Antihypertensive Therapy and Adaptive Mechanisms in Peripheral Ischemia

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In the present experiments the effect of long-term peripheral ischemia on the capillarity of two hind limb skeletal muscles was investigated in spontaneously hypertensive rats. Furthermore, the effect of antihypertensive therapy on changes in capillarity and on the previously observed hyperreactivity of the ischemic vascular bed to vasoconstrictors was investigated in perfused hind limbs of rats after long-term treatment with the angiotensin I converting enzyme inhibitors captopril (0.5 mg/kg • h) or zabicaprilate (0.025 mg/kg • h), the angiotensin II type 1 receptor antagonist losartan (0.625 mg/kg • h), or the calcium antagonist felodipine (0.042 or 0.42 mg/kg • h). Skeletal muscle ischemia in the left hind limb was induced by partial ligation of the left common iliac artery. Long-term (4 weeks) ischemia increased significantly the capillary-to-fiber ratio in the soleus muscle, whereas capillarity in the contralateral muscle was not affected. Furthermore, capillarity in the gastrocnemius muscle (type II muscle fiber part) of both the ischemic and contralateral hind limb did not change. Long-term treatment with the angiotensin I converting enzyme inhibitors during ischemia abolished the increase in the capillary-to-fiber ratio in the soleus muscle, whereas a comparable antihypertensive dose of felodipine had no effect. Greater blood pressure reductions by both losartan and felodipine prevented increases in capillarization in skeletal muscle ischemia. With respect to vascular hyperreactivity during ischemia, only treatment with losartan normalized reactivity of the ischemic vascular bed to vasoconstrictors. These data suggest that both the renin-angiotensin system, probably through the angiotensin II type 1 receptor, and hypoperfusion play a role in the adaptation mechanisms after ischemia of skeletal muscle. (Hypertension. 1993;22:780-788.)

KEY WORDS  • angiotensin converting enzyme inhibitor  • receptors, angiotensin  • calcium channel blockers  • neovascularization  • antihypertensive therapy  • ischemia

Peripheral ischemia activates several short- and long-term compensatory mechanisms, such as structural adaptations of resistance vessels and metabolic and structural adaptations of skeletal muscles. In a rat model for long-term ischemia of skeletal muscle, we previously demonstrated hyperreactivity of the total vascular bed of severely ischemic hind limbs to the vasoconstrictors angiotensin I (Ang I), angiotensin II (Ang II), and phenylephrine, whereas in a comparable ischemia model Verheyen and coworkers observed a hyperreactivity to serotonin and a thromboxane analogue. One of the mechanisms for restoring blood supply to the ischemic regions is the development of collateral vessels. Generation of new vessels by ACE inhibitors may also have an

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effect on capillary growth in skeletal muscle, because an increase in blood flow due to long-term treatment with vasodilators results in new vessel formation.11,29,30

We hypothesize that peripheral ischemia increases capillarity in skeletal muscle, probably dependent on muscle fiber type, and that different antihypertensive drugs have differential effects on changes in the vasculature due to peripheral ischemia. Therefore, in the present experiments the effect of long-term (4 weeks) ischemia of skeletal muscle on capillarity in the soleus muscle (oxidative fibers) and in the type II muscle fiber part (glycolytic and oxidative glycolytic fibers) of the gastrocnemius muscle was investigated. Also, the influence of ACE inhibition, Ang II antagonism, and calcium antagonism on possible capillary changes in ischemic and nonischemic skeletal muscles and their influence on the previously described nonselective hyperreactivity of the vascular bed of ischemic hind limbs to vasoconstrictors3 were investigated. Spontaneously hypertensive rats with induced skeletal muscle ischemia were chronically treated (4 weeks) with the ACE inhibitors captopril and zaciprilate, the Ang II type 1 (AT1) receptor antagonist losartan, or the calcium antagonist felodipine.

Methods

Animals

Male spontaneously hypertensive rats (Central Animal Facilities, University of Limburg, Maastricht, the Netherlands) were used. Starting body weights were 320 to 360 g for nonischemic (control) animals and 270 to 310 g for chronically ischemic animals (resulting in a body weight of 320 to 360 g after 4 weeks of ischemia). In dose-finding studies (angiotensin responses), starting body weights were 300 to 350 g. Animals were housed in groups of up to four rats and had free access to standard food (Hope Farms, Woerden, the Netherlands) and tap water. After implantation of arterial and venous catheters (in dose-finding studies), animals were housed separately. The experimental procedures were performed according to institutional guidelines.

Surgery and Preparations

Long-term ischemia. Long-term ischemia was induced by partial ligation of the left common iliac artery with rats under ether anesthesia.3 The abdomen was opened, and the left common iliac artery was dissected. Partial occlusion of the artery was accomplished by firmly tying a stainless steel wire (0.15 mm) against the side of the vessel with a 4-0 silk suture, resulting in complete occlusion of the vessel lumen. The stainless steel wire was then removed, leaving the silk suture ligature intact and allowing the vessel to expand by an amount equal to the diameter of the wire. In addition, the left iliolumbar artery was occluded completely with a 4-0 silk suture to avoid restoration of blood supply to the left hind limb through this vessel. After the abdomen was closed, animals were allowed to recover for 4 weeks before hind limb perfusion experiments.

Hind limb perfusion. The hind limb preparation according to Ross and Price previously used this staining method in ischemic skeletal muscles. Sections were stained for 1 hour at 37°C using a buffer (MgSO4, 6.9 mmol/L; NaBO3, 27.5 mmol/L) containing 1 mg/mL nitroblue tetrazolium and 0.2 mg/mL 4-chloro-3-indolyl phosphate p-toluidine salt and adjusted to pH 9.2 to 9.4 with boric acid. After rinsing with buffer and postfixation in sucrose-buffered Formalin (4% formaldehyde, 300 mOsm/L sucrose, pH 7.3), the sections were counterstained with eosin (0.1% wt/vol).

To distinguish between type I and type II muscle fibers, gastrocnemius muscle sections were also stained for type I muscle fibers using an indirect enzyme-labeled antibody technique. After preincubation with normal rabbit serum, alkaline phosphatase–stained sections were incubated with the monoclonal antibody RnDio (Centocor Europe, Leiden, the Netherlands; 1:10 000). Peroxidase-conjugated rabbit anti-mouse IgG (RAMPO; Dako, Glostrup, Denmark; 1:200) was used as the second layer, and 3,3-diaminobenzidine was used as chromogen.

Dose Comparison

Angiotensin responses. Chronic doses of the new ACE inhibitor zaciprilate (Servier, Neuilly, France) and the
AT₁ antagonist losartan, which are comparable with a previously used dose of captopril (0.5 mg/kg·h)²³ with respect to inhibition of pressor effects of exogenous Ang I or II, were determined. Osmotic minipumps (Alzet 2001 or 2ML1, Alza Corp, Palo Alto, Calif) were implanted subcutaneously, and animals were infused with zabicaprilate at rates of 0.0017, 0.005, or 0.017 mg/kg·h SC or with captopril at rates of 0.1 or 0.5 mg/kg·h SC for 4 to 5 days. Thereafter, animals were anesthetized with ether. The abdominal aorta was cannulated via a femoral artery for blood pressure measurements, and the vena cava inferior was cannulated via a femoral vein for intravenous bolus injections. Both catheters were guided subcutaneously to the neck, where they were exteriorized, filled with saline containing 5 to 15 IU heparin/mL, and closed with a metal plug.

After recovery for 1 day, dose-response curves for Ang I were generated in anesthetized (pentobarbital) untreated and treated animals. Changes in mean arterial pressure (MAP) after intravenous bolus injections of [Val⁵]Ang I (0.01 to 30 μg) were measured.

Both captopril and zabicaprilate increased the ED₅₀ for Ang I dose dependently after long-term treatment. However, even the highest dose tested of zabicaprilate resulted in a smaller shift in the Ang I dose-response curve than after captopril at a rate of 0.5 mg/kg·h (ED₅₀ of Ang I: untreated, 0.083±0.01; captopril, 0.700±0.21; zabicapril, 0.510±0.10 μg; n=4). Therefore, in further experiments zabicaprilate was administered at a rate of 0.025 mg/kg·h.

A chronic dose of losartan comparable with captopril (0.5 mg/kg·h) was previously determined by Smits et al.²⁴ The shift in ED₅₀ for [Val⁵]Ang II after losartan at a rate of 0.625 mg/kg·h was approximately 32-fold, whereas the shift in ED₅₀ for [Val⁵]Ang I after captopril at a rate of 0.5 mg/kg·h was approximately 17-fold in anesthetized animals.

**Blood pressure reduction.** Comparable antihypertensive doses of felodipine with the doses of the two ACE inhibitors and of losartan were determined by treatment of chronically ischemic animals with captopril (0.5 mg/kg·h), zabicaprilate (0.025 mg/kg·h, see above), losartan (0.625 mg/kg·h, see above), or felodipine (0.042, 0.125, and 0.42 mg/kg·h) for 4 weeks. Thereafter, animals were anesthetized with ether, and the left carotid artery was cannulated for blood pressure measurements. The catheter was guided subcutaneously to the neck, where it was exteriorized, filled with saline containing 5 to 15 IU heparin/mL, and closed with a metal plug. After recovery for 1 day, MAP was measured in conscious treated and untreated animals.

The blood pressure reduction after captopril and zabicaprilate was comparable in chronically ischemic rats (untreated, 193±8 mm Hg; captopril, 157±10 mm Hg; zabicaprilate, 158±9 mm Hg; n=3 to 4). In contrast, losartan resulted in a greater blood pressure reduction (138±4 mm Hg, n=3). Felodipine at a rate of 0.042 mg/kg·h resulted in a blood pressure reduction comparable to the ACE inhibitors (164±1 mm Hg, n=3), whereas felodipine at a rate of 0.42 mg/kg·h resulted in a blood pressure reduction comparable to losartan (131±3 mm Hg, n=5). Therefore, in further experiments animals were treated with two different doses of felodipine.

**Treatment**

Immediately after induction of ischemia, osmotic minipumps were implanted subcutaneously in the neck, and animals were infused for 4 weeks with comparable doses of captopril (0.5 mg/kg·h SC, Alzet 2001), zabicaprilate (0.025 mg/kg·h SC, Alzet 2ML2), losartan (0.625 mg/kg·h SC, Alzet 2002), or felodipine (0.042 and 0.42 mg/kg·h SC, Alzet 2002 and 2001) or received no treatment. Captopril was dissolved in saline, zabicaprilate and losartan in distilled water, and felodipine in polyethylene glycol (PEG 400). With rats under ether anesthesia, the osmotic minipumps were replaced every week for captopril and felodipine (high-dose) treatments or after 2 weeks for the other treatments.

**Measurements**

**Hind limb perfusion.** The animals were prepared for hind limb perfusion experiments 4 weeks after induction of long-term ischemia. The hind limb preparation was allowed to equilibrate for 30 to 45 minutes to achieve a steady-state level of perfusion pressure and flows. Dose-response curves of [Val⁵]Ang I (0.1 to 10 μg, in untreated and treated animals), [Val⁵]Ang II (0.03 to 10 μg, in treated and untreated animals), or phenylephrine (0.1 to 300 μg, in untreated and losartan-treated animals) were generated immediately after equilibration.

Each doresponse curve was generated in a separate hind limb preparation. The minimal time interval between two bolus injections of Ang I or II was 15 minutes. Before and after the generation of a dose-response curve of Ang I or II, 30 μg phenylephrine was injected for evaluation of the responsiveness of the hind limb preparation.

**Skeletal muscle.** The muscles of the ischemic upper and lower hind limb were evaluated for signs of inflammation. Two soleus muscles with signs of inflammation were excluded from further morphological experiments.

The origin of all samples for morphometry was blinded to the investigator. The number of capillaries and muscle fibers in gastrocnemius and soleus muscles of both hind limbs in control, treated, and untreated ischemic animals was counted microscopically (×250 magnification), using a graphic tablet, optically projected into a standard microscope and coupled to a computer (CAS200, Becton Dickinson, Etten-Leur, the Netherlands). In each section of soleus muscle, eight randomly chosen fields were counted (encompassing approximately 250 to 600 fibers), whereas in each section of gastrocnemius muscle, 10 randomly chosen fields only in the type II muscle fiber part were counted (encompassing approximately 300 to 500 fibers).

Furthermore, fiber size was measured in both soleus and gastrocnemius muscles of control and untreated ischemic animals; total muscle size (×25 magnification) was measured in soleus muscle (control, untreated, and treated ischemic animals) but not in gastrocnemius muscle, in which only the type II muscle fiber part was used.

**Data Analysis**

**Hind limb perfusion.** Resistance (R) was computed as \( R=P/(F_L+F_R) \), where P is perfusion pressure and \( F_L \) and \( F_R \) are flow in the left and right hind limbs, respectively. Responses were expressed as maximal changes in resistance (ΔR). In both hind limb and in vivo experiments, half-maximal effective dose (ED₅₀)
TABLE 1. Capillary-to-Fiber Ratio, Total Fiber Number, and Muscle Fiber Size in Gastrocnemius and Soleus Muscles of Left (Ischemic) and Right (Nonischemic) Hind Limbs in Control and Ischemic Animals

| Group and Parameter | Gastrocnemius | | | Soleus | |
|---------------------|---------------|---------------|---------------|---------------|
|                     | Left          | Right         | Left          | Right         |
| Control (n=9)       | 336±7         |               |               |               |
| C/F                 | 1.35±0.05     | 1.41±0.06     | 2.83±0.05     | 2.60±0.09     |
| FN                  | ND            | ND            | 2911±204      | 3076±184      |
| FS, μm²             | 3055±226      | 2981±155      | 2945±11       | 2917±67       |
| Ischemia (n=12)     | 335±5         |               |               |               |
| C/F                 | 1.50±0.05*    | 1.40±0.04     | 3.18±0.09†    | 2.82±0.05     |
| FN                  | ND            | ND            | 3309±191↑     | 2664±92       |
| FS, μm²             | 2908±61       | 3056±111      | 2372±71↑†     | 3081±78       |

BW indicates body weight; C/F, capillary-to-fiber ratio; FN, total fiber number; ND, not determined; and FS, fiber size. Data are mean±SEM.

*Significant difference from control.
†Significant difference from contralateral hind limb.

and maximal effect (ΔRmax or ΔMAPmax) of Ang I, Ang II, or phenylephrine were computed by fitting the responses to a sigmoidal curve using the equation

$$\Delta R = \frac{\Delta R_{\text{max}} \cdot D^n}{E_{D_0} + D^n}$$

where $D$ equals doses of Ang I, Ang II, or phenylephrine and $n$ is the Hill coefficient.

The degree of ischemia of the left hind limb was computed as

$$\% \text{ Residual Flow} = \frac{F_L}{F_R} \cdot 100\%$$

Skeletal muscle. Ischemic hind limbs with a residual flow greater than 40% do not have an increased reactivity of the vasculature. Therefore, only animals with a residual left flow less than 40% were used. The capillary-to-fiber ratio (C/F) was computed from the numbers of capillaries and fibers. Total number of fibers in soleus muscle was computed as

$$FN = \frac{F_{\text{mean}} \cdot S}{MS}$$

where $FN$ is total fiber number, $F_{\text{mean}}$ is fiber number (mean of eight fields), $S$ is field size ($=142.985 \ \mu m^2$), and $MS$ is total muscle size (cross section).

Statistics

Values of experiments in untreated and treated ischemic animals were compared by one-way analysis of variance and Bonferroni and Dunnett's test or Student's $t$ test for unpaired observations. Values of left and right hind limbs were compared by Student's $t$ test for paired observations. Data are expressed as mean±SEM. Differences were regarded to be statistically significant at a value of $P<.05$.

Results

Long-term Ischemia and Capillarization

C/F in the type II muscle fiber part of gastrocnemius muscles was lower than C/F in soleus muscles (type I muscle fibers) independent of ischemia, as shown in Table 1.

In soleus muscles of the ischemic hind limb, fiber size was decreased and C/F was increased significantly compared with control and contralateral (nonischemic) muscles (Table 1). Long-term ischemia had no effect on fiber size and C/F in soleus muscles of nonischemic hind limb and in gastrocnemius muscles of ischemic and nonischemic hind limb. Because of a slight difference between C/F in left and right control gastrocnemius muscles, C/F of the ischemic hind limb was significantly higher compared with control. The difference between C/F in ischemic (left) and nonischemic (right) muscles was increased significantly in soleus muscles (Fig 1). This increase in C/F in soleus muscles was the result of an increased total capillary number and did not depend on changes in total fiber number, which actually increased (Table 1).

Therapy

Hind limb experiments. Baseline resistances of both hind limbs in treated and untreated animals are summarized in Table 2. Only losartan increased baseline resistance in nonischemic hind limbs; none of the treatments affected baseline resistances of ischemic hind limbs. $ED_{50}$ for Ang I increased only slightly in

![Graph showing difference in capillary-to-fiber ratio (C/F) between left and right gastrocnemius (open bars) and soleus (hatched bars) muscles of control and ischemic animals. *P<.05.](http://hyper.ahajournals.org/)}
TABLE 2. Baseline Resistances of Left (Ischemic) and Right (Nonischemic) Hind Limbs in Treated and Untreated Animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose, mg/kg · h</th>
<th>n</th>
<th>R_L, mm Hg · min/mL</th>
<th>R_R, mm Hg · min/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>...</td>
<td>25</td>
<td>17.2±1.4</td>
<td>121.7±13.7</td>
</tr>
<tr>
<td>Captopril</td>
<td>0.5</td>
<td>21</td>
<td>14.9±1.7</td>
<td>185.7±36.8</td>
</tr>
<tr>
<td>Zabiciprilate</td>
<td>0.025</td>
<td>14</td>
<td>16.9±2.9</td>
<td>189.1±42.2</td>
</tr>
<tr>
<td>Losartan</td>
<td>0.625</td>
<td>7</td>
<td>17.1±2.3</td>
<td>171.6±33.6</td>
</tr>
<tr>
<td>Felodipine</td>
<td>0.42</td>
<td>8</td>
<td>10.6±1.9</td>
<td>184.5±38.4</td>
</tr>
</tbody>
</table>

% Indicates residual flow in left (ischemic) hind limb; R_L, baseline resistance in left hind limb; and R_R, baseline resistance in right hind limb. Values are mean±SEM.

*Significant difference from other treatments and untreated animals.

both hind limbs after long-term treatment with captopril and zabiciprilate (twofold to threefold, Table 3). As shown in Fig 2, the increase in ΔR_{max} in the ischemic hind limb (Ang I: ischemic hind limb, 56.6±13.4; nonischemic hind limb, 6.86±0.65 mm Hg · min/mL) was not significantly influenced by the ACE inhibitors (ischemic hind limb: captopril, 38.4±7.9; zabiciprilate, 55.9±10.2 mm Hg · min/mL). Furthermore, captopril and zabiciprilate had no significant effects on the dose-response curves of Ang II, although ΔR_{max} in the ischemic hind limb tended to increase (Table 3; ΔR_{max}: untreated, 47.9±13.7; captopril, 79.3±22.8; zabiciprilate, 93.1±35.8 mm Hg · min/mL).

After felodipine treatment, ED_{50} and ΔR_{max} for Ang II did not change significantly, although ED_{50} decreased in both hind limbs (Table 3), and ΔR_{max} increased in the ischemic hind limb compared with untreated animals (Fig 3; untreated: 47.9±13.7; felodipine: 123.1±35.1 mm Hg · min/mL).

In contrast to the ACE inhibitors, the AT_1 antagonist losartan completely inhibited both Ang I and II responses (ED_{50} for Ang I and II, >300 μg). Treatment with losartan resulted in a greater shift of the dose-response curves of Ang I and II in the hind limb experiments (Ang I, >300-fold; Ang II, >600-fold) than in vivo. ED_{50} for phenylephrine was not significantly different in untreated and losartan-treated animals (Table 3), whereas ΔR_{max} for phenylephrine normalized in the ischemic hind limb (Fig 4).

Capillarization. Zabiciprilate or captopril treatment had no influence on C/F in gastrocnemius muscles of ischemic and nonischemic hind limb. In contrast, C/F decreased in nonischemic and ischemic gastrocnemius muscles after treatment with felodipine (Table 4), and losartan decreased C/F only in ischemic gastrocnemius muscles (data not shown). The difference between ischemic (left) and nonischemic (right) gastrocnemius muscle did not change after treatment (Fig 5).

Antihypertensive therapy had no effect on C/F in nonischemic soleus muscles. In contrast, C/F in ischemic soleus muscles decreased significantly after losartan and the high dose of felodipine compared with untreated ischemic soleus muscles. The increased difference in capillarity of ischemic (left) and nonischemic (right) soleus muscles after long-term ischemia was abolished by zabiciprilate, captopril, losartan, and the high dose of felodipine. The abolishment was significant for captopril, losartan, and felodipine (Fig 5). Therapy had no influence on total fiber number of soleus muscles in both hind limbs (data not shown); therefore, the

Table 3: Half-Maximal Effective Dose for Angiotensin I, Angiotensin II, and Phenylephrine in Left (Ischemic) and Right (Nonischemic) Hind Limbs in Untreated and Treated Animals

<table>
<thead>
<tr>
<th>Group and Treatment</th>
<th>n</th>
<th>ED_{50} Left, μg</th>
<th>ED_{50} Right, μg</th>
<th>% Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>7</td>
<td>0.93±0.11</td>
<td>0.89±0.20</td>
<td>19.4±3.8</td>
</tr>
<tr>
<td>Captopril</td>
<td>9</td>
<td>2.13±0.49</td>
<td>2.19±0.85</td>
<td>17.7±3.1</td>
</tr>
<tr>
<td>Zabiciprilate</td>
<td>7</td>
<td>2.53±0.41*</td>
<td>1.52±0.31</td>
<td>17.7±4.6</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>8</td>
<td>0.56±0.13</td>
<td>0.44±0.07</td>
<td>16.8±2.6</td>
</tr>
<tr>
<td>Captopril</td>
<td>10</td>
<td>0.84±0.42</td>
<td>0.29±0.04</td>
<td>13.5±1.5</td>
</tr>
<tr>
<td>Zabiciprilate</td>
<td>7</td>
<td>0.54±0.17</td>
<td>0.37±0.06</td>
<td>16.0±3.8</td>
</tr>
<tr>
<td>Felodipine, high dose</td>
<td>8</td>
<td>0.25±0.04</td>
<td>0.27±0.05</td>
<td>10.5±0.8</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>10</td>
<td>24.7±5.6</td>
<td>34.9±7.4</td>
<td>16.1±1.2</td>
</tr>
<tr>
<td>Losartan</td>
<td>7</td>
<td>35.6±14.0</td>
<td>39.7±23.0</td>
<td>17.1±2.3</td>
</tr>
</tbody>
</table>

ED_{50} indicates half-maximal effective dose; and % Flow, residual flow in left (ischemic) hind limb. Values are mean±SEM. High felodipine dose was 0.42 mg/kg · h.

*Significant difference from untreated animals.
changes in C/F after therapy were not the result of changes in total fiber number.

Discussion

Development of collateral vessels is one of the mechanisms that restore blood supply to ischemic regions; therefore, we investigated the effect of long-term severe ischemia of skeletal muscle on capillarity in two different skeletal muscles (soleus and gastrocnemius muscle). Furthermore, the influences of long-term antihypertensive treatment by ACE inhibition, Ang II antagonism, or calcium antagonism on the previously observed hyperreactivity to Ang II and phenylephrine and on the possible changes in capillarity of ischemic skeletal muscle were investigated.

The data indicate that a unilateral partial ligation of the common iliac artery in the rat, which causes a flow reduction of more than 60%, results in an increase in capillarity predominantly in the slow oxidative (type I muscle fiber) soleus muscle. Furthermore, the renin-angiotensin system seems to be involved in the neovascularization in soleus muscle and in the previously observed nonselective hyperreactivity to vasoconstrictors.

The observed lack of change in capillarity in the type II muscle fiber part of the gastrocnemius muscle in the ischemic hind limb of the rat (see Fig 1) is comparable to earlier observations in total gastrocnemius muscle in patients with intermittent claudication and in tibial anterior muscle and extensor digitorum longus muscle (both predominantly type II muscle fibers) of rats. The observed increase in C/F in soleus muscle is in contrast to previous observations by Corsi et al in Sprague-Dawley rats. In their protocol they failed to observe an increase in C/F; however, their study lasted only 8 days compared with the 4 weeks in the present study. Our observations cannot be explained by a decrease in total fiber number because total fiber number was increased. This implies that the increase in C/F is due to the development of new vessels. The increase in muscle fibers has been previously demonstrated in damaged muscles after excessive training, injury, and ischemia of skeletal muscle and may compensate for a diminished force development in ischemic muscles. The greater sensitivity of soleus muscle for ischemic conditions has been described previously and may depend on the greater oxidative metabolic demand of the oxidative soleus muscle compared with the mixed glycolytic and oxidative glycolytic gastrocnemius muscle. Besides the development of new vessels, the observed reduction in muscle fiber size in soleus muscle also may restore the balance between blood flow supply and demand.

In hind limb perfusion experiments, none of the antihypertensive agents used had any significant influence on the resistance.

\[ \text{FIG 3. Line graph shows dose-response curves of angiotensin II in untreated animals (v, ischemic hind limb; n, nonischemic hind limb) and animals treated with calcium antagonist felodipine (0.42 mg/kg · h; v, ischemic hind limb; n, nonischemic hind limb).} \]

\[ \text{FIG 4. Line graph shows dose-response curves of phenylephrine in untreated animals (v, ischemic hind limb; n, nonischemic hind limb) and animals treated with angiotensin subtype 1 receptor antagonist losartan (0.625 mg/kg · h; v, ischemic hind limb; n, nonischemic hind limb).} \]
ence on baseline resistances of both hind limbs, except on the resistance of the nonischemic hind limb in losartan-treated animals (see Table 2). As the vascular bed in the perfused hind limbs has been suggested to be almost maximally dilated, the lack of significant effects on baseline resistances suggests no major influence on maximal flow capacity of the hind limbs. Furthermore, dilatation of collateral vessels as proposed in patients with intermittent claudication is not likely to be noticed in the present hind limb perfusion model, as the main collateral vessels are supposed to be located proximal to the position of the flow probes.

Although the increase in ED_{50} of Ang I was approximately 10-fold in vivo, both ACE inhibitors increased the ED_{50} of Ang I only slightly (twofold to threefold) in the hind limb perfusion experiments (see Table 3). A possible explanation for this discrepancy may be a washout of the ACE inhibitor during hind limb perfusion. Although we cannot exclude this explanation, no differences in inhibition of a high dose of Ang I at the start or end of the experiments were observed in pilot experiments. An alternative explanation may be a greater contribution of other tissue Ang II–generating enzymes in the conversion of Ang I into Ang II during long-term ACE inhibition. Possible candidates are a chymostatin-sensitive Ang II generating enzyme, tryptase, or kallikrein. An important role of these enzymes in the generation of Ang II in ischemic situations has been demonstrated in dogs with myocardial infarction and patients with congestive heart failure. These enzymes may also be responsible for the lack of effect of ACE inhibitors on the hyperreactivity to Ang I in the ischemic hind limb. This suggests no involvement of changes in tissue ACE activity in the observed hyperreactivity to Ang I.

In contrast to the ACE inhibitors, the AT(I) antagonist losartan completely inhibited the responses of Ang I and II in both perfused hind limbs (ED_{50}>300 μg). In fact, evaluation of Ang I and II responses after losartan treatment was not possible. The inhibition of Ang II responses was much more pronounced in the hind limb experiments than in vivo. This may be due to compensatory sensory reflex mechanisms present in vivo, as previously described after occlusion and compression of the hind limbs. Losartan treatment also normalized reactivity to phenylephrine in the ischemic vascular bed (see Fig 4). This cannot be explained by the systemic blood pressure reduction after long-term treatment with losartan, because the ischemic situation probably increases by an additional blood pressure reduction; moreover, a comparable antihypertensive dose of felodipine did not reduce hyperreactivity to Ang II in the ischemic hind limb. The normalized reactivity to phenylephrine after losartan treatment suggests involvement of the AT(I) receptor in the nonselective hyperreactivity during long-term ischemia.

Neovascularization in ischemic soleus muscle was blocked by ACE inhibitors, whereas a comparable (moderate) antihypertensive dose of the calcium antagonist felodipine had no effect (see Fig 5). Thus, neither calcium channel blockade nor slight blood pressure reduction is responsible for the prevention of neovascularization. Greater blood pressure reductions by losartan and felodipine also result in a prevention of neovascularization. These effects suggest that the renin-angiotensin system is involved in neovascularization and that blood pressure reductions become more important at higher doses of the antihypertensive drugs. The lack of increase in capillarity in ischemic soleus muscle after antihypertensive therapy suggests a lack of compensation of the diminished flow in the muscle and implies a deleterious long-term effect on the muscle. This is supported by the occurrence of signs of atrophy and inflammation in the region of the vastus lateralis muscle in the ischemic hind limb after treatments with all antihypertensive agents, whereas no such signs were observed in untreated ischemic animals.

The present experiments suggest involvement of the renin-angiotensin system in both nonselective hyperreactivity to vasoconstrictors and vessel growth in ischemic muscle.
emic hind limbs. This may be due to an increase in local Ang II synthesis without an increase in tissue ACE activity (see above), in AT1 receptor number, or in activation of the second messenger system of the AT1 receptor. Because the second messenger systems of the AT1 receptor and the α1-receptor are the same, a more likely explanation for the hyperreactivity to both Ang II and phenylephrine may be changes in the stimulus-contraction coupling system, resulting in an increase in intracellular calcium release. This would also explain the hyperreactivity to serotonin and a thromboxane A2 analogue observed by Verheyen et al.7,25 in a comparable ischemia model.

The observed inhibition of neovascularization in soleus muscle suggests that ACE inhibition and AT1 receptor antagonism have no long-term beneficial effect in ischemia of skeletal muscle. This is in contrast to the beneficial effects of captopril22,26 and lisinopril27 on maximal and pain-free walking distance in patients with intermittent claudication and hypertension, which is probably due to a lack of reflex vasoconstriction, as observed with the use of other antihypertensive drugs, or a preferential vasodilatation of collateral vessels by ACE inhibitors.21,22 The discrepancy between captopril treatment in patients with intermittent claudication and treatment with captopril and zopiciclarin in the present animal model may be related to the onset of therapy. In patients, captopril treatment usually starts after clinical manifestation of the disease, so capillary growth has already been initiated. In contrast, in the present experiments, treatment was started immediately after induction of ischemia, resulting in a prevention of development of capillary growth. Studies on optimal timing of the start of ACE inhibitor therapy are needed to resolve this question.

In conclusion, long-term ischemia causes an increase in capillarity, predominantly in the soleus muscle, which in spontaneously hypertensive rats is composed mainly of type I fibers. This vessel growth can be prevented by ACE inhibition and high blood pressure reduction (high doses of felodipine and losartan). Furthermore, the observed inhibition of neovascularization in soleus muscle suggests that ACE inhibition and AT1 receptor, and hyperperfusion play important roles in the adaptation mechanisms after ischemia of skeletal muscle.

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References


