Time course of vasodilatory responses in skeletal muscle arterioles: role in hyperemia at onset of exercise

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Wunsch, Stacy A., Judy Muller-Delp, and Michael D. Delp. Time course of vasodilatory responses in skeletal muscle arterioles: role in hyperemia at onset of exercise. Am J Physiol Heart Circ Physiol 279: H1715–H1723, 2000.—At the onset of dynamic exercise, muscle blood flow increases within 1–2 s. It has been postulated that local vasodilatory agents produced by the vascular endothelium or the muscle itself contribute to this response. We hypothesized that only vasodilators that act directly on the vascular smooth muscle could produce vasodilation of skeletal muscle arterioles in <2 s. To test this hypothesis, we determined the time course of the vasodilatory response of isolated skeletal muscle arterioles to direct application of potassium chloride, adenosine, acetylcholine, and sodium nitroprusside. Soleus and gastrocnemius muscles were dissected from the hindlimbs of male Sprague-Dawley rats. First-order arterioles (100–200 μm) were isolated, cannulated on micropipettes, and pressurized to 60 cmH2O in an organ bath. Vasodilatory agents were added directly to the bath, and diameter responses of the arterioles were recorded in real time on a videotape recorder. Frame-by-frame analysis of the diameter responses indicated that none of the vasodilator agents tested produced significant diameter increases in <4 s in either soleus or gastrocnemius muscle arterioles. These results indicate that, although these local vasodilators produce significant vasodilation of skeletal muscle resistance arterioles, these responses are not rapid enough (within 1–2 s) to contribute to the initiation of the exercise hyperemic response at the onset of dynamic exercise.

Exercise hyperemia; acetylcholine; potassium chloride; adenosine; sodium nitroprusside

SKELETAL MUSCLE BLOOD FLOW increases dramatically at the initiation of dynamic exercise to meet elevated metabolic demands of the tissue (1, 2, 4, 7, 14, 34, 38, 39, 41, 43, 44). Despite a long history of investigation of this phenomenon, the physiological mechanisms responsible for this marked augmentation in flow remain unclear. In both humans and animals, the rise in muscle blood flow occurs in <2 s after exercise is initiated and typically peaks in <30 s (9). Therefore, any control mechanism involved in the initiation of skeletal muscle exercise hyperemia must be present and operative during this initial 1- to 2-s period.

The initial hyperemia at the onset of exercise could result from neurohumoral factors, locally released vasodilator substances (e.g., metabolites), or mechanical factors (e.g., the muscle pump) (9, 27, 40). A neural hypothesis is attractive because the rapidity of the initial hyperemia; however, current evidence does not support a neural mechanism. For example, neither lumbar sympathectomy (32, 36), autonomic blockade (6, 38), postural manipulation of sympathetic nerve activity (22), muscarinic receptor blockade (3, 7), nor β-adrenergic receptor blockade (7) have an effect on the hyperemic response at the initiation of exercise.

Evidence regarding a locally released vasodilator mechanism is conflicting. Sheriff et al. (38) demonstrated that the increase in total vascular conductance during the first 10 s of treadmill running was independent of exercise intensity, suggesting that no link exists between increased metabolic demand and elevated vascular conductance. Rådegran and Saltin (34) reported that with a single passive leg movement, blood flow increased during muscle relaxation, indicating that the initial hyperemia is a purely mechanical phenomenon. In contrast, the experiments of Tschakovsky et al. (43) indicate that mechanical compression cannot fully account for the hyperemic response. These authors demonstrated that the hyperemic response to a single muscular contraction of the hand was 60% greater than the hyperemic response initiated by mechanical compression in the absence of muscular contraction. They concluded that metabolites or other local vasodilators must also contribute to the rapid increase in muscle blood flow at the onset of exercise. Similar conclusions (30) have been reached by using 1-s tetanic stimulation protocols in anesthetized dogs.

To mediate the hyperemia, locally released vasodilator substances must be released, diffuse to the vascular smooth muscle, and accumulate to a concentration that results in smooth muscle relaxation and vasodilation (17). All of these events must occur at least as rapidly as the onset of the hyperemic response for a particular vasodilator to contribute to the initial increase in blood flow. In the present study, we examined one component.

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of this process: the time required for resistance arterioles to dilate. In addition, because the time course of this response may differ in vessels isolated from muscles of different fiber type, we tested responses in arterioles from the soleus muscle, which is composed predominantly of slow-twitch type I fibers, and from the superficial portion of the gastrocnemius muscle, which is composed primarily of fast-twitch type II fibers (12). The main objective of this study was to use an in vitro model to determine whether the time course of vasodilation produced by candidate vasodilators follows a time course that is sufficiently rapid to potentially contribute to the initial hyperemia in skeletal muscle. We evaluated the time course of vasodilation produced by the following: 1) potassium ions, 2) adenosine, 3) acetylcholine (ACh), and 4) the nitric oxide donor sodium nitroprusside (SNP). We hypothesized that only vasodilators acting directly on smooth muscle to cause relaxation (potassium, adenosine, and SNP) would initiate vasodilation of resistance arterioles in a 1- to 2-s time frame.

METHODS

Animals. All procedures performed in this study were approved by the Texas A&M University Institutional Animal Care and Use Committee. All methods conformed to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

Eleven 6- to 8-mo-old male Sprague-Dawley rats, weighing an average of 331 ± 15 g, were obtained from Harlan (Houston, TX). The rats were housed in a temperature-controlled (23 ± 2°C) room with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum.

Microvessel preparation. The rats were anesthetized with pentobarbital sodium (60 mg/kg). The gastrocnemius-plantaris-soleus muscle group from each hindlimb was carefully dissected free and placed in a cold (4°C), filtered physiological saline solution (PSS) containing (in mM) 145 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.17 MgSO₄, 1.2 NaH₂PO₄, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 3-(N-morpholino)-propanesulfonic acid buffer. Bovine serum albumin (10 mg/ml) was added to the PSS, and pH was adjusted to 7.4. Under a dissecting microscope (Olympus SVH10), first-order (1A) arterioles were isolated and dissected free from the surrounding muscle tissue as previously described (10). The arterioles (0.6–1.0 mm in length, 90–210 μm in inner diameter) were transferred to a Lucite chamber containing PSS albumin equilibrated with room air. Each end of the arteriole was cannulated with a micropipette and secured with nylon suture. After cannulation, the chamber was transferred to the stage of an inverted microscope (Olympus IX70). The microscope was coupled to a video camera (Panasonic BP310), video micrometer (Microcirculation Research Institute, Texas A&M University), video recorder (Panasonic AG-1300), and a data-acquisition system (Macintosh/MacLab).

Each cannulation micropipette was connected to a pressure transducer and a hydrostatic pressure reservoir. Adjustment of the height of the reservoirs allowed for manipulation of intraluminal pressure. After connection to the pressure reservoirs, the arteriole was pressurized to 60 cmH₂O (44 mmHg) and checked for leaks. If no leaks were detected, the arteriole was warmed to 37°C and allowed to develop spontaneous tone during a 30- to 60-min equilibration period during which the bathing solution was replaced every 15 min.

Assessment of vasodilatory time course. A videocassette recorder was used to record the vasodilation in real time. At the time of application, a visual cue was recorded on the video cassette. After equilibration, each of the following vasodilators was added to bring the bathing solution to the following concentration: 1) potassium: 2, 5, and 10 mM, respectively; 2) adenosine: 1 × 10⁻⁶ and 1 × 10⁻⁸ M, respectively; 3) ACh: 5 × 10⁻⁸ and 1 × 10⁻⁵ M, respectively; and 4) SNP: 1 × 10⁻⁸ and 1 × 10⁻⁴ M, respectively.

An equal volume (1 ml) of the vasodilator solution, which was prewarmed to 37°C, was added to volume in the chamber (1 ml) to eliminate temperature changes and ensure immediate mixing. Images of the vessel were recorded continuously at a rate of 30 frames/s for 2 min. The vessel was washed with PSS albumin and allowed to equilibrate for 20 min between each time course determination. At the conclusion of the nitroprusside response measurements, maximal passive diameter with an intraluminal pressure of 60 cmH₂O was determined after a 45-min incubation in calcium-free PSS-albumin solution. The calcium-free PSS-albumin was similar to the PSS-albumin except that it contained 2 mM EDTA and CaCl₂ was replaced with 2.0 mM NaCl.

Analysis of vasodilatory time course. The videotapes of the vasodilatory responses were replayed frame-by-frame, and diameter measurements for each video frame were recorded using a video micrometer and MacLab software. Vasodilation was expressed as a percentage of maximal dilation according to the following formula

\[ \% \text{Relaxation} = D_i - D_m / D_m \]

where \( D_i \) is the diameter recorded at a given time point, \( D_m \) is the diameter recorded immediately before addition of the vasoactive agent, and \( D_m \) is the maximal diameter recorded.

Statistics. Thirty diameter measurements were recorded during each second of videotape. For the initial analysis, the diameter measurements for two frames were averaged to give 15 measurements/s during the first 2 s after application of the vasodilators. When no change in diameter could be detected for any of the vasodilators during the first 2 s, diameter measurements for 15 frames were averaged to give a single diameter measurement every 0.5 s. Friedman’s repeated measures ANOVA was used to determine the significance of differences between the initial diameter (before addition of the drug) and subsequent diameters at each time interval (0.5 s). The earliest time point at which a significant change in diameter was detected was designated as the time to onset of dilation (Table 1). Three-way repeated measures ANOVA was used to detect effects of vasodilator concentration and muscle fiber type during the first 15 s of the time courses. Paired t-tests were used to compare peak dilation, time to peak dilation (\( T_{\text{peak}} \)), and time to 50% of peak dilation (\( T_{50} \)) between soleus and gastrocnemius muscle arterioles and between intermediate and high drug concentrations of adenosine, ACh, and SNP. Friedman’s repeated measures ANOVA was used to compare peak dilation, \( T_{\text{peak}} \), and \( T_{50} \) among KCl concentrations. A value of \( P < 0.05 \) was required for significance.

RESULTS

Characteristics of arterioles. In soleus muscle arterioles, intraluminal diameters ranged from 93–167 μm, with an average maximal diameter of 128 ± 7 μm.

Characteristics of arterioles.
Gastrocnemius muscle arterioles were slightly larger; maximal diameters ranged from 122–207 μm, with an average maximal diameter of 166 ± 9 μm. On equilibration, soleus muscle arterioles developed 53 ± 5% tone. Gastrocnemius muscle arterioles displayed 26 ± 4% tone.

**Potassium.** Arterioles from both soleus and gastrocnemius muscles dilated significantly to all concentrations of KCl. During the initial 15 s, the time course of the response was concentration dependent, with arterioles from both soleus and gastrocnemius muscles responding more quickly to the higher concentrations of potassium. In soleus muscle arterioles, the first significant increase in diameter occurred at 9.5, 5.5, and 4.5 s in response to 2, 5, and 10 mM KCl, respectively (Fig. 1). In gastrocnemius muscle arterioles, the initial significant dilation to 2, 5, and 10 mM KCl occurred at 8.0, 7.0, and 4.5 s (Fig. 1). The \( T_{50} \) and \( T_{peak} \) induced by KCl at all concentrations were similar between gastrocnemius and soleus muscle arterioles (Table 2). In both soleus and gastrocnemius muscle arterioles, peak relaxation responses to KCl were concentration dependent; 10 mM KCl produced significantly greater dilation. At concentrations of 2 and 5 mM, KCl produced similar peak dilation in soleus and gastrocnemius arterioles; however, there was a trend toward greater peak relaxation to 10 mM KCl in soleus muscle arterioles (\( P = 0.07 \)) (Table 3).

**Adenosine.** During the first 15 s of exposure to adenosine, the overall magnitude of vasodilation was greater in arterioles from gastrocnemius muscle (\( P < 0.01 \)); however, there did not appear to be a difference in the rapidity of the response to adenosine in arterioles from soleus and gastrocnemius muscles. The onset of dilation in soleus muscle arterioles occurred at 5.5 and 12.0 s in response to \( 10^{-8} \) and \( 10^{-4} \) M adenosine, respectively. Gastrocnemius muscle arterioles responded to \( 10^{-8} \) M adenosine in 9.0 s and \( 10^{-4} \) M adenosine in 13.5 s (Fig. 2, Table 1). Early vasodilation to adenosine (0–15 s) occurred in a concentration-dependent manner. Surprisingly, the magnitude of the initial adenosine-induced vasodilation was higher in response to the lower concentration of adenosine (Fig. 2). Prolonged vasodilation \( (T_{50} \) and \( T_{peak} \)) and maximal relaxation to adenosine were similar in soleus and gastrocnemius muscle arterioles (Tables 2 and 3).

**Acetylcholine.** Early vasodilation to acetylcholine (the initial 15 s of the response) was significantly affected by both drug concentration and muscle fiber type. In gastrocnemius muscle arterioles, the initial vasodilation in response to \( 5 \times 10^{-8} \) M ACh occurred at 8 M adenosine in 9.0 s and 10 M adenosine in 13.5 s (Fig. 2, Table 1). Early vasodilation to adenosine (0–15 s) occurred in a concentration-dependent manner. Surprisingly, the magnitude of the initial adenosine-induced vasodilation was higher in response to the lower concentration of adenosine (Fig. 2). Prolonged vasodilation \( (T_{50} \) and \( T_{peak} \)) and maximal relaxation to adenosine were similar in soleus and gastrocnemius muscle arterioles (Tables 2 and 3).

**Sodium nitroprusside.** During the initial 15 s of exposure to SNP, the magnitude of the response of gastrocnemius arterioles was greater than that of soleus muscle arterioles. The early vasodilatory response also depended on the concentration of SNP employed (Tables 1 and 2); the higher concentration \( (10^{-4} \) M) produced greater vasodilation (Fig. 4). The time to onset of dilation in response to \( 10^{-5} \) M SNP occurred in soleus muscle arterioles at 4.0 s; \( 10^{-4} \) M SNP produced dilation in 5.0 s. The time to onset of dilation in gastrocnemius muscle arterioles was 8.0 and 5.0 s for \( 10^{-8} \) and \( 10^{-4} \) M SNP, respectively (Fig. 4, Table 1). No differences were detected between soleus and gastrocnemius muscle arterioles for \( T_{50} \), \( T_{peak} \), or peak vasodilation (Table 3). \( T_{50} \) and \( T_{peak} \) were similar in response to \( 10^{-8} \) and \( 10^{-4} \) M SNP, respectively; however, the higher concentration of SNP produced greater peak vasodilation in both soleus and gastrocnemius muscle arterioles (Table 3).

**DISCUSSION**

It has been hypothesized that the action of locally released vasodilators contributes to the rapid (1–2 s) hyperemic response that occurs at the onset of exercise (30, 40, 43). For a vasodilator to participate in the initial hyperemic response, the time course of the onset of the vasodilatory response must be at least as rapid as that of the hyperemic response. In this study, a 2-min time course of vasodilation in response to potassium chloroide, adenosine, ACh, and SNP was measured in soleus and gastrocnemius muscle arterioles to determine whether the time to onset of the vasodilation corresponds to the time to the onset of hyperemia. We found that all of the vasodilators studied required at least 4 s to produce a significant increase in diam-
ter. These time-course determinations indicate that the vasodilation of resistance arterioles that occurs in response to intermediate and high concentrations of potassium, adenosine, ACh, and SNP does not occur quickly enough to participate in producing the rapid muscle hyperemia at the onset of exercise.

Several studies, based on indirect evidence, have concluded that locally released vasodilatory factors help mediate the initial hyperemia during exercise. For example, Tschakovsky et al. (43) found that increases in forearm blood flow via rhythmic mechanical compression could account for only 60% of the peak flow response during normal muscle contractions. Thus it was suggested that factors other than the muscle pump mediate the remaining 40% of the peak flow response. However, it was assumed that the mechanical efficiency of externally applied rhythmic limb compression mimics the rhythmic shortening and lengthening contractions of muscle. In another study (30), blood flow to canine hindlimb muscle was reported to be elevated above baseline 1 s after the cessation of a 1-s tetanic stimulation, peaked after ~6–7 s, and remained elevated for at least 20 s postcontraction. The authors concluded that the muscle pump could account for a portion of the initial (~1 s) elevation in blood flow; the rapid rise and subsequent slow decline of the flow response followed a pattern that the authors suggested might be expected if a vasodilatory substance(s) was released from the contracting muscle and was subsequently washed away by the elevated perfusion. A somewhat different conclusion was reached in a comparable study of humans (34), where temporal changes in leg blood flow were assessed during passive and voluntary knee-extensor exercise. Results of this study indicated that during the leg exercise, the first phase of the hyperemic response (onset latency of 0.3–0.5 s) was induced by a purely muscle-mechanical factor. These authors further concluded that, on the basis of the time course of pressure changes, the second phase of the hyperemic response (onset latency of 4.2 ± 0.5 s) was induced by vasodilation, the likely result of locally

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<th>Table 2. Time to reach 50% of peak dilation and time to peak dilation</th>
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<td>Potassium, mM</td>
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<td>1 × 10⁻⁴</td>
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<td>5 × 10⁻⁸</td>
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<td>1 × 10⁻⁵</td>
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<td>Nitroprusside, M</td>
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<td>1 × 10⁻⁸</td>
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Values are means ± SE. $T_{50}$ and $T_{peak}$, time to reach 50% peak dilation and time to peak dilation, respectively.

Fig. 1. Time course of vasodilation during initial 15 s after application of 2 mM (A), 5 mM (B), and 10 mM (C) potassium chloride (KCl). Data are presented as means ± SE. Vasodilation to 5 and 10 mM KCl was significantly greater than dilation to 2 mM KCl during this time period ($P < 0.05$). No differences were detected between vasodilatory responses of gastrocnemius and soleus muscle arterioles.
released vasodilators. Thus from these studies (30, 34), the prevailing question concerns the time course of the initial vasodilatory response.

The design of the present study allowed us to consider one aspect of the vasodilatory responses that might occur in skeletal muscle resistance arterioles in vivo. We administered intermediate (the IC50) and high concentrations of candidate vasodilators and measured the rapidity of the vascular relaxation that occurred. All of the vasodilators examined produced sufficient dilation to justify their consideration as contributors to the muscle hyperemia during exercise. Potassium ions are perhaps the most likely locally released vasodilator to be involved in the initial hyperemia at the onset of exercise. Potassium ions are released into the interstitium as a result of muscle contraction when muscle cells repolarize. During tetanic

Table 3. Peak dilation in soleus and gastrocnemius muscle arterioles

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<th>Potassium, mM</th>
<th>Adenosine, M</th>
<th>Acetylcholine, M</th>
<th>Sodium Nitroprusside, M</th>
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<td>2</td>
<td>5</td>
<td>10</td>
<td>1×10^-8</td>
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<tr>
<td>Soleus</td>
<td>54±13</td>
<td>63±10</td>
<td>86±4†</td>
<td>42±12</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>50±10</td>
<td>71±9†</td>
<td>62±12†</td>
<td>61±11</td>
</tr>
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Values are means ± SE measured as % of maximal dilation. *Significant difference exists between soleus and gastrocnemius muscle arterioles; †significantly different from lowest respective dilator concentration.

Fig. 2. Time course of vasodilation during initial 15 s after application of 10^-8 (A) and 10^-4 M (B) adenosine. Data are presented as means ± SE. Vasodilatory responses were not affected by the concentration of ADP but were greater in arterioles from gastrocnemius muscle than soleus muscle (P < 0.05).

Fig. 3. Vasodilatory responses as a function of time during initial 15 s of exposure to 5×10^-8 (A) and 10^-3 M (B) acetylcholine (ACh). Data are presented as means ± SE. In gastrocnemius muscle arterioles, vasodilation to 10^-5 M ACh was significantly greater than that to 5×10^-8 M ACh (P < 0.05). Concentration of ACh did not significantly affect time course of vasodilation in soleus muscle arterioles. Vasodilatory response to ACh was greater in soleus than gastrocnemius muscle arterioles (P < 0.05).
contractions, 50–80% of peak interstitial potassium concentration can be attained in 1–2 s (20, 21). Potassium ions induce vasodilation at low concentrations through activation of inward rectifier potassium channels (31). Small increases in extracellular potassium and activation of these channels serve to hyperpolarize the smooth muscle cells, thereby inactivating voltage-gated calcium channels. Inactivation of voltage-gated calcium channels leads to a decrease in intracellular calcium and relaxation of the smooth muscle. Despite the attractiveness of this rapidly released vasodilator as a candidate for inducing the initial muscle hyperemia, we found that KCl did not initiate dilation in arterioles from gastrocnemius and soleus muscles (4, 24, 25, 36) have shown that, during submaximal exercise, adenosine is primarily formed in muscle composed of slow type I fibers, yet the rapid hyperemia occurs in muscles with few slow type I fibers. Second, in a study by Hester et al. (19), adenosine was perfused in resting canine gracilis muscle, resulting in a sevenfold increase in blood flow. After several hours of adenosine perfusion, flow gradually returned to control levels. The muscle was then stimulated to contract with continued exogenous adenosine perfusion. The resulting hyperemic response was not different from that elicited during contraction without adenosine infusion. Thus it was concluded that if endogenous adenosine release accounted for part of the muscle hyperemia, then blood flow should have been diminished during contraction with exogenous adenosine perfusion due to the saturation and desensitization of the adenosine receptors. The present study also supports the conclusion that adenosine does not function to mediate the initial exercise hyperemia in muscle composed of slow-twitch or fast-twitch fibers.

The final candidate vasodilator tested in this study was nitric oxide. We utilized ACh to determine the time course of endothelium-dependent dilation to endogenous nitric oxide and other endothelium-derived vasodilatory factors. However, local release of nitric oxide could originate from the vascular endothelium (35), as well as hemoglobin (42), skeletal muscle cells (5), and nitroxidergic neurons (8) during exercise. Therefore, SNP was also used as an exogenous donor of nitric oxide to mimic release from these other potential sources.

Evidence from hamster cremaster muscle has indicated that nitric oxide is an important contributor during the first minute of the hyperemic response (18). In contrast, a study of human forearm blood flow showed that neither nitric oxide nor ACh influenced muscle hyperemia at the onset of exercise (39). On the basis of the time to onset of the response measured for these dilators (>4 s), neither endogenous nor exogenous nitric oxide can contribute to the hyperemia at the onset of exercise in soleus or gastrocnemius muscles, although nitric oxide-induced vasodilation could contribute to prolonged exercise hyperemia. These findings suggest that even when nitric oxide is applied
directly to the smooth muscle, diffusion through the cell membrane and the transduction of signal through intracellular pathways require at least 4 s to occur.

None of the vasodilators produced a response rapid enough to contribute to the initial hyperemia (1–2 s) at the onset of exercise (1, 2, 4, 7, 14, 34, 38, 39, 41, 43, 44). It is possible that vessels in an intact muscle show a more rapid vasodilatory response. This does not appear to be the case, given that the time to onset of dilation of primary arteries and secondary arterioles after stimulation-induced contraction in spino-trapezius muscle is ≥5 s (28). Furthermore, it is possible that vasodilators could act synergistically to more rapidly induce vasorelaxation. However, these actions would have to reduce the measured in vitro response times by <50% to contribute to the initial hyperemia. In addition, within the intact exercising muscle, time would also be required for the vasodilator(s) to be released, diffuse to the vascular smooth muscle cells, and build to a concentration sufficient to induce smooth muscle relaxation. Thus it does not appear likely that intact vessels or a possible synergistic action of vasodilators could so dramatically alter the response times measured in the present study to produce a vasodilation in <1–2 s.

The possibility also exists that the concentrations of the vasodilators employed were limiting factors in the time courses of the vasodilatory responses. We measured responses to both an intermediate (the IC50) and a high concentration of each vasodilator and found that at the higher concentration employed, all of these vasodilators produced robust increases in diameter (46–86% of maximal vasodilation) in these vessels. In both soleus and gastrocnemius arterioles, the peak vasodilation to KCl, ACh, and SNP was concentration dependent. The initial responses to all of the vasodilators utilized in this study were concentration dependent; however, despite producing greater relaxation in a similar time period, even the high concentrations required at least 4 s to initiate a response. These results suggest that, although the efficacy of these vasodilators is concentration dependent, the latency of onset to dilation is determined by a factor other than concentration.

Differences in the vasodilatory responsiveness of resistance vessels from muscles composed of different fiber types could affect the time course of vascular relaxation. To address this possibility, we studied arterioles isolated from the soleus muscle, which is composed primarily of slow-twitch type I fibers, and the superficial portion of the gastrocnemius muscle, which is composed predominantly of fast-twitch type II fibers (12). We detected differences in the initial relaxation responses between soleus and gastrocnemius muscle arterioles to ACh, adenosine, and SNP, suggesting that the mechanisms of vasodilation present in these arterioles are linked to the metabolic milieu of the surrounding muscle fibers. The magnitude of early vasodilation of gastrocnemius muscle arterioles in response to adenosine and SNP was greater than that of soleus muscle arterioles, suggesting that smooth muscle sensitivity to these dilators is greater in gastrocnemius arterioles. In contrast, we found that early dilation as well as the maximal vasodilator response to the high ACh concentration (10−5 M) was greater in soleus muscle arterioles, suggesting that the capacity for endogenous production of nitric oxide was greater in arterioles from muscle composed of slow-twitch fibers. Concentration-response data generated in a previous study have indicated that both sensitivity (IC50) and maximal responsiveness to ACh was greater in isolated soleus muscle arterioles compared with arterioles isolated from gastrocnemius muscle (11). More recent data from our laboratory also show that sensitivity (IC50) of gastrocnemius muscle arterioles to SNP and adenosine surpasses that of soleus muscle arterioles (29). These data indicate that vasodilatory responsiveness within a large muscle group is heterogeneous. However, despite the differences detected in the magnitude of the early vasodilatory responses, we found that the delay in the onset of dilation was unaffected by muscle fiber type. Therefore, regardless of the presence of diverse muscles of varying fiber types that would be recruited during the initiation of exercise, it is unlikely that the vasodilators examined in this study contribute to the initial hyperemia at the onset of exercise.

We specifically chose to examine 1A arterioles because of the prominent role proximal arterioles play in the control of muscle blood flow (16, 37). Although the present study indicates that 1A arterioles do not dilate rapidly enough to contribute to the initial hyperemic response during exercise, the possibility exists that vasodilatory responses may differ among different size vessels within a vascular bed. For example, Marshall and Tandon (28) showed that terminal arterioles in spinotrapezius muscle of rats can dilate in <2 s. In their study, the time to onset of dilation was recorded in primary arteries, secondary arterioles, and terminal arterioles after four intensities of electrical stimulation-induced contractions. In all cases, the time to onset of dilation was in between 5 and 20 s except in the terminal arterioles after the highest intensity contraction, where the onset of vasodilation occurred in <2 s. Therefore, one must consider whether a rapid dilation of terminal arterioles during exercise may underlie the initial hyperemic response. Two lines of evidence argue against the involvement of terminal arteriolar vasodilation as a mechanism for the rapid hyperemia. First, the rapid terminal arteriolar vasodilation reported by Marshall and Tandon (28) occurred only after the high-intensity tetanic contraction, whereas the magnitude of the initial hyperemic response during exercise is intensity independent, similarly occurring during both low-intensity and high-intensity exercise (15, 23, 38). Second, metabolic vasodilation in skeletal muscle is initiated in terminal arterioles and ascends to the proximal resistance arteries, i.e., the large arterioles and feed arteries (16, 37). Whereas distal arterioles initiate the vasodilation in active muscle, it is the proximal resistance vessels that determine the magnitude of skeletal muscle perfusion (16, 37). Therefore, large increases in muscle
blood flow at the onset of exercise without upstream dilation of 1A arterioles is unlikely.

In summary, potassium ions, adenosine, ACh, and nitric oxide have all been proposed to be locally released vasodilators that may contribute to the initial muscle hyperemia at the onset of exercise. In this study, we determined that none of these candidates produced significant vasodilation within 1–2 s of application to isolated soleus and gastrocnemius muscle arterioles. The time course of the onset of vasodilation (≥4 s) suggests that these vasodilators do not participate in the rapid hyperemic response that occurs at the onset of exercise but may mediate further elevations in blood flow after 4–5 s of exercise.

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