, suggesting structural remodeling of the arteriolar wall. These observations indicate that chronic angiotensin converting enzyme inhibition with captopril leads to significant alterations in arteriolar structure and reactivity, and that angiotensin II may play a protective role in maintaining normal vascular structure and vasodilator reactivity in the microcirculation. Am J Hypertens 1999;12:705–715 © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: Angiotensin converting enzyme inhibition, vascular reactivity, remodeling.

The role of angiotensin II (AngII) in maintaining normal vascular reactivity is of substantial clinical importance in light of the large number of patients who are being treated with angiotensin converting enzyme (ACE) inhibitors to lower blood pressure. Recent work in our laboratory$^{1-4}$ has demonstrated that an elevated dietary salt intake impairs the relaxation of skeletal muscle resistance arteries, middle cerebral arteries, and skeletal muscle arterioles of normotensive rats in response to a variety of vasodilator stimuli acting at different sites in the various signal transduction pathways of vascular smooth muscle relaxation.

This impaired relaxation to dilator stimuli appears to be related to the AngII suppression that occurs in response to a high-salt diet. Preliminary studies by Lombard et al$^3$ and Weber et al$^1$ have demonstrated that prevention of AngII suppression by continuous infusion of a suppressor dose of AngII (5 ng/kg/min)$^5$ restores vasodilator responses that are normally lost in skeletal muscle resistance arteries and in cerebral arteries of rats eating a high-salt diet. The impaired vasodilator responses in animals consuming a high-

Received August 10, 1998. Accepted December 21, 1998.
From the Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin
This work was supported by National Institutes of Health grants # HL29587, HL37374, and HL52211. We thank the Berlex corporation for their generous donation of the iloprost used in these experiments.
Address correspondence and reprint requests to Jefferson C. Frisbee, PhD, Department of Physiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226; e-mail: jfrisbee@mcw.edu

© 1999 by the American Journal of Hypertension, Ltd.

Published by Elsevier Science, Inc.
Salt diet appear to represent an alteration in the fundamental control mechanisms of vascular relaxation, rather than a permissive effect of AngII, as acute addition of AngII to the tissue bath does not restore normal dilator responses in skeletal muscle resistance arteries of these animals (DS Weber and JH Lombard, unpublished observations). Taken together, these data suggest that chronic suppression of the renin-angiotensin system may be responsible for the impaired response of resistance arteries to vasodilator stimuli in animals eating a high-salt diet. Given this deleterious effect of AngII suppression on vasodilator reactivity in small arteries, a related question of substantial clinical importance is whether ACE inhibition also impairs dilator responses in the microcirculation.

Wang and Prewitt and Scheidegger et al reported that chronic ACE inhibition with either captopril or benazeprilat, respectively, led to significant reductions in microvessel density, maximum arteriolar diameter, and cross-sectional wall area of skeletal muscle arterioles in normotensive rats and spontaneously hypertensive rats. However, despite the functional implications of these findings, to our knowledge there are no studies addressing the effects of chronic ACE inhibition on vasodilator reactivity.

The goal of the present study was to determine the effects of chronic ACE inhibition with captopril on vasodilator responses of distal arterioles in the cremaster muscle of normotensive rats. These experiments also sought to determine mechanisms that may be responsible for any alterations in arteriolar reactivity, because each of the agonists used in the present study exerts its effect at a different site in the signal transduction pathways, leading to vascular smooth muscle relaxation. These include pathways that are either dependent or independent of the vascular endothelium, that are mediated by receptors on the vascular smooth muscle membrane itself, and that are either dependent or independent of the heterotrimeric G-proteins.

MATERIALS AND METHODS

Animal Groups and Preparation

Vasodilator reactivity studies were conducted on two groups of Sprague-Dawley rats. In all experiments, the rats (n = 16) were fed standard laboratory chow for 8 weeks. Half of the rats were given captopril (100 mg/kg/day, Sigma Chemical Co., St. Louis, MO) in the drinking water for the 8-week duration of the study. All rats drank tap water (either with or without captopril) ad libitum.

Methods and Protocols for Microcirculatory Studies

On the day of the experiment, individual rats were anesthetized with an intraperitoneal (ip) injection (60 mg/kg) of pentobarbital sodium (Veterinary Laborato-

tories, Lenexa, KS), and the trachea was cannulated to ensure a patent airway. A carotid artery and an external jugular vein were cannulated for arterial pressure recording and for intravenous infusion of supplemental anesthetic, respectively.

After the initial surgery was completed, the cremaster muscle was prepared for television microscopy. Once in place under the microscope, the tissue was continuously superfused with physiologic salt solution (PSS), equilibrated with a 5% CO2 and 95% N2 gas mixture, and maintained at 34° to 35°C as it flowed over the muscle. The ionic composition of the PSS was as follows (mmol/L): NaCl 119.0, KCl 4.7, CaCl2 1.6, NaH2PO4 1.18, MgSO4 1.17, NaHCO3 24.0, and disodium EDTA 0.03. Arteriolar diameters were determined with a videomicrometer, accurate to ± 1 μm.

Arterioles selected for these studies were distal (third- or fourth-order) arterioles of the cremaster muscle that were situated in areas of the muscle that were away from the edges of the incision and had clearly visible walls, brisk flow velocity, and active tone, as judged by the occurrence of a brisk dilation in response to topical application of 10^-4 mol/L adenosine. Arteriolar diameters were measured before and after topical application of the following agents to the cremaster muscle: the endothelium-dependent vasodilator acetylcholine (10^-9 to 10^-6 mol/L) the membrane receptor-dependent prostacyclin analog iloprost (10^-15 to 10^-9 g/mL), the Gs-protein activator cholera toxin (10^-9 g/mL), the endothelium-independent nitric oxide donor sodium nitroprusside (10^-9 to 10^-6 mol/L), and the direct adenyl cyclase activator forskolin (10^-15 to 10^-7 mol/L). The maximum arteriolar diameter was assessed by measuring the response of the vessel to superfusion with calcium-free PSS containing 10^-4 mol/L adenosine. Successive agonist challenges were applied only after the vessel had returned to its original diameter after the application of the preceding agonist, and application of the agents was randomized to prevent the occurrence of ordering effects.

Data and Statistical Analyses

All data were initially summarized as the mean ± SEM of either the absolute vessel diameter (μm) or as the change in diameter from rest in response to the agonist challenge (%). To gain insight into the magnitude of the dilation relative to the maximum diameter increase that the vessel could attain, agonist-induced dilations (determined during superfusion of preparation with normal PSS) were also expressed as a percentage of the maximum possible dilation (determined during superfusion of the preparation with Ca2+-free PSS plus 10^-4 mol/L adenosine). Analysis of variance (ANOVA) was employed to determine initial differences across experimental conditions, and Scheffé’s post-hoc test was
employed to identify significant differences between specific experimental groups. In all cases, a probability level of $P < .05$ was considered statistically significant.

RESULTS

Mean Arterial Pressure (MAP) and Body Weights

After 8 weeks of captopril treatment, MAP was significantly lower in rats receiving ACE inhibition (95 ± 6.2 mm Hg) than in the untreated control group (118 ± 5.4 mm Hg). Body weights for rats treated with captopril for 8 weeks (454 ± 16 g) were not significantly different from those of rats drinking normal tap water (463 ± 18 g).

Resting and Maximum Arteriolar Diameter

The resting diameter of distal arterioles of the cremaster muscle was not different between the control animals (20 ± 1.6 μm) and the captopril-treated rats (22 ± 1.1 μm). The maximum arteriolar diameter, measured during superfusion with calcium-free PSS containing $10^{-4}$ mol/L adenosine, was 27 ± 1.4 μm in the captopril-treated rats and 30 ± 2.1 μm in the untreated control animals. Although maximum vessel diameters were not different between the groups, the maximum dilation of the arterioles from the captopril-treated rats was significantly less than that in the untreated control animals (Figure 1).

Responses to Acetylcholine

Figure 2 summarizes the effect of chronic captopril treatment on arteriolar dilation in response to acetylcholine. In these experiments, the dilation of cremasteric arterioles to acetylcholine was significantly reduced, compared with that in the untreated controls, when the response was normalized to the resting arteriolar diameter. At the maximum acetylcholine concentration, arterioles of the control animals dilated to 31% ± 2.9% greater than the rest diameter, compared with 11% ± 1.7% in the captopril-treated rats (Figure 2a). However, when the acetylcholine-induced dilation of the arterioles was expressed as a percentage of the maximum dilation, no differences in the vessel responses were evident in the control and captopril-treated rats (Figure 2b).

Responses to Iloprost

Figure 3 summarizes the effects of captopril treatment on arteriolar dilation in response to the stable prostacyclin analog iloprost. When normalized to the resting vessel diameter, the relaxation of the arterioles in response to each concentration of iloprost was significantly less in the captopril-treated rats than in the untreated control animals. At the maximum iloprost concentration of $10^{-9}$ g/mL, arterioles of the control animals dilated to 27% ± 0.6% greater than the rest diameter, compared with 9% ± 1.0% for arterioles of the captopril-treated rats (Figure 3a). Normalization of the iloprost-induced dilation to the maximum dilation of the vessels indicated that there were no differences in arteriolar relaxation between the two experimental groups, with the exception of the response at $10^{-9}$ g/mL iloprost, where the arteriolar dilation was significantly reduced in captopril-treated animals (Figure 3b).

FIGURE 1. The maximum dilation of distal arterioles in the cremaster muscle of captopril-treated rats and untreated control animals. Data are expressed as mean percent increase (± SE) from the resting diameter to the maximum diameter (determined during superfusion with Ca$^{2+}$-free PSS containing $10^{-4}$ mol/L adenosine). Asterisk indicates a significant decrease ($P < .05$) in the maximum dilation, compared with the control group.
Responses to Cholera Toxin  Chronic ACE inhibition significantly reduced the response of the arterioles to cholera toxin, compared with the untreated control animals. This pattern was evident whether the dilator response was normalized to the resting diameter determined during superfusion with PSS. Asterisks indicate a significant decrease (P < .05) in arteriolar dilation to a given concentration of acetylcholine in the captopril-treated animals. (B) Cremasteric arteriolar responses to the acetylcholine in control and captopril-treated rats. Data are expressed as mean (± SE) response to acetylcholine, expressed as a percentage of the maximum dilation determined during superfusion with Ca^{2+}-free PSS containing 10^{-4} mol/L adenosine. No significant differences in the vessel sensitivity to acetylcholine were identified.

Responses to Forskolin  When expressed as a percentage of the resting vessel diameter, the response of cremasteric arterioles to forskolin was significantly less in the captopril-treated rats than in the untreated control animals (Figure 5a). At the maximum forskolin concentration (10^{-7} mol/L), the distal arterioles of control rats dilated to 31% ± 3.1% greater than the resting diameter, compared with 14% ± 1.0% in the rats receiving chronic ACE inhibition. When the response of
the distal arterioles to forskolin was normalized to the maximum response, no differences were identified between the control and captopril-treated groups (Figure 5b).

**Responses to Nitroprusside** Figure 6 summarizes the effect of captopril treatment on arteriolar dilation in response to sodium nitroprusside. Arteriolar reactivity to nitroprusside, normalized to the resting diameter, was significantly reduced with captopril treatment. Challenge with $10^{-6}$ mol/L nitroprusside dilated the arterioles of untreated control rats to 37% ± 2.2% greater than the rest diameter and those of captopril-treated animals to 12% ± 2.1% greater than the rest diameter (Figure 6a). Normalization of the vascular response to nitroprusside to the maximum dilation indicated that there was no difference in the arteriolar response to this agonist between the two
groups, with the exception of the response at $10^{-6}$ mol/L nitroprusside, where the arteriolar dilation was significantly reduced with chronic ACE inhibition (Figure 6b).

**DISCUSSION**

In light of the large number of patients currently receiving ACE inhibitors as an antihypertensive therapy, the role of AngII in regulating the function of the peripheral microcirculation is an issue of substantial clinical importance. However, the effects of ACE inhibition on the regulation of active tone in the microcirculation have received little attention. The goal of the present study was to determine the effects of chronic ACE inhibition on the vasodilator responses of the distal arterioles in the cremaster muscle microcirculation of normotensive rats.

**Effects on Microvessel Structure**  Captopril inhibits the conversion of Ang I to Ang II by ACE. 10 The

---

**FIGURE 4.** (A) Response of cremasteric arterioles to the $G_s$ protein activator cholera toxin ($10^{-9} \text{g} \cdot \text{ml}^{-1}$) in control and captopril-treated rats. Data are expressed as mean ($\pm$ SE) percentage increase from the resting diameter, determined during superfusion with PSS. Asterisk indicates a significant decrease ($P < .05$) in arteriolar dilation to cholera toxin in the captopril-treated animals. (B) Cremasteric arteriolar responses to cholera toxin in control and captopril-treated rats. Data are expressed as mean ($\pm$ SE) response to cholera toxin, expressed as a percentage of the maximum dilation, determined during superfusion with Ca$^{2+}$-free PSS containing $10^{-4}$ mol/L adenosine. Asterisk indicates a significant reduction ($P < .05$) in the vessel sensitivity to cholera toxin, compared with untreated controls.
results of the present study suggest that chronic ACE inhibition with captopril is associated with significant alterations in microvessel structure, which may limit the ability of the vessel to dilate, as the maximum dilation of the arterioles in response to Ca^{2+}-free PSS plus 10^{-4} mol/L adenosine was significantly reduced in animals treated with the ACE inhibitor. A possible explanation for this observation may be that the trophic effects of AngII on vascular smooth muscle may be lost during ACE inhibition with captopril, compromising the ability of the vessel to dilate as a result of alterations in microvessel structure.

The hypothesis that AngII suppression has a detrimental effect on microvessel structure has received increasing attention in recent years. Previous studies have suggested that the renin-angiotensin system may play a critical role in regulating the anatomical structure of the microcirculation under a va-
riety of conditions, including reduced renal mass hypertension, elevated dietary salt intake in normotensive animals, and in spontaneously hypertensive rats. Wang and Prewitt\textsuperscript{6,7} and Scheidegger et al\textsuperscript{8} reported that chronic ACE inhibition with captopril or with benazeprilat caused significant reductions in microvessel density, and reductions in the maximum diameter and cross-sectional wall area of arterioles in the cremaster muscle of normotensive rats and spontaneously hypertensive rats. In another study, Hernández et al\textsuperscript{5} reported that the reductions in microvessel density in the cremaster muscle of rats consuming a high-salt diet (4.0\% NaCl) are prevented by chronic infusions of subpressor doses of AngII. When combined with the studies of Rieder et al\textsuperscript{14} which demonstrated a strong correlation between the density of skeletal muscle microvessels and the activity of the renin-angiotensin system (estimated by measurements of plasma renin activity, ACE activity, and plasma AngII concentration), these results indicate

**FIGURE 6.** (A) Response of cremasteric arterioles to the nitric oxide donor sodium nitroprusside in control and captopril-treated rats. Data are expressed as mean (± SE) percentage increase from the resting diameter determined during superfusion with PSS. Asterisks indicate a significant decrease (P < .05) in arteriolar dilation to a given concentration of nitroprusside in the captopril-treated animals. (B) Cremasteric arteriolar responses to sodium nitroprusside in control and captopril-treated rats. Data are expressed as mean (± SE) response to nitroprusside, expressed as a percentage of the maximum dilation determined during superfusion with Ca\textsuperscript{2+}-free PSS containing 10\textsuperscript{-4} mol/L adenosine. Asterisk indicates a significant reduction (P < .05) in the vessel sensitivity to nitroprusside, compared with untreated controls.
that AngII suppression may be a critical element contributing to the alterations in microvessel structure that develop with any experimental protocol inhibiting the renin-angiotensin system (ie, pharmacologic inhibition of ACE, reduced renal mass (RRM) hypertension, and high-salt diet).

**Effects on Arteriolar Reactivity** Chronic elevations in dietary salt intake lead to a strong inhibition of the renin-angiotensin system. Kieder et al reported that chronic high-salt diet in RRM-hypertensive rats was associated with a 79% reduction in plasma AngII concentration, compared with reduced renal mass in rats fed a low-salt diet. In another study, Hansen-Smith et al reported that 3 days of a high-salt diet resulted in a 58% reduction in plasma AngII concentration in normotensive rats, compared with controls eating a low-salt diet (0.4% NaCl). Although we are not aware of any measures of plasma AngII concentration in rats receiving captopril treatment, significant reductions in AngII have been demonstrated in clinical trials for humans receiving captopril as an antihypertensive therapy. These reductions in AngII were associated with increases in the levels of renin and Ang I (precursors for Ang II formation via ACE), indicating successful enzyme inhibition with captopril. If chronic ACE inhibition with captopril leads to reductions in circulating AngII that are comparable to its role in maintaining vasodilator reactivity during peri-renal hypertension, and high-salt diet.

Analysis of the reactivity data, normalized to the maximum attainable diameter that occurs during superfusion with Ca\(^{2+}\)-free PSS containing 10\(^{-4}\) mol/L adenosine. However, it is possible that captopril-induced alterations in the composition of the arteriolar wall (eg, changes in collagen and elastin distribution) could lead to a reduced distensibility of the vessel wall and a nonspecific reduction in the ability of the vessel to increase its diameter after relaxation of the vascular smooth muscle.

The present findings suggest that the impaired vasodilator responses in the captopril-treated animals are not solely a function of structural remodeling of the arterioles that physically hinders vessel dilation, but instead represent a more generalized defect in the dilator mechanisms. This conclusion is based on our observation that the maximum dilation of arterioles in response to these agonists does not approach the maximum attainable diameter that occurs during superfusion with Ca\(^{2+}\)-free PSS containing 10\(^{-4}\) mol/L adenosine. However, it is possible that captopril-induced alterations in the composition of the arteriolar wall (eg, changes in collagen and elastin distribution) could lead to a reduced distensibility of the vessel wall and a nonspecific reduction in the ability of the vessel to relax in response to vasodilator stimuli.

Analysis of the reactivity data, normalized to the maximum dilation, indicated that chronic ACE inhibition had no effect on vascular reactivity to acetylcholine and forskolin and minimal effects on vascular reactivity to iloprost and sodium nitroprusside. These interactions with these earlier studies, the present experiments suggest that angiotensin II suppression may be a critical element regulating the alterations in microvessel structure and function that develop with RRM-hypertension and high-salt diet.

**Interaction Between Remodeling and Reactivity** A central issue regarding the impaired vasodilator responses in arterioles of the captopril-treated rats is the mechanisms underlying the impaired relaxation. Specifically, is the reduced dilator response due to an impaired function of active vasodilator mechanisms, to structural alterations in the arteriolar wall that impair the ability of the vessel to dilate, or both?

Compared with the vessels of the untreated animals, cremasteric arterioles of captopril-treated animals exhibited severely blunted responses to all of the vasodilator agonists in the present study. These observations could suggest that chronic ACE inhibition impairs vasodilator responses throughout the signal transduction pathway, including membrane receptors on the vascular endothelium (acetylcholine) and smooth muscle (iloprost), G-proteins (cholera toxin), and the adenyllyl and guanylyl cyclase (forskolin and nitroprusside) stages. Alternatively, vascular relaxation could be impaired at a point downstream from the site of action of each of these vasodilator mechanisms, eg, at the level of intracellular calcium regulation, or the regulation of K\(^{+}\) channels in the cell membrane. In addition, the reduced dilation of the vessel in response to the various vasodilator stimuli could result from structural changes in the arteriolar wall that can physically impair the ability of the vessel to increase its diameter after relaxation of the vascular smooth muscle.
observations suggest that, in captopril-treated animals, the extent to which the mechanisms of dilation are compromised generally occurs in proportion to the remodeling of the arteriole, leading to the observed similarity in vascular reactivity to acetylcholine, iloprost, forskolin, and nitroprusside. This contrasts with the significant reduction in the arteriolar reactivity to choler toxin with captopril treatment. It is apparent that the extent to which dilator mechanisms to cholera toxin challenge are compromised in the captopril-treated animals is disproportionate to the remodeling of the arteriole, leading to the demonstrated reductions in the reactivity.

Possible Role of Bradykinin in Contributing to Altered Vascular Reactivity in Captopril-Treated Rats
As reviewed by Luscher, ACE is identical to kininase II, which degrades the potent endogenous vasodilator bradykinin. Therefore, one of the additional consequences of the ACE inhibition is to increase the local bradykinin concentrations, enhancing a powerful endothelium-dependent vasodilator mechanism. These increased bradykinin levels could potentially enhance any relaxing effect of the vasodilator agonists used in the present study. However, despite the possibility that the endogenous levels of bradykinin may have been elevated during captopril treatment, the vasodilator responses to each of the agonists used in the present study were reduced. These observations suggest that elevated bradykinin levels are not sufficient to override the detrimental effects of ACE inhibition on vasodilator reactivity in the captopril-treated animals. Whereas previous studies demonstrating that angiotensin II infusion restores vasodilator reactivity suggest that the effects of captopril are mediated via inhibition of ACE, the potential effects of elevated bradykinin on vasodilator reactivity are unknown, and represent a potentially interesting area of investigation.

Conclusions The results of the present study indicate that chronic treatment of normotensive rats with the angiotensin converting enzyme inhibitor captopril leads to significant alterations in the structure of skeletal muscle arterioles and in the responses of the vessels to endothelium-dependent and -independent dilator agonists. Based on the integration of the results of the present experiments with those of existing studies, further investigation into a number of specific areas seems warranted. These include an investigation of the specific mechanisms responsible for the altered microvascular structure and reactivity; the qualitative differences between captopril administration and elevated dietary salt intake in terms of the regulation of vascular structure and reactivity; the mechanisms by which angiotensin II may exert its protective role in maintaining microvascular structure and function; and the interrelationships between ACE inhibition and endogenous bradykinin activity in terms of arteriolar reactivity. Finally, recent studies in our laboratory have indicated that short-term (3-day) elevations in dietary salt intake lead to an impaired reactivity of microvessels to vasodilator agonists. Given that continuous infusion of subpressor doses of AngII restores the normal vasodilator reactivity in resistance arteries of normotensive rats eating a short-term high-salt diet, future experiments employing short-term captopril treatment may be helpful in determining the effect of short-term ACE inhibition in contributing to the impaired vascular relaxation in skeletal muscle arterioles and resistance arteries, and elucidating the mechanisms by which vasodilator reactivity is impaired by ACE inhibition in these vessels.

REFERENCES