Time course of vasodilation at the onset of repetitive skeletal muscle contractions

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Armstrong ML, Dua AK, Murrant CL. Time course of vasodilation at the onset of repetitive skeletal muscle contractions. Am J Physiol Regul Integr Comp Physiol 292: R505–R515, 2007. First published August 24, 2006; doi:10.1152/ajpregu.00381.2006.—To characterize the vasodilatory response in the transition from a single skeletal contraction to a series of contractions, we measured the response of hamster cremaster muscle arterioles associated with four to five skeletal muscle fibers stimulated to contract for one, two, three, or four contractions (250-ms train duration) at 4-s intervals [15 contractions per minute (CPM)] for up to 12 s, at stimulus frequencies of 4, 10, 20, 30, 40, 60, and 80 Hz. To investigate the contribution of contraction frequency, we stimulated muscle fiber bundles at 30 or 60 CPM for 12 s at stimulus frequencies of 4, 20, and 60 Hz. Arteriolar diameters at the site of overlap with the stimulated muscle fibers were measured before and after each contraction. At 15 CPM at 4, 20, and 60 Hz, we observed a peak change in diameter following the first contraction of 1.1 ± 0.1, 1.6 ± 0.2, and 2.1 ± 0.2 μm that almost doubled in response to the second contraction (2.0 ± 0.1, 3.0 ± 0.1, and 3.8 ± 0.1 μm, respectively), but there was no further dilation following the third or fourth contraction. A similar response occurred at all stimulus and contraction frequencies tested. At 30 and 60 CPM at 60 Hz, the plateau after two contractions was followed by a further increase in diameter to a second plateau at 7–8 s. Therefore, the vasodilatory response in the transition from single to multiple contractions had components that were stimulation parameter dependent and independent and showed a plateauing behavior indicative of rapid changes in either the nature and/or concentration of vasodilators released or changes in vascular reactivity.

stimulus frequency; arteriole; muscle contraction; contraction frequency

THE PATTERN OF CHANGE IN BLOOD flow in response to skeletal muscle contractions is a multiphase process with an initial, rapid increase at the onset of contractions, followed by a slower change to a steady state over time (for example, Refs. 4, 9, 35, 40, 47, 54, 59). While vascular compression was thought to be the major process in developing the initial, rapid hyperemia (for review, see Ref. 55), rapid vasodilation has been conclusively identified as an important component of this response (21, 30, 33, 41, 57). The identity of the dilators responsible for the rapid dilation remain unclear. Since Gaskell (19), it has been hypothesized that the vasodilation responsible for the changes in blood flow in response to skeletal muscle contraction is due, in part, to dilatory products released from the active skeletal muscle cells themselves. Potential vasodilators released as a result of skeletal muscle activation and metabolism have been identified (for review, see Refs. 12, 27), but no single vasoactive substance has emerged as having a consistent, predominantly large contribution, especially at the onset of exercise.

The reasons for the lack of identity of a single, prominent dilator may be twofold. First, the complex nature of the pattern of changes in flow and vasodilation at the onset of muscle contraction indicates that there may be multiple vasodilators or multiple processes involved very early in the hyperemic process. Second, these processes may not be constant, changing with changes in stimulation parameters of the skeletal muscle cells themselves. Skeletal muscle cell activation and metabolism (processes of ATP utilization and restoration) have been hypothesized to be the source of the dilators responsible for active hyperemia; thus, if activation and metabolism change, by changing muscle stimulation parameters, then the dilators produced may also change. Thus different experiments that use different stimulation parameters may indeed identify different vasodilatory processes responsible for changes in diameter and changes in flow. Both blood flow patterns and arteriolar vasodilatory patterns have been shown to differ, depending on the stimulation parameters used to contract the skeletal muscle cells. After a single contraction, the vasculature dilatory response pattern has been observed to change with changes in stimulus frequency (30) and with longer train durations (20, 30, 57) and has been influenced by the number of fibers recruited (22, 33) and contraction intensity, a derivative of stimulus frequency and number of motor units recruited (13, 54). Which stimulation parameters have primary influences on the dilatory patterns in the transition from a single contraction to multiple contractions and what the effects of contraction frequency will be are unknown. Close observation of the vasculature and the early dilatory responses at the onset of repetitive muscle contraction and how they change with changing stimulation parameters will identify key time points of change and key stimulation parameters that determine the vasodilation. This is a necessary first step in identifying the critical dilators and processes involved in the early stages of active hyperemia.

The goal of this study was to characterize the arteriolar vasodilatory behavior in response to muscle contraction in the transition from a single contraction to multiple repetitive contractions and to test whether these early events of dilation change with changes in stimulation parameters. We tested the hypothesis that the vasodilation in response to muscle contraction would be rapid and the pattern of dilation would be dependent on both stimulus frequency and contraction frequency. We directly stimulated a small bundle of skeletal muscle fibers (four to five) in an anesthetized hamster cremaster muscle in situ overlying an arteriole (maximum diameter...
METHODS

All experimental procedures were approved by the institutional animal care and use committee and were conducted in accordance with the guidelines of the Canadian Council on Animal Care as set out in the Guide to the Care and Use of Experimental Animals.

Experimental Model

Adult male Golden hamsters (100–130 g) were anesthetized with pentobarbital sodium (70 mg/kg ip) and tracheotomized. Catheters were placed in the left femoral artery and vein to monitor mean arterial pressure and for supplemental pentobarbital administration, respectively. Supplemental pentobarbital was given as needed during surgery and constantly infused throughout the experimental protocol (10 mg/ml saline, 0.56 ml/h). Hamster esophageal temperature was maintained at 37°C via convective heat from a coiled water-filled glass tube (42.0°C) secured under the hamster.

The right cremaster was prepared for in situ microscopy as previously described (3, 31). The cremaster is a thin muscle consisting of five to six layers of skeletal muscle fibers and is an extension of the muscles of the abdominal wall that holds the testes and epididymus in place. The muscle consists of ~70% fast-oxidative glycolytic fibers, 15% fast glycolytic fibers, and 15% slow oxidative fibers (39) and would be involved a range of activity states, such as fast, higher force activities, such as quick retraction of the testes into the abdomen, as well as lower force, more tonic activities produced by postural muscle. Briefly, the cremaster was isolated, cut longitudinally, separated from the testis and epididymis, and gently spread over a semicircular lucite platform. The edges of the tissue were secured with insect pins to maintain tension but not stretch the muscle. Once exposed, the cremaster muscle was constantly superfused with a bicarbonate-buffered salt solution containing (in mmol/l) 131.9 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, 30 NaHCO₃, and 0.3 mg/l tubarine (curare), equilibrated with gas containing 5% CO₂–95% N₂ (pH 7.35–7.45). Cremaster muscle temperature was maintained at 34.0°C.

For both protocols, arteriolar diameter at the site of overlap with the transverse arteriole per preparation was used to collect data, and each experiment, arteriolar diameters were recorded after 2 min of superfusion of the preparation with 10⁻⁴ M sodium nitroprusside (considered to produce maximal diameter).

Experimental Protocols

The nervous system stimulates skeletal muscle fibers using a range of stimulation parameters to produce a wide range of movements. Physiologically, the nervous system activates skeletal muscle cells through discrete, short-duration bursts of multiple stimuli (trains), producing tetanic contractions. Contractile activity is altered by changing the number of stimuli within a train (stimulus frequency), duration of a train (train duration), the number of trains per minute (train frequency or contraction frequency in CPM), and the total duration of the contraction period over time. Figure 1 illustrates each of these stimulation variables.

Protocol 1. Muscle fiber bundles were stimulated to contract with a 250-ms train duration for one, two, three, or four contractions at 4-s intervals (15 CPM) for up to 12 s, at stimulus frequencies of 4, 10, 20, 30, 40, 60, and 80 Hz. Fused tetanus and maximal force generation for hamster cremaster muscle occurs at ~30 Hz (31). For each experiment, the order of stimulus frequencies was randomized.

Protocol 2. To determine the effects of contraction frequency, contraction frequencies >15 CPM were used as they are more relevant to movement. We stimulated muscle fiber bundles with a 250-ms train duration once every 2 s (30 CPM) or once every second (60 CPM) for 12 s at stimulus frequencies of 4, 20, and 60 Hz. These stimulus frequencies were chosen as they produce the three distinct patterns of dilatation to a single contraction, a single rapid dilatation at 4 Hz, a biphasic dilatation at 20 Hz, and a single longer lasting dilatation at 60 Hz. For each experiment, the order of stimulus frequencies was randomized.

For both protocols, arteriolar diameter at the site of overlap with contracting skeletal muscle fibers was continuously recorded for 1 min before each stimulation bout, during stimulation, and for 2 min following stimulation. A 5-min rest period was allowed between each stimulus frequency.

Data Analysis and Statistics

All data are reported as means ± SE. Baseline diameter was defined as the diameter just before muscle stimulation. Only one arteriole per preparation was used to collect data, and n indicates the number of arteriolar preparations recorded.
number of arterioles observed. All experiments were videotaped and analyzed offline. Images were digitized, and arteriolar diameters measured via Image-Pro Plus software. Arteriolar diameter was measured each second during stimulation and for 10 s following the last contraction, after which diameter was measured every 5 s until 1 min and then at 90 and 120 s. For data collected over time, group means were compared with a repeated-measures ANOVA. Baseline and maximal diameters were compared with an ANOVA. When the ANOVAs identified significant differences, a protected least significant difference was used post hoc to determine significant differences between specific points. Linear and nonlinear (using classical equations to describe a hyperbola) regression analysis was used to determine the nature of the relationships between stimulation parameters and changes in diameter (49). Differences were considered significant when \( P < 0.05 \).

RESULTS

The initial baseline and maximum diameters of the transverse arterioles used in this study are presented in Table 1. Within protocol 1, the baseline diameters before each stimulus frequency were not significantly different from each other, thus diameter before 4-Hz stimulation is shown as representative. Within both protocols, baseline diameters did not differ significantly from each other, but the maximum diameters of the vessels between groups did differ.

**Protocol 1**

The differences in the pattern of dilation resulting from changes in stimulus frequency in response to one, two, three, and four contractions at 4, 20, and 60 Hz are shown in Fig. 2. Figure 2A shows the magnitude of the dilation (0.7 ± 0.2 \( \mu \)m peak at 2 s) in response to 4 Hz with a train duration of 250 ms, thus resulting in one impulse delivered. At 20 Hz, five impulses were delivered, but the resulting dilation (peak diameter 1.5 ± 0.2 \( \mu \)m peak at 3 s) was not five times larger than the dilation resulting from a single impulse. At 60 Hz, 15 impulses were delivered, but the resulting dilation (2.4 ± 0.2 \( \mu \)m peak at 3 s) was not 15 times larger than dilation, resulting from a single impulse. Therefore, the magnitude of the dilation did not correlate directly with the number of impulses delivered per train.

Figure 2, B, C, and D, shows the dilations resulting from two, three, and four contractions, respectively, delivered at 4-s intervals (15 CPM). The biphasic nature of the dilation at 20 Hz appears to disappear following multiple contractions, possibly as the second dilation from the first contraction appears as the second, third, and fourth contraction are given, making the peaks less distinct.

Figure 3 shows the peak diameter change within 4 s of each contraction for experiments involving two, three, and four

Table 1. Baseline arteriolar diameters and sodium nitroprusside-induced maximal arteriolar diameters for vessels in protocol 1 and protocol 2

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<th>Protocol 1</th>
<th>Protocol 2</th>
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<td></td>
<td>15 CPM</td>
<td>30 CPM</td>
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<td>Contraction</td>
<td>4 Hz*</td>
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<td>Stimulus frequency</td>
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<td>Contraction number</td>
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<td>1</td>
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<tr>
<td>4</td>
<td>(n = 9)</td>
<td>(n = 8)</td>
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<tr>
<td>Initial baseline diameter*, ( \mu )m</td>
<td>16.9 ± 1.7†</td>
<td>16.6 ± 2.3</td>
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<td>Maximal diameter, ( \mu )m</td>
<td>34.6 ± 1.9</td>
<td>37.2 ± 3.0</td>
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<td>(n = 8)</td>
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*No. of animals. CPM, contractions per minute. *Baseline diameters were not significantly different across stimulus frequencies within each contraction number protocol; thus baseline diameter for 4 Hz was used as representative. †Values are average ± SE. ‡Significant difference from 15 CPM, 4 contractions. §Significant difference from 15 CPM, 1 contraction.
contractions. These data show that the dilation after the second contraction was two times that of the first contraction (Fig. 3A).

With three contractions (Fig. 3B), the dilation resulting from the second contraction was also two times that of the first, but there was no significant increase in dilation following the second contraction. A similar trend occurred for four contractions, whereby the magnitude of the dilation resulting from the second contraction was two times the dilatory response of the first, but there was no significant increase in dilation following the third or fourth contraction (Fig. 3C).

Protocol 2

The time course of the change in arteriolar diameter following one (A), two (B), three (C), or four contractions (D) at 4 Hz (○), 20 Hz (■), and 60 Hz (▲) at 4-s intervals (protocol 1). The arrows and dashed lines indicate when each skeletal muscle contraction occurred. Significant dilations occurred following each contraction protocol, and a significant biphasic dilation occurred following one contraction (A) at 20 Hz, where the change in diameter at 3 and 15 s was significantly different from the change in diameter at 9 s.

Fig. 2. The time course of the change in arteriolar diameter following one (A), two (B), three (C), or four contractions (D) at 4 Hz (○), 20 Hz (■), and 60 Hz (▲) at 4-s intervals (protocol 1). The arrows and dashed lines indicate when each skeletal muscle contraction occurred. Significant dilations occurred following each contraction protocol, and a significant biphasic dilation occurred following one contraction (A) at 20 Hz, where the change in diameter at 3 and 15 s was significantly different from the change in diameter at 9 s.

change in diameter decreased significantly. At 30 CPM, the rate of change in diameter was $2.4 \pm 0.1 \mu m/s$ until the first plateau was reached at 3 s. From 3 to 6 s, the rate of change in diameter decreased significantly to $0.4 \pm 0.2 \mu m/s$. Following this apparent plateau, there was a second increase in the rate of change in diameter to $3.8 \pm 0.4 \mu m/s$ up to 8 s. A second plateau was observed from 8 to 11 s, where the rate of change in diameter significantly decreased to $0.8 \pm 0.5 \mu m/s$. There is another increase in diameter following 11 s, but whether this is representative of an increase to a third plateau is unknown, as contractions were stopped at 12 s.

At 60 CPM, the rate of change in diameter was $1.7 \pm 0.1 \mu m/s$ until the first, less well-defined plateau was reached between 2 and 4 s, where the rate of change of diameter decreased significantly to $0.4 \pm 0.2 \mu m/s$. This was followed by a second increase in the rate of change of diameter to $3.3 \pm 0.2 \mu m/s$ until 7 s, where a second apparent plateau occurred. From 7 to 10 s, the rate of change of diameter significantly decreased to $0.7 \pm 0.3 \mu m/s$. There is another increase in diameter after 10 s, but whether this is representative of an increase to a third plateau is, again, unknown, as contractions were stopped at 12 s.

The trend observed in Fig. 2, whereby there appears to be no further dilation following the second contraction at 15 CPM at
all stimulus frequencies, is maintained at higher contraction frequencies. Figure 5 shows the change in diameter following each of the first four contractions at 30 and 60 CPM. We observed the same trend toward an early plateau following two contractions at all stimulus frequencies. Although not significant, the change in diameter at 30 CPM at 4 Hz (Fig. 5A) for contraction 2 (1.1 ± 0.2 µm) was almost double that observed in contraction 1 (0.6 ± 0.1 µm). The significant increase in diameter of the fourth contraction observed at 60 Hz at both 30 and 60 CPM may be the beginning of the further increase to a second plateau observed in Fig. 4.

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**Fig. 3.** The peak change in arteriolar diameter within 4 s of each contraction (250-ms train duration) during the two- (A), three- (B), and four-contraction (C) protocol. Shaded bars indicate the change in diameter following one contraction; open bars, two contractions; hatched bars, three contractions; solid bars, four contractions. *Contraction 2 was significantly larger than contraction 1. *Contraction 3 was significantly larger than contraction 2. In C, the vasodilation to contraction 4 was not significantly different from dilation at contractions 2 or 3 at any stimulus frequency.

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**Fig. 4.** The time course of the change in arteriolar diameter during muscle stimulation at 15 CPM (A), 30 CPM (B), and 60 CPM (C), at stimulus frequencies of 4 Hz (●), 20 Hz (■), and 60 Hz (○). The dashed lines indicate when each skeletal muscle contraction occurred. *Plateau regions at 60 Hz at 30 and 60 CPM, where the rate of change in diameter is significantly lower than the rate before it.
Figure 6 illustrates the relationship between the magnitude of the change in dilation and contraction frequency at four different time points. These data show that there is little relationship between vasodilation and contraction frequency at low stimulus and contraction frequencies, but that there is a large change in dilation associated with high stimulus and contraction frequencies over time. We did not observe a systematic doubling of the vasodilatory response with a systematic doubling of contraction frequency at any stimulus frequency at any time in the contraction bout, i.e., the dilation at 60 CPM was not twice the dilation observed at 30 CPM, and the dilation at 30 CPM was not twice the dilation observed at 15 CPM.

Figure 7 plots the peak change in diameter for both protocol 1 (Fig. 7A) and protocol 2 (Fig. 7B) with reference to stimulus frequency. Figure 7A shows the significant increases in diameter as stimulus frequency increased, but whether the relationships are linear or curvilinear is not clear. We found that, with a single contraction, the relationship better fit a curve ($r^2 = 0.97$) than a line ($r^2 = 0.89$). Following two contractions, the relationship better fit a line ($r^2 = 0.94$) than a curve ($r^2 = 0.65$). With three contractions, the relationship better fit a curve ($r^2 = 0.87$) than a line ($r^2 = 0.81$), and with four contractions, the relationship fit both almost equally (line: $r^2 = 0.89$; curve $r^2 = 0.88$). At higher contraction frequencies, there was the development of nonlinear relationship between peak diameter and stimulus frequency (Fig. 7B).
contraction and thus does not influence the interpretation of the data. Therefore, differences in maximal diameter between experimental groups were not considered to impact the change in dilation that occurred in response to muscle contraction.

**DISCUSSION**

The purpose of this study was to establish the pattern of dilatory responses that occur in the transition from a single contraction to a short series of repetitive contractions and to understand the effects of stimulation parameters on this transition pattern. Our primary findings were as follows. 1) The dilatory response for the second contraction was twice that of the first, regardless of stimulation parameters, indicating that the dilatory complement for contraction 1 was similar to the dilatory complement for contraction 2. 2) The dilatory response to contractions over time plateaued following two contractions at all stimulus frequencies and all contraction frequencies, indicating that the dilatory mechanisms that initiate rapid dilation are fully expressed by two contractions by mechanisms that are not influenced by contraction or stimulus frequency. 3) There was a subsequent increase in vasodilatory activity to a second plateau at ~8 s at high stimulus (60 Hz) and contraction frequencies (30 and 60 CPM), indicating a significant change in dilatory mechanisms with these frequencies. Therefore, the early events of dilation in response to muscle contraction show a rapid increase in dilation in which the very early processes are both independent and dependent of stimulation parameters.

**Experimental Considerations**

We found no significant differences in baseline diameters, but there were significant differences in maximal diameters, which could lead to a difference in the dilatory potential (dilatory potential = maximum diameter − baseline diameter) of the vessels. Differences in maximal diameter may be due to the position of the arteriolar observation site: it will be larger if close to its parent feed arteriole and smaller nearer the terminal branches (32). Previously, we have found a very low correlation between the potential for the vessel to dilate and the change in diameter induced by different stimulation parameters (31) under experimental conditions where blood vessels were not stimulated to dilate maximally. In the present experiments, blood vessels did not dilate maximally with three exceptions: one of the nine vessels stimulated at 20 Hz at 30 CPM, five of the eight vessels at 60 Hz at 30 CPM, and two of the nine vessels at 60 Hz at 60 CPM produced maximum dilations by 13 s. The trends in the changes in diameter over the first 13 s, where maximal diameter was yet attained, contains accurate information regarding how the blood vessels respond to muscle contraction and thus does not influence the interpretation of the data. Therefore, differences in maximal diameter between experimental groups were not considered to impact the change in dilation that occurred in response to muscle contraction.

**Effects of Stimulation Parameters**

Stimulation parameters that are physiologically relevant to movement and locomotion encompass a large, varied range. The stimulus frequencies within a train used by α-motoneurons are controversial. External stimulation of a motor unit to produce maximal force requires stimulus frequencies up to 100 Hz, while the discharge rates of motor units during high force contractions are lower, up to 50 Hz (for review, see Ref. 17). Physiological train durations are short, usually under 750 ms, depending on the nature and the speed of the activity (7, 36, 48). Contraction frequencies will vary dramatically, depending on the movement involved; for reference, the contraction frequency of muscles of normal walking gait range between 45 and 70 CPM for muscle that contract once per gait cycle (58). These independent stimulation parameters are used to derive force generated by a single motor unit, and, if more force is required, then either a higher stimulus frequency can be used within a single motor unit, or more motor units can be recruited, or both. Our model is meant to simulate the contraction of part of a motor unit, albeit not spatially accurate and not accurate with respect to the order of fiber-type recruitment. Our model simulates how a blood vessel overlapping a few fibers in a motor unit respond to skeletal muscle cell activation and what changes in dilation occur when stimulation parameters of that motor unit change. The assumption is once the dilators and processes responsible for initiating dilation are identified and resolved within a motor unit, then, when more motor units are recruited, to generate more force, they will use the same dilatory mechanisms as identified in the partial “motor unit” model. Therefore, generating more dilation by generating more force will not necessarily be due to different dilatory mecha-
The effects of contraction intensity were maintained, and there was less muscle contraction affected by contraction intensity over 24–30 s. The muscle contraction was not affected by contraction frequency (46, 47). We observed that the rapid blood flow at the onset of contraction could be similar. Shoemaker et al. (46, 47) found that the rapid dilator complement from 15 to 30 CPM, while the dilators released at 30 CPM would be twice the dilation observed at 15 CPM. A doubling in diameter could not happen between 30 and 60 CPM. The dilation at 60 CPM should be twice the dilation observed at 15 CPM, and, surprisingly, no greater dilation occurred with further increases to 60 CPM. This indicates that a constant vasodilator complement was not released per contraction. If vasodilators are released per contraction and their effects remain constant, then we would predict that the dilation observed at 30 CPM would be twice the dilation at 15 CPM and the dilation at 60 CPM should be twice the dilation observed at 30 CPM. A doubling in diameter could not happen between 30 and 60 CPM, as the vessel is near maximally dilated at 30 CPM, and we observed a dilation that more than doubles between 15 and 30 CPM. This indicates a significant shift in dilator complement from 15 to 30 CPM, while the dilators released at 30 and 60 CPM may be similar. Shoemaker et al. (46, 47) observed that the rapid blood flow at the onset of muscle contraction was not affected by contraction frequency and more affected by contraction intensity, over 24–30 s. The effect of contraction intensity was maintained, and there was a greater effect of contraction frequency. Similarly, we observed a greater effect of contraction frequency over time.

Contraction frequency is a major determinant of ATP utilization in skeletal muscle. The rate of energy turnover has been shown to increase as contraction frequency increases (18, 50). Multiple, intermittent contractions are more energetically demanding (6, 11, 50) and have a higher oxygen uptake (51) than a continuous contraction, and the difference in ATP cost has been observed within 5 s of the initiation of contraction (34). The rate of ATP hydrolysis has been shown to decrease as train duration increases (24, 37, 50), as the force generation phase is thought to be more metabolically costly than the force maintenance phase due to the high cost of activating and relaxing muscle. Indeed, Hamann et al. (23) found that flow to a muscle was greater with shorter train durations and increased contraction frequency, contraction conditions that correlated with a significantly higher \( \text{O}_2 \) consumption. Thus contraction frequency is a dominant factor in dictating ATP utilization and muscle metabolism over very short timeframes. Therefore, there is the potential for contraction frequency to alter the nature and/or the quantity of metabolic dilators in the transition from a single to multiple contractions. Conversely, stimulus frequency does not appear to have a large impact on ATP utilization when forces are similar (38) or different (37, 50), although submaximal stimulus frequencies have been reported to be more efficient (1). Although stimulus frequency may not have significant effects on muscle metabolism and, therefore, metabolic vasodilators, stimulus frequency may be critical in expressing vasodilators from processes of activation, such as acetylcholine and potassium, which may potentially be more critical in the early stages of hyperemia.

**Implications for Vasodilator Release and Flow Control**

Our data indicate that there are multiple processes involved in initiating vasodilation. As stimulus frequency increases, the dilatory response to a single contraction changed from a single rapid peak to a biphasic dilation to single, prolonged dilation. Common among all frequencies was a rapid dilation, within a second of contraction. Therefore, products of activation are primary candidates, such as potassium and acetylcholine (57). The second dilation, occurring at 20, 30, and 40 Hz, and the prolonged dilation at higher frequencies could be the result of a metabolic process (i.e., adenosine), as its production and/or release may be slower. It does appear to be a different vasodilator, as its time to onset of dilation, dilatory effect, and its degradation are all slower than the dilator responsible for the initial, rapid peak. The larger, prolonged vasodilation at higher stimulus frequencies may be the production of more of the dilators responsible for the dilations at lower stimulus frequencies, or there may be a third dilator produced altogether. These primary (rapid) and secondary (slower) processes are similar to changes in flow observed over time, which are also characterized by a rapid increase in flow followed by a subsequent slower change to a level that is related to different indexes of metabolism.

The dilator complement for a single contraction appears to be the same for the second contraction, regardless of stimulus parameters. The initial, early dilatory plateau observed upon the initiation of muscle contraction indicates that the first dilatory processes are fully expressed by two contractions.
Again, this pattern occurred, regardless of stimulus or contraction frequency. Multiple dilatory plateaus within 10 s were observed, induced with both high-stimulus and contraction frequencies. Distinct, single-plateau regions have been observed previously in the microvascular responses to muscle contraction following a single contraction (20, 57) and multiple contractions (20, 31). Our data are surprisingly consistent with plateau regions observed in the changes in blood flow in the rest-to-work transition within 10 s of the initiation of repetitive muscle contraction (9, 45, 53) (for review, see Refs. 4, 55) and reports of multiple plateaus in the change in flow within 10 s of the onset of contraction (59). Saunders and Tschakovsky (41) have also observed a plateau occurring following two contractions and early plateaus whose timing is independent of contraction intensity (40).

For the patterns in the change in dilation to be similar to patterns in the change in flow is not necessarily expected, as the changes in diameter measured in one microvessel at a specific vascular level would not necessarily be expected to reflect the behavior of all vessels. The expectation that dilation changes will reflect flow changes by calculating radius to the fourth power is too simplistic a model for how flow changes within a tissue. Changes in flow are the composite result of changes that initiate vasodilation plus, at least, ascending conducted responses initiated by skeletal muscle contraction to open the proximal vascular bed (5, 16, 32, 42), flow-dependent and wall shear stress-dependent responses (although controversial) (26, 29, 44), and mechanical effects due to vascular compression (for review, see Ref. 55). Our model is designed to mimic the response of part of a motor unit, optimal for investigating the initiators of vasodilation and the relationship between skeletal muscle contraction and cells of the vasculature. The processes in models that observe the vasculature response to stimulation of more muscle fibers, or the whole muscle, would be expected to comprise more and varied conducted response components arising from various levels of the vascular tree. The processes involved in models that measure flow under submaximal force-generating conditions may have less of a vascular compression component than those generating more maximal forces. These models set up a continuum in which each of the processes of vasodilation will play a differential role. While there are some major differences between the vasodilation observed in a single vessel and the changes on flow observed in vivo [i.e., the duration of the dilation to a single contraction observed in this study is much longer than the duration of elevated blood flow in vivo (8, 13, 21, 33)], there are similarities as discussed above. The transverse arteriole used in the present study is part of the terminal vasculature that is thought to control the distribution of flow to active skeletal muscle cells (15, 25, 52) and the part of the vascular bed that is more responsive to skeletal muscle contraction (15, 28, 57). Thus the changes in diameter at the transverse arteriolar level do appear to reflect some of the early and the important components of the processes responsible for the changes in blood flow at the onset of muscle contraction.

Plateau regions between intervals of increased vasodilatory activity could be the result of shifts in either the amount or nature of the dilator produced or changes in the reactivity of the vasculature. If skeletal muscle activation and metabolism are the source of the vasodilators, then processes deriving these dilators would need to change significantly over a very short period of time and in response to a very limited number of contractions. Significant changes in dilator release due to activation, for example, acetylcholine from the neuromuscular junction and/or potassium release as a result of skeletal muscle repolarization, would not be expected to be significantly altered following two contractions. Nor would we expect significant changes in the metabolic processes that derive ATP in response to two contractions. A plateau in vascular diameter is consistent with the release of stored vasodilator, release of all stored dilator occurring within two contractions, for example, and a plateau resulting from more dilator needing to be synthesized for further dilation.

Plateau regions in the progression of vasodilation could also be produced by changes in vascular smooth muscle responsiveness to the dilator produced. Changes in smooth muscle reactivity could be due to changes in the smooth muscle’s ability to dilate as the smooth muscle cell changes length. Smooth muscle cells that are not at optimal length for force development will develop less force to the same stimulus than when they are at optimal length (14, 43). We have previously discussed that the length of the vascular smooth muscle does not significantly alter the vessel’s reactivity to skeletal muscle contractile activity. Changes in smooth muscle reactivity could also be the result of saturation of receptor-mediated processes or dilators whose effect on vascular smooth muscle or endothelial cells is transient. Some of the proposed “metabolic dilators” act through receptor-mediated processes (such as acetylcholine and adenosine), and there are some dilators whose effect on vascular smooth muscle is very transient [i.e., potassium (10)]. Further understanding of the vasodilatory plateaus will be aided by the identity of the vasodilators themselves.

In summary, we have characterized the dilatory behavior of the vasculature of the early dilatory events in the transition from a single contraction to a series of multiple contractions and show that there are multiple dilatory processes that comprise the initial, rapid vasodilatory response to muscle contraction. Our data imply that the vasodilatory complement at the onset of contraction was similar for the first two contractions and fully expressed within two contractions by processes independent of contraction and stimulus frequency. Subsequent changes in the dilatory processes resulting in a second plateau at 8 s were observed and induced by high-contraction and stimulus frequencies. This plateauing behavior indicates rapid changes in either the nature and/or concentration of vasodilators released or changes in vascular reactivity. The characterization of the dilatory patterns associated with stimulation patterns gives clear direction on which time points and which stimulation conditions are important in determining the identity of the dilators and processes responsible for the early stages of active hyperemia.

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REFERENCES
6. Bergstrom M and Hultman E. Energy cost and fatigue during intermit-
11. Chasiotis D, Bergstrom M, and Hultman E. ATP utilization and force during intermit-
16. Duling BR and Berne RM. Propagated vasodilation in the microcircu-
21. Hamann JJ, Buckwalter JB, and Clifford PS. Vasodilatation is oblig-
22. Hamann JJ, Buckwalter JB, Clifford PS, and Shoemaker JS. Is the blood flow response to a single contraction determined by work per-


