Matrix metalloproteinase activity is required for activity-induced angiogenesis in rat skeletal muscle

T. L. HAAS,1 M. MILKIEWICZ,2 S. J. DAVIS,1 A. L. ZHOU,2 S. EGGINTON,2 M. D. BROWN,2 J. A. MADRI,1 AND O. HUDLICKA2
1Department of Pathology, Yale University, New Haven, Connecticut 06515; and 2Department of Physiology, University of Birmingham, Birmingham B15 2TT, United Kingdom

Received 22 September 1999; accepted in final form 17 March 2000

Matrix metalloproteinase activity is required for activity-induced angiogenesis in rat skeletal muscle. Am J Physiol Heart Circ Physiol 279: H1540–H1547, 2000.—Proteolysis of the capillary basement membrane is a hallmark of inflammation-mediated angiogenesis, but it is undetermined whether proteolysis plays a critical role in the process of activity-induced angiogenesis. Matrix metalloproteinases (MMPs) constitute the major class of proteases responsible for degradation of basement membrane proteins. We observed significant elevations of mRNA and protein levels of both MMP-2 and membrane type 1 (MT1)-MMP (2.9 ± 0.7- and 1.5 ± 0.1-fold above control, respectively) after 3 days of chronic electrical stimulation of rat skeletal muscle. Inhibition of MMP activity via the inhibitor GM-6001 prevented the growth of new capillaries as assessed by the capillary-to-fiber ratio (1.34 ± 0.08 in GM-6001-treated muscles compared with 1.69 ± 0.03 in control 7-day-stimulated muscles). This inhibition correlated with a significant reduction in the number of capillaries with observable breaks in the basement membrane, as assessed by electron microscopy (0.27 ± 0.27% in GM-6001-treated muscles compared with 3.72 ± 0.65% in control stimulated muscles). Proliferation of capillary-associated cells was significantly elevated by 2 days and remained elevated throughout 14 days of stimulation. Capillary-associated cell proliferation during muscle stimulation was not affected by MMP inhibition (80.3 ± 9.3 nuclei in control and 63.5 ± 8.5 nuclei in GM-6001-treated animals). We conclude that MMP proteolysis of capillary basement membrane proteins is a critical component of physiological angiogenesis, and we postulate that capillary-associated proliferation precedes and occurs independently of endothelial cell sprout formation.

Activity-induced angiogenesis occurs in adult skeletal and cardiac muscles, and this response is essential for maintaining adequate tissue function in the face of chronic increases in metabolic demand (18, 31). The predominant involvement of hemodynamic and mechanical stimuli, and the lack of evidence for a significant inflammatory response (18, 23), distinguish activity-induced angiogenesis from angiogenesis occurring during tumor growth or wound healing. Chronic indirect electrical stimulation of the muscle provides a good model of activity-induced angiogenesis because hemodynamic and mechanical stimuli are thought to provide the major angiogenic stimuli, whereas an inflammatory component is minimal (10, 27). Evidence to date suggests that not only the initial stimuli involved but also the course of events that occurs in activity-induced angiogenesis may differ significantly from the course of inflammatory-mediated angiogenesis. First, in skeletal muscles of which activity is increased by chronic electrical stimulation, endothelial cell proliferation occurs at a very early stage (17) that precedes detectable sprout formation (16) rather than being associated with sprout elongation. Second, proteolysis of the basement membrane, detected by transmission electron microscopy as breaks in the basal lamina, has been observed very rarely (16), whereas, during tumor growth and wound healing, venules and capillaries often are completely devoid of electron-dense basal lamina (9, 15, 30, 34). These observations raise key questions as to whether proteolysis is essential in the process of physiological angiogenesis and whether,
this case, endothelial cell proliferation precedes or follows proteolysis and/or sprout formation.

Proteolysis of basement membrane proteins is most frequently performed by matrix metalloproteinases (MMPs), a diverse family of proteases each with a specificity for multiple matrix proteins (3). MMP activity is known to be involved in tumor and inflammation-mediated angiogenesis (11, 31), but it is not known whether MMP production and/or activity is modulated during activity-induced angiogenesis. Two particular MMPs, MMP-2 (gelatinase A) and membrane type 1 (MT1)-MMP, were shown to play an essential role in an endothelial cell culture model of extracellular matrix-induced angiogenesis (13) and have been implicated in tumor angiogenesis (20, 33, 35). Coordinated expression of the two MMPs is frequently observed, and MT1-MMP is known to activate MMP-2 (28, 33). On the basis of these findings, we used the chronic stimulation model to address the questions as to whether MMP-2 and MT1-MMP are upregulated during activity-induced angiogenesis and whether MMP activity is a requisite for this type of adaptive angiogenesis. We found significantly increased production of both MMPs as a result of chronic muscle stimulation and that chronic inhibition of MMP activity prevented the growth of new capillaries. This study defines a critical role for MMP activity in the activity-induced formation of new capillaries and suggests that endothelial cell proliferation occurs independently of proteolysis and sprout formation.

**METHODS**

**Animal model.** Rat hindlimb skeletal muscles (ankle flexors: the tibialis anterior (TA); extensor digitorum longus (EDL); and extensor hallucis proprius (EHP) muscles) were subjected to increased activity induced by chronic electrical stimulation as previously described (27). Briefly, animals were implanted unilaterally with electrodes in the vicinity of the common peroneal nerve, providing stimulation of the TA, EDL, and EHP muscles via the peroneal nerve. The implanted electrodes never came in direct contact with the muscle groups studied and thus did not cause trauma to the analyzed muscles. The implantation was performed under fluothane anesthesia in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986. Animals received analgesic (buprenorphine, 2.5 mg in 0.1 ml) twice daily for the first 2 days after the operation. Electrical stimulation, 8 h/day for up to 14 days, commenced for the first 2 days after the operation. Animals received daily intraperitoneal injections of 150 μl DMSO. Control unstimulated rats received daily doses of DMSO (n = 4) or GM-6001 (n = 4) for 7 days at the same doses used for stimulated rats. Neither treatment regimen exerted any apparently adverse effects on gross body weight, wet muscle mass, or muscle ultrastructure.

**Histology and immunohistochemistry.** Serial cryostat sections (8 μm) were stained for alkaline phosphatase using an indoxyltetrazolium method to depict all anatomically present capillaries (41) and for proliferating cell nuclear antigen (PCNA; clone pc10, DAKO) using standard immunohistochemical techniques. The counting of capillaries and PCNA-positive nuclei was performed using an Olympus microscope equipped with a drawing arm. Two to four fields (0.20 mm² each at ×200 magnification) were used for counting capillaries in each muscle, and four fields (0.05 mm² at ×400) were used for counting PCNA-positive nuclei in each muscle. Three to four muscles were analyzed for each intervention. The same fields identified in the alkaline phosphatase-stained sections were used to assess whether the position of PCNA-positive nuclei colocalized with the alkaline phosphatase staining and, hence, the site of a capillary. This enabled calculation of the density of proliferating nuclei linked with capillaries (PCNAcap/mm²) or other interstitially located nuclei not associated with capillaries (PCNAint/mm²) (measured in PCNAcap/mm² or PCNAint/mm², respectively). The number of muscle fibers also was counted, and capillary supply was expressed as the capillary-to-fiber ratio (CFP).

**MMP analysis.** Frozen EDL muscles were powdered and then homogenized in TRIzol (GIBCO BRL), and RNA was isolated. Total cellular RNA (20 μg) from each sample was size fractionated and Northern blotted as previously described using cDNA probes specific for rat MMP-2 (from D. H1541
Proteins were isolated by homogenization of the powdered EDL muscle in Triton X-100-containing lysis buffer. The protein concentration of the homogenate supernates was quantitated by bicinchoninic acid solution (Pierce). Western blotting using anti-MMP-2 and anti-MT1-MMP (Chemicon) antibodies was performed on 20\(\mu\)g of each sample, using methods previously described (13). Gelatin zymography analysis was performed on 15\(\mu\)g of each sample as previously described (13). Films and zymographic gels were scanned, and band densitometry performed using Biomax software. Northern blots were normalized to 28S rRNA, and Western blots were normalized to vimentin to control for loading. Immunofluorescence staining was performed on 10-\(\mu\)m-thick frozen sections of EDL and TA muscles using polyclonal antibodies against MMP-2 (Chemicon) and MT1-MMP (Chemicon; provided by S. Weiss, Univ. of MI, Ann Arbor, MI), detected using Texas red secondary antibodies, and viewed with a Zeiss microscope using a \(\times40\) objective. Images were captured using a SPOT charge-coupled device camera (Diagnostic Instruments) and Adobe Photoshop software. Compilations were made using Freehand software.

Statistical analysis. Data are presented as means \(\pm\) SE, with samples from a minimum of three rats included in each data point. Capillary density and proliferating cell data were analyzed by factorial ANOVA, with significance ascribed as \(P < 0.05\). MMP mRNA and protein levels were analyzed using Student’s unpaired \(t\)-test, assuming equal variance among samples, with significance also ascribed as \(P < 0.05\).

### Table 1. Proliferation precedes increase in capillary supply during chronic stimulation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2</th>
<th>3</th>
<th>7</th>
<th>8</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/F ratio</td>
<td>1.29 ± 0.04</td>
<td>1.43 ± 0.09</td>
<td>1.43 ± 0.06</td>
<td>1.67 ± 0.05*</td>
<td>1.86 ± 0.11*</td>
<td>2.07 ± 0.12*</td>
</tr>
<tr>
<td>PCNA(_{\text{cap}})</td>
<td>5.1 ± 3.4</td>
<td>107.8 ± 4.4*</td>
<td>65.5 ± 20*</td>
<td>61.6 ± 7.7*</td>
<td>38.5 ± 3.8*</td>
<td>44.0 ± 4.2*</td>
</tr>
<tr>
<td>PCNA(_{\text{int}})</td>
<td>10.3 ± 1.3</td>
<td>46.2 ± 2.2*</td>
<td>46.6 ± 19*</td>
<td>60.4 ± 5.1*</td>
<td>19.5 ± 10</td>
<td>14.0 ± 3.4</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE and are expressed as number of positive cells per millimeter squared; \(n = 3\) rats. C/F ratio, capillary-to-fiber ratio; PCNA\(_{\text{cap}}\) and PCNA\(_{\text{int}}\), proliferating cell nuclear antigen (PCNA) associated with nuclei linked to capillaries and interstitially located nuclei not linked to capillaries, respectively. *\(P < 0.05\) compared with control.

**Fig. 1.** Basement membrane disturbances in chronically stimulated muscle. A: electron micrograph of a capillary from a control unstimulated extensor hallucis proprius (EHP) muscle showing thin endothelial cell layer with smooth luminal and abluminal surfaces surrounded by an intact basement membrane (open arrows) that appears as a thin electron-dense band encircling the capillary. The cellular process of a pericyte (*) is apparent on the inside of the capillary basement membrane (bar = 0.5 \(\mu\)m). B: capillary from 3-day-stimulated EHP muscle showing abluminal cytoplasmic projection into the interstitium (sprout). The basement membrane is intact around the capillary (open arrow) but absent at the tip of the sprout (filled arrows). The adjacent cell (*) likely is a fibroblast (bar = 0.2 \(\mu\)m).
RESULTS

Chronic electrical stimulation of EDL muscle induced a significant 30% increase in the C/F ratio after 7 days of stimulation, and this was increased further at the 14-day-stimulation time point (Table 1), which was similar to previous findings (27). The increased C/F ratio strongly indicates a growth of new vessels because the methodology used identifies all anatomically present capillaries. The number of proliferating capillary-associated nuclei was increased 20-fold in muscles that had been stimulated for only 2 days, and, although this number gradually decreased, it remained significantly elevated at 14 days of stimulation (Table 1). In contrast, the number of proliferating interstitially located nuclei (those not associated with capillaries, most likely fibroblasts) was increased four- and sixfold at 2 and 7 days of stimulation, respectively, but returned to control levels by 14 days of stimulation.

Basement membrane disruptions were detected by electron microscopy as breaks in the normally contiguous basement membrane surrounding the capillary endothelium. In control unstimulated muscles, there were no capillaries with a sprouting endothelium or a loss of basement membrane (Fig. 1A). In 3- and 7-day-stimulated muscles, sprouting endothelial cells were apparent (Fig. 1B). Basement membrane disruption did not occur around the parent capillaries except in locations showing signs of budding (irregularity of the endothelial cell abluminal surface and microfilament reorganization). After 3 days of stimulation, 1.2 ± 0.7% of all capillaries examined exhibited budding sprouts with a disrupted basement membrane. After 7 days of stimulation, 2.5 ± 1.0% (P < 0.05 compared with control) of all capillaries had budding sprouts with a detectable loss of basement membrane investiture. Examination of muscle sections by both light and electron microscopy showed no evidence for accumulation of inflammatory cells surrounding the capillaries or in the interstitial spaces of stimulated muscles, nor was there morphological evidence of muscle damage, a finding consistent with previous observations using the same experimental system (5, 16, 27).

MMP-2 mRNA was detectable in control and contralateral unstimulated EDL muscles but was increased significantly by 3 days of stimulation. Levels peaked at 3 days of stimulation, remained elevated at 8 days of stimulation, and were not significantly higher than control levels by 14 days of stimulation (Fig. 2A and Table 2). The MMP-2 protein level, as assessed by Western blotting, increased slightly over the time course of stimulation, and a greater proportion of MMP-2 was detectable in the 62-kDa (active) form (Fig. 2B). MT1-MMP mRNA, also detectable in control and unstimulated muscles, was increased significantly after 4 days of stimulation, with levels continuing to increase until 7 days of stimulation, and remaining elevated after 14 days of stimulation (Fig. 2A and Table 2). The MT1-MMP protein levels increased over the time course of stimulation and also remained elevated at 14 days of stimulation (Fig. 2B). Immunofluorescent staining localized the MMP-2 and MT1-MMP proteins to capillaries as well as to larger arterioles/venules. Although detectable in control unstimulated muscles (Fig. 2, C and E), higher intensities of both

![Fig. 2. Matrix metalloproteinase (MMP)-2 and membrane type 1 (MT1)-MMP mRNA and protein increase with chronic stimulation.](http://ajpheart.physiology.org/)

**A**. Representative Northern blots show a time course-related increase for MMP-2 and MT1-MMP mRNA in stimulated extensor digitorum longus (EDL) muscle, with minimal levels detectable in unstimulated control (day 0) or contralateral muscles. Detection of the ribosomal 28S RNA [by radioactive probe (left) and by ethidium bromide staining (right)] was used to normalize band intensities according to loading. **B**. Western blotting detects increases in MMP-2 and MT1-MMP protein over a time course of EDL stimulation. A longer exposure of the MMP-2 blot shows that the amount of active MMP-2 (62 kDa) also increases over time of stimulation. Vimentin staining was used to demonstrate equivalent protein loading in each lane. C-F: Immunofluorescence of MMP-2 and MT1-MMP proteins to capillaries as well as to larger arterioles/venules. Although detectable in control unstimulated muscles (C and E), higher intensities of both
Gelatin zymography was used to quantitate the levels of pro- and active MMP-2 protein. The total MMP-2 protein levels increased 1.5-fold over the duration of stimulation, and, as was observed in Western blots, the percentage of active MMP-2 also increased twofold (Fig. 3 and Table 2). Stimulation also significantly increased the production of a 100-kDa MMP (Fig. 3 and Table 2). Stimulation also significantly increases in MMP-2 protein, both latent (72 kDa) and active (62 kDa) forms, detectable as bands of clearing within the stained gel. A high-molecular-weight (MW) MMP-2 (~100 kDa) also increases during EDL stimulation. MW standards (Std) are indicated on the left side of the gel.

The broad-specificity MMP inhibitor GM-6001 inhibited new capillary formation. In GM-6001-treated 7-day-stimulated animals, the C/F ratio in EDL muscle was not increased significantly above the C/F ratio for vehicle-treated unstimulated muscles (1.34 ± 0.08 and 1.24 ± 0.06, respectively) (Fig. 4). In contrast, EDL muscle from 7-day-stimulated rats treated with vehicle showed the expected significant increase in the C/F ratio (1.69 ± 0.03). By electron microscopy, extending capillary sprouts were not detectable in EHP muscles of GM-6001-treated rats. Furthermore, the capillaries in GM-6001-treated muscles had significantly fewer focal regions (budding sprouts) with a disturbed basement membrane compared with capillaries in DMSO-treated muscles (0.27 ± 0.27 vs. 3.72 ± 0.65%, respectively; P < 0.05, n = 3 rats). Capillaries in GM-6001-treated muscles also appeared to be invested with a thicker than normal basement membrane (Fig. 5).

Endothelial cell activation, marked by cell thickening and the increased appearance of synthetic organelles, is thought to occur early in the angiogenic response. There was no observed difference between DMSO- and GM-6001-treated animals in the number of visibly activated endothelial cells, implying that the responsiveness of the endothelium to the angiogenic stimulus was not altered by inhibition of MMP activity. Similarly, despite the lack of new sprout formation associated with GM-6001 treatment, chronic stimulation induced proliferation of both capillary-associated and interstitial cells, with no significant differences between GM-6001- and vehicle-treated rats (Table 3). Thus MMP inhibition blocked stimulation-induced increases in the C/F ratio but did not exert an inhibitory effect on cellular activation and proliferation.

![Fig. 3. Increased production of multiple MMPs is observable by gelatin zymography. The time course of stimulation results in increases in MMP-2 protein, both latent (72 kDa) and active (62 kDa) forms, detectable as bands of clearing within the stained gel. A high-molecular-weight (MW) MMP-2 (~100 kDa) also increases during EDL stimulation. MW standards (Std) are indicated on the left side of the gel.](image)

![Fig. 4. Mean capillary-to-fiber (C/F) ratio does not increase in EDL muscle treated with MMP inhibitor GM-6001. Comparisons were made of C/F ratio in EDL from stimulated and nonstimulated rats treated with DMSO (vehicle) or GM-6001. C/F ratio is expressed as means ± SE (n = 4 rats). *Only C/F ratio significantly different (P < 0.05) from nonstimulated, DMSO-treated animals.](image)
DISCUSSION

Chronic electrical stimulation of hindlimb muscles induces a robust angiogenic response detectable as an increase in the C/F ratio. We present the novel findings that the process of activity-stimulated angiogenesis in skeletal muscle involved an increased production of MMP-2 and MT1-MMP and, furthermore, that MMP activity was a requisite for new capillary formation.

Notably, capillary-associated cell proliferation preceded and occurred independently of MMP activity and new capillary growth.

Although production of proteolytic enzymes is well known to be associated with inflammatory responses, the involvement and the requirement for proteolysis of basement membrane components during activity-induced angiogenesis previously had not been tested. We first demonstrated that indications of the regional absence of the basement membrane, possibly due to proteolysis, are detectable at sites of budding endothelial cells but not observed around the parent capillaries (Fig. 1). A similar observation was reported, but not quantified, in an earlier study (16). Notably, we found that inhibition of MMP activity through use of the broad-spectrum MMP inhibitor GM-6001 prevented the growth of capillaries, as assessed by the C/F ratio. The failure of capillary growth in these muscles may be attributable to the lack of visible sprout formation (Fig. 5), implying that proteolysis of the basement membrane by MMPs is a critical element in sprout formation.

The significant increases in MMP-2 mRNA, MT1-MMP mRNA, and protein detectable after 4 days of stimulation are consistent with these particular proteases playing a major role in the process of angiogenesis. The involvement of MMP-2 and MT1-MMP has already been demonstrated previously (13, 20) using cell culture and tumor growth models but has not been shown for activity-induced angiogenesis. It is generally considered that production of MT1-MMP is required for activation of MMP-2 (28), and, in our study, we observed a correlation between increased levels of MT1-MMP and increased processing of MMP-2 to the active form (Fig. 2 and Table 2). Although it is clear from our data that MMP-2 and MT1-MMP are produced, we do not yet know whether the distribution of these MMPs correlates with the electron microscope-visualized pattern of basement membrane disruption. However, proteolysis and the localization of the membrane-bound MT1-MMP and MMP-2 (28) to the extending tips of migrating cells have been noted previously (26, 29), indicating that proteolysis can be finely localized to subregions of the cellular membrane. Further studies may provide evidence as to whether the tight control of proteolytic enzymes is required for the appropriate organization of capillary networks.

The MMP-2 and MT1-MMP proteins were detected in capillaries as well as in larger blood vessels using immunofluorescence, but the cell type(s) producing these proteases remains to be determined. Both pro-

Table 3. Proliferation is not affected by inhibition of MMP activity

<table>
<thead>
<tr>
<th></th>
<th>Unstimulated</th>
<th>7-Day Stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMSO GM-6001</td>
<td>DMSO GM-6001</td>
</tr>
<tr>
<td>PCNA&lt;sub&gt;cap&lt;/sub&gt;</td>
<td>16.4 ± 1.85</td>
<td>80.3 ± 9.3</td>
</tr>
<tr>
<td>PCNA&lt;sub&gt;int&lt;/sub&gt;</td>
<td>7.7 ± 0</td>
<td>44.9 ± 5.0</td>
</tr>
</tbody>
</table>

All values expressed as means ± SE; n = 4 rats. *Values not significantly different from DMSO-treated animals.
teases are produced not only by endothelial cells but also by vascular smooth muscle cells, fibroblasts, and (perhaps) pericytes, and it is quite possible that during chronic stimulation more than one of these cell types participates in MMP production. It also is possible that activity of MMPs other than MMP-2 and MT1-MMP, such as the 100-kDa MMP protein detected by zymography, may be required for the process of sprout formation. Similarly, it has been reported (6) that endothelial cell-stimulating angiogenesis factor (ESAF), which can activate prostromelysin-1 and procollagenase (25), is elevated during chronic electrical stimulation and thus may play a role in the initiation of proteolysis. Because there currently are no inhibitors specific for particular MMPs, it is not yet possible to test the roles of individual MMPs using this model.

Early increases in proliferation were seen both for capillary-associated and interstitial nuclei, as has been noted previously using 5-bromo-2'-deoxyuridine (BrdU) labeling (17). Interestingly, PCNAcap staining remained elevated during the 14-day stimulation period (suggesting early as well as continuing phases of proliferation), whereas PCNAint increased rapidly but declined to baseline by 14 days of stimulation (suggesting only an early proliferative response in the interstitial cells). PCNAcap may represent not only endothelial cell proliferation but may also include those fibroblasts or pericytes in close association with the capillaries; a previous study (39), utilizing in vivo BrdU labeling coupled with electron micrographic analysis, reported equal numbers of BrdU-positive endothelial cells and capillary-associated fibroblasts after 2 days of stimulation but almost entirely endothelial cell labeling at day 7. The significant finding of the present study is that the MMP inhibitor GM-6001 did not inhibit cell proliferation (either in capillary-associated or interstitial cells), arguing that MMP activity is not required for, but rather occurs downstream of, cellular activation and proliferation processes. This finding is consistent with observations made by a previous study (2) that reported GM-6001 successfully inhibited the migration of vascular smooth muscle cells in vivo without affecting the proliferation rate. Thus, on the basis of the early increases in capillary-associated cell proliferation (Table 1) and because GM-6001 successfully inhibited sprout formation without inhibiting cell proliferation, we postulate that endothelial (and possibly fibroblast or pericyte) cell activation and proliferation temporally precede sprout formation in this model of activity-induced angiogenesis.

Increased blood flow to the contracting muscle increases shear stress and transmural wall pressure, and the contracting myocytes themselves cause stretch and deformation of the interstitial proteins and the neighboring capillaries. These stimuli are thought to play major roles in the initiation of angiogenesis during chronic muscle activity. The intracellular signaling events coupling these stimuli with endothelial cell activation in vivo have not been defined, although several mediators are likely to be involved. The production of vascular endothelial growth factor, stimulated either by hypoxia or by mechanical stretch (4, 24, 32), has been correlated with the initiation of angiogenesis in coronary and skeletal muscles (37, 38) and is a strong candidate for signaling the initiation of endothelial cell proliferation. Hemodynamic and mechanical forces also are known to stimulate the production and release of nitric oxide (7), and the production of nitric oxide has been shown to play an important role in activity-induced angiogenesis (19), indicating a potential involvement of this signaling molecule as well.

Our report documents a time course of early increases in proliferation with a temporally delayed increase in production of MMP-2 and MT1-MMP. This suggests that, although proliferation likely is mediated by immediate-early growth response genes (21), enhanced production of MMPs may lie downstream of the initial signaling events. Endothelial cell transcriptional upregulation of MT1-MMP in response to changes in the extracellular matrix environment is mediated by the transcription factor egr-1 (14). Egr-1 is known to be upregulated in endothelial cells as a result of both fluid shear stress and mechanical stretch (22), thus implicating this transcription factor as a likely candidate for inducing the production of MT1-MMP during activity-induced angiogenesis in vivo.

This study points to several potential differences between the processes of activity-induced angiogenesis and inflammation-mediated angiogenesis. Electron microscopic observations indicate that proteolysis during adaptive angiogenesis appears localized to the sprouting tips of endothelial cells. In contrast, proteolysis is reported to occur around tumor capillaries and the sprouts of tumor capillaries, both of which frequently appear entirely devoid of the electron-dense basement membrane (30). Proliferation has been noted to occur only in sprouts during inflammation-mediated angiogenesis, whereas we have seen clear evidence of capillary-associated proliferation before the formation of sprouts. Indeed, this proliferation is maintained in the absence of sprout formation. On the basis of these differences, it is likely that the intracellular signaling mechanisms driving angiogenesis also differ, perhaps as a result of disparate initiating stimuli. Further analysis of the signaling pathways responsible for initiating the responses of proliferation and proteolysis during activity-induced angiogenesis will be useful in understanding the physiological maintenance of the capillary number. Identification of the signaling pathways used for the hemodynamic or mechanical stimulation of angiogenesis may have a therapeutic use in some pathologies, when ischemia alone fails to elicit compensatory capillary growth.

This work was supported by National Heart, Lung, and Blood Institute Grant F32-HL-09983 and by a Research Career Enhancement Award from the American Physiological Society (to T. L. Haas); National Heart, Lung, and Blood Institute Grant R01-HL-51018 (to J. A. Madri); and a donation from Dr. J. Barclay (to O. Hudlicka).
REFERENCES


