Short-Term Angiotensin Converting Enzyme Inhibition Reduces Basal Tone and Dilator Reactivity in Skeletal Muscle Arterioles

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Alterations in resting tone, maximum diameter, and dilator reactivity to acetylcholine (ACH) and sodium nitroprusside (SNP) were assessed in cremaster muscle microvessels of Sprague-Dawley rats receiving angiotensin converting enzyme (ACE) inhibition with captopril for 4 days and in untreated time-control rats. The transilluminated in situ cremaster muscle was superfused with physiologic salt solution (PSS) and viewed via television microscopy; arteriolar diameter was measured using a videomicrometer. Before agonist challenge, resting arteriolar diameter was significantly increased in captopril-treated rats. Although maximum arteriolar diameter (determined during superfusion of the cremaster muscle with Ca²⁺-free PSS containing 10⁻⁴ mol/L adenosine) was not altered with ACE inhibition, the maximum possible arteriolar dilation was reduced in captopril-treated rats. Captopril administration reduced both ACH- and SNP-induced dilation of cremasteric arterioles compared with responses in control rats, although this was partially a function of the reduced capacity for dilation, primarily to SNP. These observations indicate that short-term ACE inhibition reduces both resting tone and agonist-induced dilator responses of skeletal muscle arterioles. Am J Hypertens 2000;13:389–395 © 2000 American Journal of Hypertension, Ltd.

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Previous studies in our laboratory have demonstrated that chronic elevations in dietary salt intake and reduced renal mass (RRM) hypertension in rats impairs the relaxation of skeletal muscle resistance arteries, middle cerebral arteries, and skeletal muscle arterioles in response to dilator stimuli.¹⁻³ This impaired relaxation may be related to reductions in plasma angiotensin II (AngII) levels occurring with a high-salt diet⁴ and RRM-hypertension,⁵ as preliminary studies have indicated that prevention of AngII suppression by continuous infusion of a low dose of AngII⁶ restores the dilator responses that are normally lost in cerebral arteries and in skeletal muscle resistance arteries of rats eating a high-salt diet.⁷ More recently, Frisbee et al⁸ demonstrated that chronic angiotensin converting enzyme (ACE) inhibition with captopril reduced the dilator reactivity of distal arterioles of the rat cremaster muscle in a manner similar to that with chronic RRM-hypertension.³ Taken together, these data suggest that chronic reduction of plasma AngII levels with a high-salt diet, RRM-hypertension, or pharmacologic inhibition of ACE may contribute to alterations in the
reactivity of resistance arteries and skeletal muscle arterioles to vasodilator stimuli.

Preliminary studies investigating the time course of these alterations in arteriolar reactivity with a high-salt diet and RRM-hypertension demonstrate that these changes develop rapidly, in as little as 3 days. In light of these findings that experimental conditions reducing AngII levels rapidly depress microvessel reactivity, and the fact that large numbers of clinical patients are currently receiving ACE inhibitors as an antihypertensive therapy, the goal of the present study was to determine the effects of short-term pharmacologic ACE inhibition on the dilator reactivity of skeletal muscle arterioles in normotensive rats.

MATERIALS AND METHODS

Animal Groups and Preparation The present studies were conducted on two groups of age-matched, normotensive male Sprague-Dawley rats. Four days before the experiment, one group of rats (n = 7) was administered captopril (100 mg · kg$^{-1}$ · day$^{-1}$, Sigma Chemical Co., St. Louis, MO) in their drinking water, while the other group (n = 7) served as untreated time-control animals. All rats drank tap water (with or without captopril), ad libitum.

Methods for Microcirculatory Studies On the day of the experiment, rats were anesthetized with an intraperitoneal injection (60 mg · kg$^{-1}$) of pentobarbital sodium (Veterinary Laboratories, Lenexa, KS), and the trachea was cannulated to insure a patent airway. A femoral artery and an external jugular vein were cannulated for arterial pressure recording and for infusion of supplemental anesthetic, respectively.

After this initial surgery, the in situ cremaster muscle was prepared for television microscopy, fully detailed previously. Once completed, the tissue was continuously superfused with physiologic salt solution (PSS), equilibrated with a 5% CO$_2$/95% N$_2$ gas mixture and maintained at 34–35°C as it flowed over the muscle. The ionic composition of the PSS was as follows (mmol/L): NaCl 119.0, KCl 4.7, CaCl$_2$ 1.6, NaH$_2$PO$_4$ 1.18, MgSO$_4$ 1.17, NaHCO$_3$ 24.0, and disodium EDTA 0.03.

In a clearly visible region of the cremaster muscle, a second-order arteriole (diameter~60 μm) was identified. This vessel was tracked along its length to the point at which capillaries arose from terminal arterioles. Once capillaries arising from this arteriolar network were identified, the section of arteriole lying immediately proximal to the capillaries was selected for analysis. For all experiments, microvessel location (immediately proximal to the capillaries) was the primary selection criterion and vessel diameter was not a factor. The ultimate selection of arterioles was subject to the following additional requirements: location in a region of muscle that was away from any incision, clearly discernible vessel walls, brisk flow velocity, and active tone, as indicated by the occurrence of a brisk dilation in response to topical application of $10^{-4}$ mol/L adenosine.

Arteriolar diameter was measured before and after topical application of the endothelium-dependent vasodilator acetylcholine (ACH; $10^{-5}$–$10^{-6}$ mol/L) and the endothelium-independent nitric oxide donor sodium nitroprusside (SNP; $10^{-9}$–$10^{-6}$ mol/L). Maximum arteriolar diameter was assessed by measuring the vascular response to superfusion with Ca$^{2+}$-free PSS containing $10^{-4}$ mol/L adenosine. Successive challenges were applied only after the vessel had returned to its original diameter after application of the preceding agonist challenge. The application of the agents was randomized to prevent the occurrence of ordering effects, and to control for any time-dependent changes in vascular reactivity during the course of the experiment. After the initial equilibration period, the total duration for an individual experiment was approximately 60 min.

Data and Statistical Analyses All data are expressed as mean ± SEM. Student’s t test, comparing control versus captopril-treated animal groups, was used to determine differences in all parameters not involving repeated measures. All vascular reactivity data were initially summarized as the change in the absolute vessel diameter (μm) in response to the agonist challenge. To gain insight into the magnitude of the dilation relative to the maximum response, agonist-induced responses were also normalized to the maximum possible dilation (determined during superfusion of the preparation with Ca$^{2+}$-free PSS plus $10^{-4}$ mol/L adenosine). Analysis of variance (ANOVA) with Scheffé’s post-hoc test was employed to determine differences in the agonist-induced vascular reactivity across experimental groups and between captopril-treated and control rats at specific agonist concentrations.

All ACH and SNP dose-response curves in control and captopril-treated rat groups were fitted with the following regression equation (least-squares analysis; $r^2 > 0.90$):

$$y = \alpha + \beta(x),$$

where y represents the increase in arteriolar diameter from rest to challenge with an agonist at a specific concentration, α is an intercept term, and x is the logarithm of the agonist concentration. β represents the slope of the dose-response curve (ie, the rate of change in arteriolar diameter for a logarithmic change in agonist concentration). The differences between slope coefficients for agonist-induced responses in the control and captopril-treated rat were determined
with a Student’s *t* test. In all cases, a probability level of *P* < .05 was considered to be statistically significant.

**RESULTS**

**Mean Arterial Pressure and Body Weight** Mean arterial pressure in rats receiving captopril treatment (102 ± 5 mm Hg) was not different from that in untreated control rats (110 ± 7 mm Hg). Body weight was not different between control (248 ± 9 g) and treated rats (241 ± 7 g).

**Resting and Maximum Arteriolar Diameter** In the present study, the resting diameter of cremasteric arterioles before agonist challenge was significantly increased in captopril-treated rats (21.4 ± 0.9 μm) compared with control animals (18.3 ± 0.8 μm), whereas maximum arteriolar diameter was not different between treated (28.1 ± 0.9 μm) and control rats (29.6 ± 1.3 μm). Combining these measurements, the maximum possible arteriolar dilation in control animals (11.3 ± 0.9 μm) was significantly greater than that in captopril-treated rats (6.7 ± 0.6 μm).

**Responses to Acetylcholine** In both animal groups, ACH caused a brisk dilation of cremasteric arterioles from the rest condition, although this response was greater in control rats. Figure 1a presents the responses of cremasteric arterioles to challenge with ACH in control and captopril-treated rats. Across the agonist concentration range used in the present study, the microvessel dilation to ACH in captopril-treated rats was reduced, compared with responses in control animals. As a result of this inhibition, the slope of the dose-response curve describing the ACH-induced arteriolar responses was reduced in rats receiving ACE inhibition (1.1 ± 0.2 μm/log [ACH] [mol/L]), compared with control rats (1.9 ± 0.3 μm/log [ACH] [mol/L]). However, when arteriolar responses to ACH were normalized to the maximum possible dilation, no differences between the arteriolar responses to ACH were identified between animal groups (Figure 1b).

**Responses to Sodium Nitroprusside** As with ACH, challenge with SNP caused a brisk arteriolar dilation from rest in all rats, although the maximum response (to 10⁻⁶ mol/L SNP) was significantly greater in control rats (9.6 ± 0.7 μm) than in rats receiving ACE inhibition (6.0 ± 0.5 μm). Figure 2a presents the effect of short-term captopril administration on cremasteric arteriolar dilation to SNP in the present study, where the microvessel responses to SNP were reduced in captopril-treated rats compared with responses in control animals. In response to the ACE inhibition, the slope of the dose-response curve describing arteriolar responses to SNP was significantly lower in captopril-treated rats (1.5 ± 0.2 μm/log [SNP] [mol/L]), compared with control rats (2.5 ± 0.3 μm/log [SNP] [mol/L]). Normalization of SNP-induced reactivity to the maximum possible dilation eliminated the differences in the cremasteric arteriolar responses between treated and control rats (Figure 2b).
DISCUSSION

Given the large number of patients receiving ACE inhibitors as antihypertensive therapy, the regulation of microvascular function under these conditions is an issue of substantial importance. Previous studies have demonstrated that chronic ACE inhibition with captopril reduces dilator reactivity and the maximum possible dilation of rat skeletal muscle arterioles in a manner similar to that for chronic high-salt diet and RRM-hypertension. Conditions causing a substantial reduction in plasma AngII levels, in light of recent studies demonstrating the extremely rapid development of altered arteriolar reactivity with a high-salt diet and RRM-hypertension, the goal of the present study was to determine the effects of short-term ACE inhibition with captopril on vascular reactivity in cremasteric arterioles of normotensive rats.

Captopril inhibits the conversion of AngI to AngII through ACE. However, as ACE is identical to kininase II (responsible for degradation of bradykinin to inactive compounds), pharmacologic agents inhibiting ACE also inhibit kininase II. Therefore, interpretation of results from the present study must address the inhibition of both enzymes. Further, in the present study, ACE inhibition decreased basal vascular tone (ie, increased resting arteriolar diameter) and had a tendency to decrease maximum arteriolar diameter, although the latter change was not significant. The combination of these effects on arteriolar diameter reduced the maximum possible dilation in treated rats, compared with control animals. As a result, alterations in vascular reactivity may reflect either reduced net capacity for dilation (which would be agonist independent) or intrinsic differences in vascular sensitivity to the agonist itself.

Effects on Maximum Microvessel Diameter

In agreement with our earlier study investigating the effects of chronic ACE inhibition on the microcirculation, the present study indicates that short-term pharmacologic ACE inhibition does not significantly reduce the maximum attainable microvessel diameter. This conclusion contrasts with previous studies demonstrating that experimental conditions that should reduce plasma AngII levels (ACE inhibition and chronic and short-term high-salt diet and RRM-hypertension) lead to a reduction in maximum microvessel diameter. Although an explanation for the contrasting observations between the present study and previous investigations is not readily apparent, alterations in maximum arteriolar diameter with ACE inhibition in the present experiments and in the previous study of Frisbee et al., though of insufficient magnitude to be statistically significant, are qualitatively similar to those in previous studies.

Preliminary studies investigating the role of bradykinin during alterations in vascular structure with ACE inhibition suggest that any effects of increased bradykinin levels during ACE inhibition depend on vascular diameter. Benetos et al. determined that the prevention of aortic collagen deposition with ACE inhibition in spontaneously hypertensive rats (SHR) was independent of blood pressure and kininase II.
inhibition, and was solely the result of altered interaction of AngII with AT_1 receptors. However, in the microcirculation, Gohlke et al^19 demonstrated that the increase in cardiac capillary length density with ACE inhibition in SHR was due primarily to the potentiation of endogenous bradykinin, as B_2 receptor blockade eliminated this response. Clearly, further study is warranted to investigate the roles of AngII and bradykinin in regulating vascular structure during ACE inhibition.

**Effects on Resting Arteriolar Diameter** Short-term ACE inhibition increased the resting diameter of cremasteric arterioles compared with values in control rats. This observation was not a function of the vessel selection procedure, as the selection of vessels for observation was based upon the location of the arterioles within the network (ie, immediately proximal to the capillaries), and vessel diameter was not a factor in selecting arterioles for study. Previous studies suggest that the increased resting diameter with pharmacologic ACE inhibition was most likely mediated via captopril-based inhibition of kininase II, rather than a reduction in plasma AngII levels. Ekelund^20 investigated the effects of local ACE inhibition on vascular tone in cat gastrocnemius muscle. Close arterial infusion of enalaprilat elicited a dilation throughout the vascular network that was eliminated by infusion of Hoe 140 (a bradykinin B_2 receptor antagonist), whereas infusion of losartan (an angiotensin AT_1 receptor antagonist) did not alter vascular tone. These results suggested that ACE plays a significant role in regulating basal vascular tone through degradation of endogenous bradykinin, rather than generation of AngII.

**Effects on Arteriolar Reactivity** When expressed as the raw change in vessel diameter (D \( \mu m \)) to either ACH or SNP, the slopes of the agonist dose-response curves were reduced in treated rats, compared with those in control animals, indicating reduced reactivity to both dilator agents. However, after normalization of vascular responses to the maximum dilation (to Ca\(^{2+}\)-free PSS with 10\(^{-4}\) mol/L adenosine) no differences were identified between the animal groups.

Previous studies indicate that high-salt diet and RRM-hypertension rapidly reduce microvascular reactivity to endothelium-dependent dilator stimuli.\(^9\) Results from the present study demonstrate that vasodilator responses of cremasteric arterioles to ACH during pharmacologic ACE inhibition follow a similar pattern in terms of altered reactivity. This conclusion, however, contrasts with that from the study by Berkenboom et al,\(^21\) where ACE inhibition potentiated endothelium-dependent responses to ACH in aortae of normotensive rats. It may be that during short-term ACE inhibition, the loss of vascular reactivity due to lowered plasma AngII levels may be more significant than any concurrent amplification of endothelium-derived nitric oxide pathways in determining net arteriolar reactivity to ACH. It is also evident that the effects of ACE inhibition on vascular reactivity to endothelium-dependent agonists may be a function of vessel diameter.

Compared with vessels of control rats, cremasteric arterioles of captopril-treated rats exhibited blunted responses to both dilator agonists in the present study. One possible interpretation of this finding is that short-term ACE inhibition may impair dilator mechanisms throughout the signal transduction pathway of vasorelaxation. Alternatively, the reduced dilation of the vessel to the dilator stimuli could result from the reduced capacity for dilation, largely the result of the elevated resting microvessel diameter. Finally, vascular relaxation could be impaired at a point downstream from the site of action of SNP, eg, intracellular Ca\(^{2+}\) regulation or K\(^+\) channel regulation in the cell membrane, although this final speculation lies outside the scope of the present study.

The present findings suggest that the impaired vascular relaxation to ACH and SNP are most likely a result of both a defect in dilator mechanisms (primarily to ACH) and a reduced capacity for dilation that physically limits the amplitude of the vasorelaxation (in the case of SNP). This speculation is based on several observations. First, the dilator response of arterioles to ACH, which was reduced when it was expressed as the raw change in vessel diameter (Figure 1a), did not approach the maximum possible dilation in either control or captopril-treated rats. However, when normalized to the maximum possible dilation, the ACH-induced response in treated rats was not different from that in control animals (Figure 1b). The combination of these results suggests that the relaxation of the vessel was not impaired by the reduced capacity for dilation. Instead, we believe that mechanisms of ACH-induced vasodilation may have been impaired in parallel with alterations in the maximum possible response. In contrast, arteriolar reactivity to SNP approached the maximum possible levels in both control and captopril-treated rat groups (Figure 2b), suggesting that mechanisms of dilation to SNP are unaffected by short-term ACE inhibition and that the reduced reactivity primarily reflects the lowered capacity for dilation.

When integrated with results from our previous study investigating the effects of chronic ACE inhibition via captopril,\(^8\) the results from the present study may have significant implications for patients receiving ACE inhibition as an antihypertensive therapy. With ACE inhibition, two primary effects on the microcirculation develop: a gradual reduction in the di-
lator reactivity of skeletal microvessels, and a reduced passive microvessel diameter. The severity of these alterations appears to depend on the duration of ACE inhibition, as these effects are exacerbated with prolonged captopril treatment in rat skeletal muscle. It is generally accepted that captopril treatment in human hypertensives results in an improved vascular resistance in muscular vascular beds such as the calf and forearm. However, in patients receiving chronic ACE inhibition, the loss of responsiveness to vasodilator stimuli and the reduced maximum capacity for arteriolar dilation during captopril treatment could have a negative impact on tissue perfusion and skeletal muscle performance during conditions when a strong microvessel dilation is needed (eg, exercise). In this respect, it appears that studies of the sensitivity of peripheral vascular beds to vasodilator stimuli in patients receiving ACE inhibitors may be an important area for future investigation.

Conclusions The results of the present study suggest that short-term treatment of normotensive rats with captopril lowers the basal tone of skeletal muscle arterioles, ultimately reducing the maximum capacity for vasodilation. In addition, ACE inhibition reduces dilator reactivity of skeletal muscle microvessels to ACH in normotensive rats, in contrast to previous studies in larger conduit arteries. Finally, the present study suggests that the mechanisms involved in the vasodilator response to SNP may remain largely unaffected by short-term ACE inhibition, as vascular responses to SNP approached the maximum possible response in both captopril-treated and control animals. Rather, the reduced arteriolar response to SNP (as raw diameter) may have been due to a reduced capacity for dilation, as a result of the reduced basal tone that occurred in response to short-term captopril treatment. Further investigation into mechanisms regulating microvascular tone, structure, and reactivity during pharmacologic ACE inhibition (eg, the role of ACE/AngII versus kininase II/bradykinin) represent exciting areas for future study.

REFERENCES


