Stimulation characteristics that determine arteriolar dilation in skeletal muscle

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Murrant, Coral L. Stimulation characteristics that determine arteriolar dilation in skeletal muscle. Am J Physiol Regul Integr Comp Physiol 289: R505–R513, 2005; doi:10.1152/ajpregu.00571.2004.—To determine the skeletal muscle stimulation parameters that are most important in establishing vasodilation in the microvasculature, I tested whether arteriolar diameter during 2 min of repetitive, short-duration, tetanic skeletal muscle contractions increased with changes in stimulus frequency, stimulation train duration, and contraction frequency. To test this, the diameter of transverse arterioles approximately perpendicular to small bundles of cremaster muscle fibers in situ of anesthetized Golden Syrian hamsters was used as a bioassay system. Arteriolar diameter was measured before and during different stimulation patterns that consisted of a contraction frequency [6, 12, or 24 contractions per minute (cpm)], a stimulation train duration (250, 500, or 750 ms) and a stimulus frequency (4, 8, 10, 15, 20, 30, 40, 60, and 80 Hz). The magnitude of the dilation significantly increased with stimulus frequency but not in a simple linear manner. The average rate of increase was 0.32 ± 0.02 μm/Hz from 4 to 20 Hz and 0.09 ± 0.02 μm/Hz from 30 to 80 Hz. The magnitude of the dilation increased significantly with the contraction frequency where the dilation at 6 cpm was significantly smaller than the dilation at 24 cpm across all stimulus frequencies. Changing the train duration from 250 to 750 ms did not significantly affect the magnitude of the dilation. These observations suggest that stimulation parameters are important in determining the magnitude of the microvascular dilation and that the magnitude of the dilation was dependent on both the contraction frequency and stimulus frequency but was independent of train duration.

contraction frequency; stimulus frequency; train duration; muscle contraction; hyperemia

LOCOMOTION IS PRODUCED by the nervous system through discrete bursts (trains) of multiple action potentials in the alpha motor neuron that produces tetanic contractions in skeletal muscle. Contractile activity of skeletal muscle can be altered by changing four independent variables: 1) the stimulus frequency within a train, 2) duration of a train, 3) the contraction frequency (the number of trains per minute), and 4) the duration of the contraction period. Changing these independent variables will alter many dependent variables such as developed force or tension. For example, changing the stimulus frequency from 1 to 100 Hz will increase the amount of developed force from the minimal force produced by a single twitch to the submaximal force generated by an unfused tetanic contraction to the maximum force produced by a fused tetanic contraction. Through stimulation of skeletal muscle, the nervous system is creating the contractile activity and therefore setting the metabolic demand of the skeletal muscle cells.

Skeletal muscle metabolism has been hypothesized to be the source of dilators responsible for the vasodilation and resulting changes in blood flow in response to muscle contraction (for a review, see Refs. 11, 14, 22). On the basis of this hypothesis, changes in blood flow or indexes of blood flow have been investigated as to their relation to the metabolic demand of active skeletal muscle. Indexes of metabolism such as oxygen consumption (8), contraction strength, (2, 12, 19, 39), running speed (23), power (30), and muscular work (1, 20) have been shown to be linearly related to blood flow. Changing metabolic demand by altering basic stimulation parameters such as stimulus frequency was first shown to be related directly to the magnitude of blood flow by Anrep and von Saalfeld (2). More recently, the magnitude of blood flow (4, 16) and microvascular dilations that are responsible for these changes in blood flow (17, 24, 27, 28) have also been related to stimulus frequency. Metabolism of a skeletal muscle fiber can be altered by changing the muscle’s basic stimulation characteristics; stimulus frequency, duration of the train of stimuli, and contraction frequency. Each index of metabolism used to relate metabolism to hyperemia is determined by these basic stimulation parameters. For example, work is derived from tension (derived from the number of fibers recruited and stimulus frequency) and train duration, and these parameters that derive work have been shown to be more directly related to changes in blood flow to contracting muscle than work itself (2, 19). Therefore, the basic stimulation characteristics of skeletal muscle may be the primary determinants of the dilatory responses to skeletal muscle activity. However, there is a distinct lack of information regarding how basic stimulation parameters affect the vasculature.

In the present study, I sought to determine which stimulation characteristics of skeletal muscle were most influential in determining vasodilation in the microvasculature. To investigate this issue, a small bundle of skeletal muscle fibers in hamster cremaster muscle in situ was repetitively contracted with the chosen stimulation pattern for 2 min. The stimulation pattern was changed by systematically changing the parameters that comprise a tetanic contraction (contraction frequency, train duration and stimulus frequency). Stimulation parameters that are physiologically relevant to movement and locomotion were used where possible; this encompassed a range of stimulus frequencies within a train and short-train durations, under 1,000 ms depending on the nature of the activity (18, 31, 35, 40).

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.

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Preparations

Blood-perfused cremaster muscle in situ. Adult male Golden hamsters (100–130 g) were anesthetized with pentobarbital sodium (70 mg/kg ip) and tracheotomized. Catheters were placed in the right femoral artery and right femoral 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, 26). 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. After the surgery, all preparations were allowed to equilibrate for 45–60 min before data collection. The cremaster microvasculature was visualized by transillumination with a tungsten lamp and with an Olympus BX51WI microscope using a 20× long working distance water immersion objective [numerical aperture (na) 0.50] and a ×1.6 magnification changer. The microscope image was displayed via a video camera (Hitachi VK-C370) on a monitor and recorded on a videotape recorder (Sony, SVO-9600MD). Final magnification of the site was ×2000. Diameter measurements were reproducible to ±0.3 µm, which is ∼1–2% of the expected diameter. Transverse arterioles of ∼40 µm maximum diameter were observed. Transverse arterioles were identified as previously described (24). Briefly, capillaries were initially identified, and traced back to their inflow arteriole. The inflow arteriole was traced back to its vessel of origin, and this branch arteriole was, in turn, traced back to its arteriole of origin. If more than 2 branches arose from that vessel, it was considered a transverse arteriole (which typically has 3–8 branches) (38). Our only selection criterion required that muscle fibers associated with the transverse arteriole run approximately perpendicularly to the vessel. This architecture is common and can be found in all areas of the tissue preparation.

Muscle fiber bundles (3–5 fibers) were stimulated directly using a platinum wire microelectrode (tip diameter ~25 µm), which was placed in close proximity to, but not touching, muscle fibers running approximately perpendicularly to the arteriole. The microelectrode was positioned at least 1000 µm away from the chosen site of the arteriole/stimulated muscle fiber intersection. Curare was added to the superfusate to ensure direct electrode stimulation of the skeletal muscle cells and not nervous stimulation of the muscle fibers. Blocking nicotinic cholinergic membrane receptors on the skeletal muscle ensured that if a motor nerve were stimulated by the electrode, then the resulting acetylcholine release would not stimulate skeletal muscle cell contraction. Therefore the addition of curare isolated any muscle contraction to the control of the electrode only. The ground electrode was placed in the superfusate around the outer rim of the tissue support pedestal. Each stimulus was a square wave pulse of 0.4-ms duration and 4–10 V (Grass S48 stimulator, Quincy, MA).

The overall state of vascular responsiveness in each preparation was assessed. Only the data collected on preparations that clearly displayed arteriolar constriction to 10% oxygen and dilatation to 10⁻⁴ M sodium nitroprusside were kept for further analysis (~4% of all preparations were discarded). After each experiment, arteriolar diameters were recorded after 2 min of superfusion of the preparation with 10⁻⁴ M sodium nitroprusside (considered to be maximal diameter).

Hamster cremaster muscle in vitro. To establish the force-frequency relationship for the hamster cremaster muscle, hamsters were overdosed with pentobarbital sodium, and the left cremaster was isolated, cut longitudinally, separated from the testis and epididymus, and cut into strips. The muscle strips were tied at each end with 4.0 surgical silk and arranged in the stimulating apparatus. The distal tie was attached to a fixed anchor and the proximal tie to a hollow, stainless steel rod. The stainless steel rod was attached to a force transducer [either model FT03, Grass Medical Instruments (Quincy, MA) or model 60–2995, Harvard Apparatus (Southmatic, MA)]. The force transducer was supported by a ratchet system, which allowed the transducer to be raised and lowered for the adjustment of the length of the muscle. The stimulating apparatus was placed in an organ chamber filled with Krebs-Henseleit solution, heated to 27°C, and aerated continuously with 95% O₂-5% CO₂. The Krebs-Henseleit solution contained in mMol/l: 118 NaCl, 4.69 KCl, 1.18 KH₂PO₄, 2.52 CaCl₂, 1.18 MgSO₄·7H₂O, 24.76 NaHCO₃, 11.1 glucose, and 10 U/l insulin with tubarine (curare) added to 0.3 mg/l. The pH of the solution ranged from 7.35 to 7.45.

Muscle strips were stimulated through stainless steel electrodes using a Model S80 stimulator (Grass Instruments, Quincy, MA). Each square wave pulse was 5 ms long and of supramaximal stimulation voltage (100 V). All contractions were isometric, and the length of each muscle was set for maximal tetanic force development at 40 Hz. Data were collected and analyzed using the MP100WSW data acquisition system and AcqKnowledge III software (Biopac Systems, Goleta, CA) on an IBM computer. Experiments were conducted after a 45–60 min equilibration period. At the end of each experiment, muscle length and wet mass were measured.

Experimental Protocols

Blood-perfused cremaster muscle in situ. Arteriolar diameter at the observation site was continuously recorded for 1 min prior to muscle stimulation, for 2 min during muscle stimulation and for 2 min after muscle stimulation. Muscles were stimulated using a stimulation pattern consisting of a contraction frequency, a train duration and a stimulus frequency (Table 1). Within any given experiment, a train duration and a contraction frequency were selected and the stimulus pattern consisting of a contraction frequency, a train duration and a stimulus frequency (Table 1). Within any given experiment, a train duration and a contraction frequency were selected and the stimulus pattern consisted of a range of stimulus frequencies 1, 5, 10, 20, 30, 40, 60, and 80 Hz, in random order, for train durations of 250, 500, or 750 ms (n = 8). Muscles were then contracted with a train duration of 300 ms at 40 Hz once every 90 s for 20 min to verify that muscle force production was stable and muscle strips were not damaged.

Table 1. Combinations of stimulation pattern sets used to induce microvascular dilation

<table>
<thead>
<tr>
<th>Train Duration, ms</th>
<th>Contraction Frequency, Stimulus Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>6, 4, 10, 20, 30, 40, 60, 80</td>
</tr>
<tr>
<td>250</td>
<td>12, 4, 10, 20, 30, 40, 60, 80</td>
</tr>
<tr>
<td>500</td>
<td>6, 4, 10, 20, 30, 40, 60, 80</td>
</tr>
<tr>
<td>500</td>
<td>12, 4, 8, 15, 20, 30, 40</td>
</tr>
<tr>
<td>500</td>
<td>24, 4, 8, 15, 20, 30</td>
</tr>
<tr>
<td>750</td>
<td>6, 4, 10, 20, 30, 40, 60, 80</td>
</tr>
</tbody>
</table>
Data Analysis and Statistics

All data are reported as means ± SE. Arteriolar diameters are expressed as change in diameter (diameter-baseline diameter) in micrometers. 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 arterioles observed. All in situ experiments were videotaped and analyzed off line. Images were digitized and arteriolar diameters were measured via Image-Pro Plus software. Diameter was measured before stimulation, every 10 s during the 2 min of stimulation and every 30 s for 2 min after stimulation.

In hamster cremaster muscle strip experiments in vitro, force developed by each contraction was normalized to mN/mm². Normalization of force to mN/mm² involves dividing the force developed in grams by the weight of the muscle to calculate a force per cross-sectional area [mN/mm² = grams × (muscle length/muscle weight) × gravity × muscle density]. The cremaster muscle is a sheet muscle ~5 to 6 muscle cell layers thick with fibers oriented in many different directions; thus, when muscle strips are removed, their ends tied, and are then stimulated to contract, not every layer contributes to the generation of force, but all the muscle layers contribute to the muscle weight. The mass of the active, force-generating fibers is therefore being overestimated, and normalized force is being underestimated. If only one or two muscle cell layers are correctly oriented to contribute to measured force, normalized force may be underestimated by 4–5 times. Given the error in estimating the force generated by these muscles, the rate of decrease in developed force in the control contraction period was used as a marker of the viability of the preparation rather than the absolute amount of force developed.

Group means were compared with a repeated measures ANOVA. If significance were found at the P < 0.05 level, a protected least squares difference test was used post hoc to analyze the data further. 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 (36).

RESULTS

Blood-perfused cremaster muscle in situ.

The baseline and maximum diameters of the transverse arterioles in the various stimulation pattern protocols are presented in Table 2. Within a given stimulation pattern, the baseline diameters were not significantly different from each other with two exceptions, within the 250-ms, 12-contractions per minute (cpm) group and at 500 ms, 24 cpm (see Table 2). Maximum sodium nitroprusside-induced diameters were not significantly different from each other with one exception, maximal diameter for 500 ms, 12 cpm, was significantly larger than all other groups. Figure 1A shows that there was little correlation ($r^2 = 0.001$) between baseline diameter and the ability of the arteriole to change in diameter in response to muscle contraction at 2 min for all data at 40 Hz. To further assess any relation between the ability of the vessel to dilate and baseline vessel conditions, the change in diameter was plotted against an indicator of the potential for the vessel to dilate (maximum diameter-baseline diameter) for all data at 40 Hz (Fig. 1B). Again, there was very little correlation between the two ($r^2 = 0.23$). This observation was similar for all stimulus frequencies. Thus, neither the baseline diameter nor the potential for the vessel to dilate were primary determinants of the change in dilation that occurred in response to muscle contraction.

Table 2. The baseline diameters of the arterioles before stimulation and their maximum diameter

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Baseline Diameter (μm)</th>
<th>Maximum Diameter, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 ms</td>
<td>6 cpm</td>
<td>12 cpm</td>
</tr>
<tr>
<td>4</td>
<td>13 ± 2</td>
<td>13 ± 1.0</td>
</tr>
<tr>
<td>8</td>
<td>16 ± 2</td>
<td>16 ± 1.1</td>
</tr>
<tr>
<td>10</td>
<td>16 ± 2</td>
<td>16 ± 1.2</td>
</tr>
<tr>
<td>15</td>
<td>16 ± 2</td>
<td>16 ± 1.2</td>
</tr>
<tr>
<td>20</td>
<td>13 ± 2</td>
<td>16 ± 1.1</td>
</tr>
<tr>
<td>30</td>
<td>14 ± 2</td>
<td>17 ± 1.1</td>
</tr>
<tr>
<td>40</td>
<td>14 ± 2</td>
<td>17 ± 1.1</td>
</tr>
<tr>
<td>60</td>
<td>14 ± 2</td>
<td>17 ± 1.2</td>
</tr>
<tr>
<td>80</td>
<td>15 ± 2</td>
<td>18 ± 3</td>
</tr>
</tbody>
</table>

Maximum diameter was established by 10–4 M sodium nitroprusside for each stimulation pattern set used. Each stimulation pattern consisted of a train duration (250, 500, or 750 ms) at a contraction frequency of 6, 12, or 24 contractions per minute (cpm). Within each train duration, stimuli were delivered over a range of stimulus frequencies (Hz). n, number of preparations in each group; §Significantly different from other maximum diameters. ‡Significantly different from 4 Hz 250 ms 12 cpm. $Significantly different from 30 and 60 Hz 250 ms 12 cpm. *Significantly different from 20 and 40 Hz 250 ms 12 cpm.

It was necessary, for proper interpretation of the data that none of the stimulation parameters induce a maximum diameter change in the blood vessels. If maximum diameter is achieved, then our bioassay system loses the ability to detect further changes in vasodilation. Therefore, the stimulation parameters were chosen to avoid producing a maximum dilation in the arterioles. This was true in all stimulation pattern protocols with two exceptions, three of the seven vessels at 500 ms 24 cpm, 30 Hz and two of seven vessels contracting at 40 Hz produced 99 or 100% of the maximum diameter recorded for that vessel seen on Fig. 1B. Data from these vessels were included, as the maximum was only reached by 110 or 120 s, so the trends in the changes in diameter over time still contain useful and accurate information. Figure 1B shows a line indicating the maximum dilation possible for each vessel under each stimulation pattern set and that all dilations at 40 Hz, except the two exceptions mentioned above, fall below this maximum. Three vessels stimulated at 30 Hz reached maximal diameter, but all other vessels at all other frequencies were below their maximum dilatory potential. Thus we did not systematically reach maximum dilation. This emphasizes that the primary criteria for the muscle stimulation patterns was met and does not impact on the interpretation of the data.

The dilation that occurred over the 2-min stimulation period for each stimulus frequency for each stimulation parameter set is shown in Fig. 2. It is interesting to note that at least 40% of the total dilation occurred within the first 10 s in 43% of the arterioles and in 83% of the vessels 25% or more of the total dilation occurred within the first 10 s. Therefore, in most cases, there is a fast dilatory component early in the stimulation bout followed by a slower phase of dilation, secondary to this initial fast phase.
Figure 3 shows the relationship of the change in diameter over a range of frequencies, while changing individual stimulation variables. Figure 3A shows that the change in diameter over a range of stimulus frequencies at 20 s and 120 s of stimulation with train duration of 750 ms at 6 cpm followed a similar pattern of dilation at a given stimulus frequency, the only difference being that the magnitude of the dilation was larger at 120 s. Given the similarities of the dilatory patterns at times early and late in the contraction period, the remainder of the data will focus on values at 120 s of contraction.

Figure 3B shows the relationship between the change in diameter and stimulus frequency at different train durations and contraction frequencies. The relationships have an initial component from 4 to 20 Hz (250 ms, 6 cpm; 0.28 ± 0.03 μm/Hz; 500 ms, 6 cpm; 0.33 ± 0.03 μm/Hz; 750 ms, 6 cpm: 0.30 ± 0.06 μm/Hz; 250 ms, 12 cpm: 0.37 ± 0.05 μm/Hz) that was more than twice the rate of the secondary component from 30 to 80 Hz (250 ms, 6 cpm: 0.10 ± 0.03 μm/Hz; 500 ms, 6 cpm: 0.08 ± 0.03 μm/Hz; 750 ms, 6 cpm: 0.12 ± 0.03 μm/Hz; 250 ms, 12 cpm: 0.05 ± 0.005 μm/Hz). These data also show that there is very little effect of train duration on the rates of change in diameter as the rates of both the fast and the slow components do not significantly differ between any of the durations at 6 cpm.

A more direct indication that train duration had little effect on the magnitude of the dilation is shown in Fig. 3C. Figure 3C shows the relationship between frequency and the change in dilation at different train durations when the contraction frequency is constant at 6 cpm. Although some of the stimulus frequencies at 500 ms differ significantly from both 250 and 750 ms, the pattern and magnitude of the dilation between 250 and 750 ms do not differ. Thus the amount of dilation did not systematically differ when train duration was altered.

Figure 3D shows the effects of changing contraction frequency, with train duration constant (500 ms). The change in diameter across all stimulus frequencies at 6 cpm were significantly different than 24 cpm, and both showed an initial component from 4 to 20 Hz (500 ms, 6 cpm: 0.33 ± 0.03 μm/Hz; 500 ms, 24 cpm: 0.64 ± 0.09 μm/Hz) and a slower secondary component from 20 to 40 Hz (500 ms, 6 cpm: 0.02 ± 0.14 μm/Hz; 500 ms, 24 cpm: 0.01 ± 0.0001 μm/Hz). This differed from the dilatory pattern at 12 cpm, which was linear across all stimulus frequencies (slope = 0.38 ± 0.03 μm/Hz).

Figure 4 shows the relationship between stimuli per minute and change in diameter. This presentation allows for the comparison of the dilatory response across a wide range of possible stimulation patterns. The number of stimuli per minute was calculated as the product of the duration, stimulus frequency, and the contraction frequency. A direct comparison of the magnitude of the dilation associated with twitch unfused tetanic and fused tetanic contractions is highlighted in this figure. The type of contraction was determined by the in vitro force-frequency relationship. Five stimulation patterns, 250 ms at 4 Hz at 6 and 12 cpm and 500 ms at 4 Hz at 6, 12, and 24 cpm resulted in a series of single, individual twitch contractions, while the rest of the points represent unfused (below 30 Hz) or fused (30 Hz and above) tetanic contractions.

The relationship between stimuli per minute and dilation (Fig. 4) was curvilinear ($r^2 = 0.79$), indicating that as the number of stimuli per minute increased, the magnitude of the change in dilation increased. There was a much larger diameter change in response to lower stimuli per minute (unfused tetanic contractions) than at higher stimuli per minute (fused tetanic contractions).

Hamster cremaster muscle strips in vitro. In vitro cremaster muscle strip initial developed force averaged 94 ± 11.4 mN/mm², and the rate of decrease in force during the 20-min control contraction protocol was 0.22 ± 0.07 mN-mm⁻²·min⁻¹. This rate of decrease in force was similar to that of other isolated muscles contracting in vitro under similar stimulation conditions (25).

The in vitro relationship between force and frequency for the cremaster muscle showed that this muscle produced maximal force (a fused tetanic contraction) at ~30 Hz. There was no significant difference of the force developed between different train durations at similar frequencies (data not shown).
DISCUSSION

The purpose of this study was to determine which stimulation and contraction characteristics of skeletal muscle were most influential in determining vasodilation of the microvasculature. By systematically changing the stimulation parameters that generate a tetanic contraction, it was determined that the contraction frequency, the stimulus frequency and the total contraction period time are the most important variables in establishing the amount of dilation that occurs in the microvasculature. The amount of dilation was not largely influenced by the train duration. Among the parameters that did determine vasodilation, their effects were not constant; for example, submaximal stimulus frequencies were more influential in determining the magnitude of the change in dilation than maximal stimulus frequencies. This implies that the type of contraction may influence the magnitude of arteriolar dilation, or the amount of force generated may influence the magnitude of the dilation as unfused tetanic contractions generate less force than fused tetanic contractions.

Microvascular response to muscle contraction. In this bioassay system, the initiation of the arteriolar dilations observed represent the production and release of dilatory signals by

![Graphs showing changes in arteriolar diameter with different stimulation parameters](image-url)
active skeletal muscle fibers. Because so few muscle fibers are stimulated to contract and because of the distance between the arteriole and the contracting muscle fibers (arteriole is either above or below the contracting muscle fibers), there are no vascular compressive forces exerted on the arteriolar or the venous vasculature; thus the muscle pump does not influence flow or dilation in this preparation. There may be a small role for flow-induced dilatory modulation of the vessel diameter over time, but only once the whole arteriole has dilated. If flow-induced dilation made a significant contribution to the dilations observed in this study, then there should be a time period at which the relationships between contraction parameters and vascular diameter should have a step increase, as new dilators are introduced, but this did not occur. Therefore, any contribution of flow-induced dilation was considered small and the majority of the dilatory response observed was attributed to products released from active skeletal muscle cells.

The nonlinearity of the dilations over time (2-min stimulation period) was expected. The changes in dilation over time are the result of the rate of production of dilators and their rate of removal either by degradation or removal by blood flow. Thus over time an equilibrium or steady state dilation would be reached. What was not expected, given this scenario, was the large percentage of the total dilation that occurred within the first 10 s. This large, early dilation may indicate that dilation is determined by certain factors early in the contraction bout but by different factors, as the muscle continues to contract over time. A variety of patterns of changes in blood flow have been observed (5), and these patterns were dependent on the contractile activity of the skeletal muscle. Large changes in flow were observed at the onset of contraction followed by either an increase, a decrease, or no change in flow depending on the contractile activity of the muscle. Similar patterns to each of these flow patterns were mimicked in the current experiments by the arteriolar dilatory response depending on the stimulation pattern of the muscle. Further studies will be required to better understand how blood vessels respond to muscle contraction within the first 10 s and what determines the pattern of dilation over time.
The relationship between vasodilation and stimulus frequency was not a simple linear relationship but a more complex curvilinear one. Vasodilation was dependent more on submaximal stimulus frequencies (below 30 Hz) than maximal stimulus frequencies. Given the metabolic hypothesis, whereby vasodilators are hypothesized to be by-products of skeletal muscle metabolism, it would be expected that if metabolism increased (i.e., an increase in ATP utilization) with an increase in stimulation frequency, then the amount of dilator produced should increase. Therefore, if a constant amount of vasodilator or vasodilators are released per stimulus or per action potential, then a positive, linear relationship between stimulus frequency and vessel diameter would be expected. The present data show that this occurred at stimulus frequencies up to 30 Hz but at higher stimulation frequencies (above 30 Hz) the slope of the relationship decreased dramatically. The change in the relationship between vasodilation and stimulus frequency coincides with the stimulus frequency at which this muscle produces a fused tetanic contraction and maximal force.

The nonlinearity of the relationship between vasodilation and a wide range of stimulus frequencies could indicate that 1) there is not a constant release of dilator per stimulus (per action potential), 2) the type of dilator changes once the skeletal muscle tetanic contraction becomes fused and generates maximal force, and 3) changes in reactivity of the vascular smooth muscle occur. The change in vascular smooth muscle reactivity could be due to changes in the smooth muscle’s ability to dilate as the smooth muscle cell changes length (the length-tension relation) (13, 34). Figure 1A shows a lack of relationship between the change in diameter to skeletal muscle contracting at 40 Hz and the baseline diameter of the vessels (from 5 to 30 μm). This figure demonstrates that a 25 μm difference in microvascular diameter (larger than the dilations induced in this study) does not significantly alter the vessels reactivity to skeletal muscle contractile activity. Thus it would appear that the length-tension relation of smooth muscle does not significantly impact the dilatory response to skeletal muscle contractile activity or the shape of the relationship between vasodilation and stimulation parameters. Changes in reactivity of the smooth muscle could also result from the saturation of processes such as receptor-mediated responses. Understanding the impact of this on the relationship between vasodilation and stimulus parameters would first require the identity of the vasodilators themselves. Further studies will be required to determine the cause of the nonlinearity of the relationship between stimulus frequency and vasodilation.

Contraction frequency was also a large contributor in determining the magnitude of the dilation. Given the metabolic hypothesis, if metabolism increased due to an increase in contraction frequency, then the amount of dilator produced should increase. There is evidence that the rate of ATP hydrolysis increases with a step increase in contraction frequency (15, 37), but how ATP hydrolysis changes over a range of contraction frequencies is unknown; thus, on the basis of metabolism, how contraction frequency effects dilator production is unknown. The relationship between contraction frequency and vasodilation may be influenced by the time between contractions. At lower contraction frequencies, there is more time between contractions, therefore, more time for removal of the dilatory signal produced from the first contraction before the next contraction. At higher contraction frequencies the subsequent production of dilatory signal will be arriving at the blood vessel before the diameter has returned to precontraction values. Thus the relationship between contraction frequency and dilation is a complex function of multiple variables.

Train duration had very little impact on the magnitude of the microvascular dilation. If vasodilators are produced on the basis of the number of stimuli delivered, then at any given stimulus frequency, the longer the train of stimuli (train duration), the more stimuli would be delivered, thus longer train durations should produce larger dilations. This did not occur. Train duration had an inconsistent effect on the magnitude of the dilation, but the dilation in response to contractions of 250- and 750-ms-train duration were not significantly different. Thus train duration is not a large contributing factor in determining dilator production. There are reports to suggest that the duration of a contraction is not linearly related to metabolic cost of the contraction. Studies have repeatedly shown that brief, intermittent contractions produced a greater ATP turnover than longer sustained contractions (7, 9, 21, 29, 32, 33). It has been demonstrated that the rate of ATP hydrolysis decreased as the train duration increased (33). If metabolism is not directly related to train duration, then the amount of dilator produced via metabolism may not be directly related to train duration, and therefore, we would not expect to see dilation increase with increases in train duration.

In the present experiments, twitch (250 ms, 4 Hz), unfused tetanic contractions (4–20 Hz at most train durations), and fused tetanic contractions (30 Hz and above at any train duration) were used to stimulate the microvasculature. This range incorporated stimulation patterns that are physiologically relevant; however, a difficulty lies in comparing these different contraction types. For example, when comparing the amount of dilation at 20 Hz for 250, 500, and 750 ms, the result is 4, 10, and 15 stimuli per contraction which produce ~40, 55, and 70% of maximal developed force, thus comparing points where the force generated, and possibly the metabolic state of the muscles, were very different. One approach to compare the different stimulation patterns is to plot the dilation as a function...
of the stimuli delivered per minute regardless of how they were delivered (6). The overall relationship between stimuli per minute and dilation was curvilinear whereby the slope of the relationship changes as stimuli per minute increases. Thus lower stimuli per minute were more instrumental in determining the magnitude of the change in dilation than high stimuli per minute; greater changes in dilation occurred with unfused tetanic contractions, but once the contraction was fused no further dilation occurred. This pattern is similar to the relationship between dilation over a range of stimulus frequencies, indicating, again, a difference in the vasodilatory response from unfused and fused tetanic contractions (or between submaximal and maximal force production). Vasodilatory responses have been shown to be determined by the total tension developed by muscle cells (39), tension being altered by changing stimulus frequencies from submaximal up to maximal, but the dilatory response to frequencies above maximal were not tested. Data presented here would predict that using frequencies above those necessary to produce maximal force would not produce a further increase in vasodilation. Whether force generation is a primary determinant driving vasodilation could not be tested with the current model but certainly requires further investigation.

Implications for dilator production. If skeletal muscle metabolism is responsible for producing the vasodilators during hyperemia, then by altering the stimulus and contractile parameters of skeletal muscle, metabolism will also be altered, thereby potentially altering the vasodilators produced. Different metabolic byproducts may be produced as a result of the changes in metabolism that occur with different stimulation parameters. Possibly the most powerful parameter to change metabolism would be the duration of the contraction period. At the onset of contraction, the production and maintenance of ATP come from creatine phosphate; after ~30 s of contraction, the primary source of ATP generation is from glycolysis and the longer the contraction bout continues, the greater the reliance on aerobic metabolism. Thus the potential for metabolic byproducts to change over time is dramatic. Other stimulation parameters such as contraction frequency (7, 9, 15, 29, 37) and train duration (9, 21, 29, 33) have been shown to have a significant impact on ATP hydrolysis and metabolism. It has even been suggested that metabolism fluctuates within the duration of a single contraction (10). In the present study, the nonlinearity of the relationships between contraction frequency and dilation and stimulus frequency and dilation may indicate that under specific stimulation conditions, and, therefore, metabolic conditions, the type of dilator is different. Therefore, depending on the metabolic profile produced within the muscle (determined by the stimulation pattern), it is possible for one metabolic vasodilator to be predominantly produced, but there may be other stimulation conditions in which it is not. Thus, to identify the dilators produced by active muscle fibers, careful consideration must be given to the stimulation and contraction parameters that determine metabolism.

In conclusion, this study has identified that the stimulation variables that have the greatest influence on vasodilation are the contraction frequency, submaximal stimulus frequencies, and the total contraction period duration, while train duration has little impact. These results suggest that magnitude of the dilation produced by skeletal muscle contraction cannot simply be described by a constant release of dilator per stimulus or per contraction and cannot be predicted by the number of stimuli (action potentials) or the contraction frequency alone. Evidence does support the idea that the type of contraction, unfused vs. fused tetanic contractions, is also a potential determinant of the magnitude of the vasodilation induced by muscle contraction. This may imply that force is also a determinant of the amount of vasodilation, as unfused tetanic contractions produce less force than tetanic contractions, but further studies are required to better understand this relationship.

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