Rapid dilation of arterioles with single contraction of hamster skeletal muscle

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Submitted 28 February 2005; accepted in final form 3 August 2005

VanTeeffelen, Jurgen W. G. E., and Steven S. Segal. Rapid dilation of arterioles with single contraction of hamster skeletal muscle. Am J Physiol Heart Circ Physiol 290: H119–H127, 2006. First published August 12, 2005; doi:10.1152/ajpheart.00197.2005.—Skeletal muscle blood flow increases rapidly with exercise onset, but little is known of where or how the rapid onset of vasodilation (ROV) is governed within the microcirculation. In the retractor muscle of anesthetized hamsters (n = 26), we tested the following: 1) where in the resistance network ROV occurred, 2) how microvascular responses were affected by the duration of contraction, and 3) whether ROV involved muscarinic receptor activation. Single tetanic contractions were evoked using supramaximal field stimulation (100 Hz) to depolarize motor end plates. In response to a 200-ms contraction, red blood cell (rbc) velocity (V_{rbc}) in feed arteries (FA; rest: 17.8 ± 2 mm/s) increased within 1 s; a transient first peak (P1; 50 ± 7% increase) occurred at ~5 s; and a second peak (P2; 50 ± 15% increase) occurred at ~15–20 s. For vasodilation, P1 increased in frequency from proximal FA (2/7) and 1A arterioles (2/7) to distal 2A (4/7) and 3A (7/8) arterioles (P < 0.05). Relative to resting (and maximal, 10 μM sodium nitroprusside) diameters, P1 increased from proximal FA (3 ± 2% from 57 ± 5 μm) to distal 3A (27 ± 6% from 14 ± 1 μm) vessel branches (P < 0.05). P2 was manifest in all vessels and increased relative to resting diameters from FA (11 ± 3%) to 3A (36 ± 6%) branches (P < 0.01). Extending a contraction from 200 to 1,000 ms (tension × time integral from 17 ± 2 to 73 ± 4 mN·mm²/s) increased P1 and P2 for V_{rbc} and for diameter (P < 0.05) while reducing the time of onset for P2 (P < 0.05). Superfusion with atropine (10 μM) attenuated P1 of vasodilation (13% decrease) to 3A (36 ± 6%) branches. We conclude that ROV in the hamster retractor muscle is initiated in distal arterioles, increases with the duration of muscle contraction, and involves muscarinic receptor activation.

EXERCISE ONSET produces a rapid increase in blood flow to active skeletal muscle (4, 27, 34, 40). The nature of this flow increase is a topic of longstanding debate. Both the mechanical effect of muscle contraction (the muscle pump) and active dilation of resistance vessels are documented (33, 37, 42); however, the contribution of each mechanism to the immediate hyperemia remains controversial (41). The muscle pump promotes muscle blood flow through enhancing the arteriovenous pressure gradient by lowering postcapillary blood volume and hydrostatic pressure (8, 18, 35). In contrast, the alternative mechanisms underlying the rapid, active vasodilation (i.e., relaxation of vascular smooth muscle) have remained elusive (41). The rapid onset of vasodilation (ROV) has been related to the number of skeletal muscle fibers recruited as well as the work they have performed (1, 6, 13, 19, 40), suggesting a role for vasodilators produced upon neuromuscular activation. Though cholinergic, neurally mediated vasodilation remains controversial (1–4, 7, 24, 36), atropine-sensitive vasodilation has been shown in the human forearm (29), and findings from the hamster retractor muscle have implied that acetylcholine released from activation of motor end plates could “spillover” to initiate the functional dilation of arterioles and trigger ascending vasodilation of feed arteries (45).

A major limitation in understanding ROV is the paucity of information from intravital studies of the microcirculation in skeletal muscle. In the hamster cremaster (9) and retractor muscles (15, 45), a latency of ≥5 s for the onset of vasodilation has been reported in response to rhythmic contractions, but these studies were not specifically designed to resolve ROV. Nevertheless, more rapid (~1 s) onset of arteriolar dilation has been reported after single contractions in hamster cremaster (9, 22) and rat spinotrapezius muscles (20). In dogs (1, 23, 24) and in humans (6, 13, 19, 37, 40), muscle blood flow can increase within seconds after a single contraction. In the human forearm, the role of the muscle pump was challenged by finding that muscle blood flow also increased rapidly under conditions in which the pump was rendered ineffective by eliminating the hydrostatic gradient for venous pooling (37, 40). The observation that muscle blood flow continued to increase until a peak was attained nearly 5 s after the contraction further indicated that “active” vasodilation was occurring. A key role for an active vasomotor response is strengthened by finding that the prevention of vasodilation through intra-arterial K+ infusion abolished the hyperemic response to a tetanic contraction in the dog hindlimb (12).

The goal of the present study was to evaluate the behavior of the microvascular resistance network in response to a single contraction of the hamster retractor muscle. This preparation was used to enable concomitant evaluation of active tension produced by skeletal muscle fibers and the corresponding vasomotor response from proximal feed arteries through distal arterioles (15, 43, 45). As a dynamic index of the integrated vascular response from proximal feed arteries through distal arterioles (15, 43, 45), we tested the hypotheses that ROV: 1) varies with vessel branch location in the resistance network; 2) increases with the number of skeletal muscle fibers recruited as well as the work they have performed (1, 6, 13, 19, 40), suggesting a role for vasodilators produced upon neuromuscular activation. Though cholinergic, neurally mediated vasodilation remains controversial (1–4, 7, 24, 36), atropine-sensitive vasodilation has been shown in the human forearm (29), and findings from the hamster retractor muscle have implied that acetylcholine released from activation of motor end plates could “spillover” to initiate the functional dilation of arterioles and trigger ascending vasodilation of feed arteries (45).
duration of contraction; and 3) involves the activation of muscarinic receptors.

METHODS

Animal Care and Preliminary Surgery

All procedures were approved by the Institutional Animal Care and Use Committee of The John B. Pierce Laboratory and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male golden hamsters (85–115 g, n = 26; Charles River Breeding Laboratories, Kingston, NY) were maintained at 24°C on a 14 h:10 h light-dark cycle and were provided rodent chow and water ad libitum. Surgical procedures typically required ~3 h and were performed using a stereo microscope. Hamsters were anesthetized with pentobarbital sodium (60 mg/kg ip) and tracheotomized with polyethylene tubing (PE-90) to ensure airway patency. Cannulas (PE-50) were secured (4–0 silk suture) in the left femoral vein to maintain anesthesia and fluid balance during experiments (10 mg/ml pentobarbital sodium in isotonic saline; infused at 410 µl/h) and in the right carotid artery to monitor systemic blood pressure, which remained stable (90–100 mmHg) during experiments (44). Depth of anesthesia was maintained according to stability of blood pressure, spontaneous rate of ventilation, and lack of withdrawal to toe pinch. Esophageal temperature was monitored with a thermocouple wire and maintained at ~37°C using conducted and radiant heat. At the end of the experimental procedures, the hamster was given an overdose of pentobarbital intravenously.

Retractor Muscle Preparation

The anesthetized hamster was positioned on a custom-built transparent acrylic platform, and the right retractor muscle was prepared as described (44). Briefly, an incision 2–3 cm long was made through the overlying skin. The exposed tissue was superfused continuously at ~5 ml/min with fresh bicarbonate-buffered physiological salt solution (pH 7.4) of the following composition (mM): 131.9 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, and 18 NaHCO₃. The physiological salt solution was equilibrated with 95% N₂-5% CO₂ (pH 7.4, further maneuvers were done at 35°C); reagents were obtained from J. T. Baker (Phillipsburg, NJ) or Sigma-Aldrich (St. Louis, MO). The effluent solution was aspirated continuously with a vacuum line. Overlying connective tissue was removed by dissection, the muscle was severed from its origin and insertion, and the same protocol was performed on each vessel. Muscle contraction was controlled using stimulation parameters that we have described (15, 43).

Muscle Fiber Activation and Tension Production

Platinum electrodes (50 × 3 × 0.2 mm) were positioned along either side of the muscle. Muscle contractions were evoked using supramaximal field stimulation to depolarize motor end plates (140 V, ~120 mA, 0.1-ms pulse duration) delivered via a stimulus isolation unit (SIU-5; Grass Instruments, Quincy, MA) driven by a monopolar square-wave stimulator (S48; Grass) (15, 44). As performed in these experiments, electrical field stimulation did not activate perivascular nerves, alter vasomotor tone, or interfere with vasomotor responses (15, 43–45). Optimal muscle length (Lₒ) for producing twitch tension was determined, and the muscle was equilibrated for ~60 min; all further maneuvers were done at Lₒ (15, 43–45). Muscle tension was recorded using a load beam (LCL-113G, Omega; Stamford, CT; resolution: ±0.1 g) mounted on the caudal micrometer spindle and coupled to an amplifier (TBM-4, World Precision Instruments; Sarasota, FL). Peak active tension was defined as the difference between maximal developed tension and resting tension. At the end of an experiment, muscle length at Lₒ was measured (~±0.1 mm), the muscle was severed from its clamps and weighed (~±0.1 mg), and cross-sectional area was calculated to determine the specific active tension per area of muscle cross section (mN/mm²) with muscle density equaling 1.056 mg/mm³. The tension × time integral (TTI; mN/mm² × s) above the resting baseline was calculated as an index of the total active tension developed during a contraction (15, 43).

Video Microscopy

Vessels were observed using brightfield microscopy [Zeiss ACH/APL condenser; numerical aperture (NA), 0.32] and a Leitz UM32 (NA, 0.20) objective. The projected image was captured by a CCD video camera (C2400; Hamamatsu, Japan) and displayed on a video monitor (PVM 1343 MD; Sony, Japan). Total magnification at the monitor face was ×860. Internal vessel diameter (D) was measured with a video caliper at the widest point of the vessel lumen or column of red blood cells; the caliper was calibrated against a stage micrometer (resolution: ±2 µm). The absolute change in diameter was calculated as the difference between the peak response diameter and the preceding resting diameter. As an index of the total vasomotor response to a contraction, the diameter × time integral (DTI; µm × s) above the resting baseline was calculated (15, 43).

To provide a dynamic index of the integrated blood flow response into the muscle, centerline V̇rbc was monitored in proximal FA using an optical Doppler velocimeter (Microcirculation Research Institute, College Station, TX). Tissue movement precluded diameter and V̇rbc measurements during muscle contraction; therefore, these measures were obtained before and immediately after muscle stimulation (15, 44, 45). Data were acquired at 100 Hz using a MacLab system coupled to a Macintosh IVRX computer.

Experimental Protocols

Our goal was to investigate the effect of single muscle contractions on vasomotor activity throughout the resistance network of skeletal muscle. With the use of the retractor muscle preparation, the proximal FAs and primary arterioles (1A), which control total blood flow into the muscle, were studied along with distal second- (2A) and third-order (3A) arterioles, which govern the distribution and magnitude of blood flow within the muscle. During an experiment, a single tetanic contraction was produced while vessel diameter (all branches) or V̇rbc (FA only) responses were measured. The preparation was allowed to recover until resting diameter was restored (~2 min), another contraction was produced, and responses were reevaluated. A total of 40 vessels were studied in 26 hamsters. When multiple vessels were studied in a given preparation, they were from different branch orders, and the same protocol was performed on each vessel. Muscle contraction was controlled using stimulation parameters that we have defined for this preparation (15, 43–45). Preliminary experiments indicated that stimulation at 100 Hz (which elicits peak tetanic tension) consistently produced robust increases in V̇rbc in FA in response to a single tetanus. Therefore, 100 Hz was used throughout each of the three experimental protocols performed in this study. At the end of the experiments, maximum vessel diameters were measured during equilibration with sodium nitroprusside (SNP, 10 µM) in the superfusion solution (15, 43–45).

Protocol 1. The change in V̇rbc in FA reflects integrated vasomotor responses throughout the resistance network. Thus dilation of branches downstream from FA will increase total network conductance and produce a corresponding increase in V̇rbc, particularly when FA diameter remains constant (see RESULTS). Although the actual site(s) of vasodilation is unknown under these conditions, FA blood
flow and wall shear rate will increase in proportion to $V_{rbc}$. To evaluate where ROV actually occurred, diameter responses to single contractions (200 ms) were evaluated in 1A, 2A, and 3A arteriolar branches as well as in the parent FA.

**Protocol 2.** The effect of changing the duration of a contraction on ROV was evaluated by varying the stimulus train (randomized across preparations) from 200 to 1,000 ms using 200-ms intervals. Responses of $V_{rbc}$ in FA or the diameter of proximal (FA and 1A) versus distal (2A and 3A) branches of the resistance network were measured. For additional perspective, a 100-ms train was also evaluated in light of $V_{rbc}$ dynamics.

**Protocol 3.** The activation of muscle fibers as performed here (i.e., indirect stimulation) occurs through acetylcholine release at motor end plates as confirmed by inhibition with $d$-tubocurarine, a nicotinic receptor antagonist (15, 43). From the observed ROV (end plates as confirmed by inhibition with $d$-tubocurarine, a nicotinic receptor antagonist (15, 43)) we tested whether muscarinic cholinergic receptors were stimulated to produce vasodilation (45). Thus microvascular responses to single contractions (200 ms) were compared before and after the addition of atropine sulfate (a muscarinic receptor antagonist) to the superfusate (10 $\mu$M; Sigma-Aldrich). To control for the effect of time, separate muscles were stimulated in an identical manner without exposure to atropine.

**Data Presentation and Statistical Analyses**

Representative traces are shown to illustrate characteristic responses of $V_{rbc}$ and diameter. Responses to single contractions were typically biphasic in nature (see RESULTS). The time-to-peak of the second component was determined for $V_{rbc}$ in FA and for diameter in respective vessel branches. To facilitate comparisons across experiments and vessel branch orders, peak vasodilator responses were expressed relative to respective resting baseline diameters and to maximum diameters obtained with SNP. To evaluate the overall effects of atropine in the resistance network, only vessels in each branch order that exhibited a rapid dilation were considered and the analysis was pooled across branch orders.

Statistical analyses were performed using SigmaStat (version 3.0; SPSS, Chicago, IL). The effect of branch order on the frequency of vasodilatation to a 200-ms contraction (protocol 1) was evaluated using a $\chi^2$-test. The effect of branch order on vasodilator responses was tested by a one-way analysis of variance on pooled (responding + nonresponding) vessels. The effect of contraction duration on the vasodilator response (protocol 2) was evaluated with $\chi^2$-tests and with repeated measures analyses of variance on pooled vessels. When significant main effects were obtained, post hoc comparisons were performed using Tukey or Dunn’s tests. The effect of atropine on diameter and $V_{rbc}$ responses (protocol 3) was evaluated with paired $t$-tests. Results were accepted as statistically significant with $P \leq 0.05$.

**RESULTS**

**Protocol 1: A Single Contraction Evokes Biphasic Dilations in Distal Arterioles**

A single tetanic contraction for 200 ms consistently produced robust, biphasic increases in the $V_{rbc}$ of FA. The increase began immediately after contraction and reached an initial peak within 5 s (Fig. 1A, left); $V_{rbc}$ then returned to baseline values within ~10 s. With the muscle at rest, a second increase in $V_{rbc}$ ensued, which peaked at 15–25 s after contraction. Hereafter, the initial peak increase is referred to as P1 and the delayed peak as P2. The change in diameter of individual responding vessels typically mimicked $V_{rbc}$ responses in FA, with a transient dilation (P1) present immediately after muscle contraction followed by a delayed second peak (P2; Fig. 1A, right). Visual inspection confirmed that vasodilatation was underway by the end of contraction (time = 0 s); the apparent 2- to 3-s delay in dilation onset for P1 (e.g., Fig. 1A, right) reflects the time required to refocus and adjust the video caliper.

The dilator response of individual vessels varied among preparations (e.g., Fig. 3A, which illustrates that P1 and P2 were not consistently separated by a complete return to resting baseline) and the peak response varied across branch orders (Fig. 1B). The P1 for diameter was typically absent or small in FA and 1A while distinctly present in 2A and 3A. Thus the proportion of vessels with a P1 response increased from proximal (FA) to distal (3A) branches ($P < 0.05$; Fig. 1B, top left). In accord with the increase in frequency of a P1 response from proximal to distal vessels, the magnitude of P1 pooled across vessels within a given branch order increased from proximal to distal branches ($P < 0.05$, closed bars). This gradient in responsiveness was apparent when expressed relative to resting baseline diameter (Fig. 1B, top left) as well as to maximum diameter (Fig. 1B, top right). When only responding vessels were considered, P1 was not significantly different across branch orders (Fig. 1, open bars). The P2 for dilation occurred in all vessel branch orders (Fig. 1B, bottom) and increased from proximal to distal branches ($P < 0.01$) when expressed relative to baseline diameter (Fig. 1B, bottom left). When expressed relative to maximum diameter, P2 was not significantly different across branches (Fig. 1B, bottom right).

**Protocol 2: $V_{rbc}$ and Diameter Increase With Duration of Contraction**

For tetanic contractions at 100 Hz, peak active tension remained constant (Fig. 2A, left), whereas TTI increased significantly ($P < 0.001$) with train duration (Fig. 2A, right). Representative records and summary data in Fig. 2B illustrate that as the duration of contraction increased from 100 to 1,000 ms, the P1 and P2 $V_{rbc}$ responses in FA increased ($P < 0.05$ and $P < 0.001$, respectively) and P2 occurred earlier (for 100-, 200-, 400-, 600-, 800-, and 1,000-ms contractions, P2 occurred at 22.8 ± 1.4, 19.5 ± 1.5, 16.1 ± 0.6, 16.4 ± 1.0, 12.8 ± 0.6, and 13.8 ± 1.6 s, respectively; $n = 3–9$ for each duration; $P < 0.001$).

Increasing contraction duration resulted in the appearance of a P1 vasodilator peak for some experiments (see Fig. 2C, left) in which P1 was absent after a 200-ms contraction. The proportion of vessels with a P1 response was greater in distal (2A + 3A) arterioles versus proximal (FA + 1A) vessel branches ($P < 0.001$). Pooled across all vessel branches, P1 dilation increased with contraction duration whether expressed relative to baseline (Fig. 2C, top center; $P < 0.05$) or to maximum diameters (Fig. 2C, bottom center; $P < 0.05$). In addition, P1 dilations were greater in distal versus proximal branches when expressed relative to baseline ($P < 0.001$) but not to maximum diameters.

For P2, dilation was present in all vessel branches and its magnitude increased with contraction duration from 200 to 1,000 ms when expressed relative to baseline ($P < 0.001$) and to maximum ($P < 0.001$) diameters (Fig. 2C, right). Across vessel branch orders, post hoc comparisons revealed greater P2 dilations for train durations ≥400 ms versus 200 ms ($P < 0.05$). Consistent with P1 responses, the P2 dilations were...
Fig. 1. Vasodilator responses to single tetanic contractions increase with branch order. A, left: representative record of red blood cell (rbc) velocity (V_{rbc}) in a feed artery (FA) (top trace) and active tension (T) of the retractor muscle (bottom trace) with single contraction at 100 Hz for 200 ms. Right: representative recording of diameter in a third-order arteriole with similar contraction. Time = 0 indicates end of contraction. Note biphasic responses (P1, P2) in V_{rbc} and diameter. B, top: P1; bottom: P2. Left: summary data for peak V_{rbc} in FA expressed relative to resting baseline V_{rbc} (17.8 ± 2 mm/s) and for peak dilation expressed relative to resting baseline diameter [FA: 57 ± 5; first-order arteriole (1A): 30 ± 6; second-order arteriole (2A): 20 ± 2; third-order arteriole (3A): 14 ± 1 μm]. Right: summary data for peak dilation expressed relative to maximum diameter (FA: 90 ± 9; 1A: 61 ± 6; 2A: 51 ± 4; 3A: 29 ± 3 μm). Top left: total number of vessels studied in each branch order is given below the x-axis label and applies to all panels; open bar, number of respective vessels responding for P1 (also applies to right panel). For P1 dilation, summary data are presented for all vessels pooled (solid bars = responding + nonresponding vessels) and for responding vessels only (open bars), P2 occurred in all vessels. #P < 0.05 from proximal (FA) to distal (3A) vessels (main effect of branch order pooled for responding and nonresponding vessels).

Protocol 3: Vasodilation to Single Contraction Involves Muscarinic Receptor Activation

The effect of muscarinic receptor blockade was evaluated for seven experiments in which the vasomotor response clearly exhibited both P1 and P2 components. Addition of atropine to the superfusate had no effect on resting vessel diameters (Fig. 3A). However, atropine altered vasomotor responses to contraction significantly. Across vessel branches, the DTI was diminished by ~45% (Fig. 3B, left; P < 0.05) and was attributable to a ~75% reduction in P1 (P < 0.05), whereas P2 was sustained (Fig. 3B, right).

Peak active tension was 85 ± 5 mN/mm² before and 73 ± 5 mN/mm² during atropine (P < 0.05). In separate vessels evaluated for time controls (n = 7), stimulation a second time without atropine treatment was also associated with a significant reduction in peak tension (90 ± 10 vs. 83 ± 9 mN/mm²; P < 0.05), which was not significantly different from that observed during exposure to atropine. However, at similar time points, there was no attenuation of P1 dilation (relative to baseline: first contraction, 1.20 ± 0.04; second contraction, 1.20 ± 0.05), of P2 dilation (relative to baseline: first contraction, 1.32 ± 0.04; second contraction, 1.42 ± 0.05), or of DTI (first contraction, 97 ± 28 μm × s; second contraction, 125 ± 40 μm × s) in the time controls.

DISCUSSION

Muscle blood flow increases rapidly upon the initiation of contractile activity, but the mechanism has remained controversial due to the paucity of direct observations that a rapid “active” dilation of resistance vessels actually occurs. In the present study, the hamster retractor muscle preparation was utilized to obtain concomitant measures of active tension production and vasomotor responses throughout the resistance network. Our principal findings are 1) that a single tetanic contraction elicits an immediate elevation of muscle blood flow (inferred from the increase in V_{rbc} in feed arteries) that was associated with a ROV that occurred primarily in distal (2A and 3A) arterioles; 2) that ROV increased with contraction duration; and 3) that ROV involved the activation of muscar-
Rinic receptors. Moreover, ROV was followed by a delayed vasodilation that encompassed proximal (FA/H11001 A) as well as distal (2A/H11001 A) vessel branches. These direct observations of the microcirculation in the hamster retractor muscle indicate that the initiation of contractile activity is associated with a rapid relaxation of arteriolar smooth muscle that is mediated by acetylcholine. This response enables oxygen delivery to increase with minimal delay upon the initiation of contractile activity in skeletal muscle.

Rapid Onset of Vasodilation

The \( V_{\text{rc}} \) and diameter could not be determined continuously because of displacement of the observed vessel during muscle contraction, which resulted in a brief delay in obtaining these measurements upon cessation of the contraction. As shown in Fig. 1A, this delay was <1 s for \( V_{\text{rc}} \), whereas 2–3 s were typically needed to refocus and adjust the video calipers. Therefore, our initial evaluation centered on \( V_{\text{rc}} \) in FA because an increase in this variable is the most sensitive index of vasodilation occurring within the resistance network, particularly when diameter was constant at this proximal site, which was typically the case for the rapid (P1) response (Figs. 1 and 2). Indeed, the rapid response of \( V_{\text{rc}} \) in FA to a single contraction in protocol 1 was confirmed by our visual inspection that dilation of distal arterioles was occurring within the first second following a single contraction, particularly as the duration of contraction increased (Fig. 2).

Analogous to the present experimental paradigm, studies of muscle blood flow in humans have used Doppler ultrasound to measure \( V_{\text{rc}} \) in the brachial artery in response to brief con-
tractions of forearm muscles (3, 13, 37, 40). As observed here in FA, \( V_{\text{inc}} \) in the brachial artery was found to increase within 1–2 s after a single 1-s contraction. The conclusion that ROV occurred in humans was based on finding that reducing the effectiveness of the muscle pump (e.g., by elevating the forearm above the heart to eliminate venous pooling) did not abolish the immediate increase in muscle blood flow in response to contraction (37, 40, 42). Furthermore, as also observed in canine hindlimb preparations (12, 24), blood flow to the forearm remained elevated for \( >10 \) s after the contraction with a peak attained at \( \sim 5 \) s. In the present study, direct observations in the hamster retractor muscle confirmed that “active” dilation of arterioles was present on cessation of contraction and continued to increase while attaining \( P_1 \) (see representative records; Figs. 1–3). The presence of ROV varied across vessel branch orders, being confined primarily to distal (2A and 3A) arterioles (Figs. 1 and 2) despite the ability of all vessels to dilate in response to SNP. These findings are consistent with earlier observations in the rat spinotrapezius muscle, where 1-s tetanic contractions elicited greater relative dilations of terminal arterioles compared with proximal 1A and 2A branches (20). In the hamster cremaster muscle, recent studies have demonstrated ROV in transverse arterioles (of similar diameter to the distal arterioles studied here) in response to brief (250 and 500 ms) trains of muscle fiber contraction (22). Collectively, these findings indicate that distal arterioles are the site(s) at which ROV is initiated and that single contractions are an effective paradigm for discerning such responses. Functionally, a rapid and intense contraction of skeletal muscle occurs in response to injury or attack.

The rapidity with which vasodilation was initiated and its increase with contraction duration (Fig. 2) implies the release of a vasodilator that is related directly to neuromuscular activation. We found that the ROV was suppressed by atropine (Fig. 3), indicating that the activation of muscarinic receptors on arteriolar endothelial cells by acetylcholine was involved. Moreover, our time controls confirmed that ROV was intact despite a reduction in peak tension that occurs over time (15).

The apparent physiological source of acetylcholine in skeletal muscle is the motor end plate. We have previously reported that acetylcholine “spillover” from neuromuscular junctions could activate muscarinic receptors on nearby microvessels and give rise to vasodilation even when muscle fiber contraction was inhibited by nicotinic receptor blockade with \( d \)-tubocurarine (45). These direct observations in the hamster retractor muscle contrast with findings in humans and animals in which atropine did not alter immediate exercise hyperemia (2–4, 36), as well as those in which motor nerve activation following neuromuscular blockade did not increase blood flow (7, 24). Nevertheless, atropine-sensitive vasodilation has been shown in the human forearm (29).

Our findings in the hamster may be explained by differences in the topology of motor innervation between the retractor muscle, which has neuromuscular junctions dispersed throughout the muscle compared with forearm or locomotor muscles, where motor end plates are confined to the central region of the muscle (31). In the hamster cremaster muscle, which also has a disperse motor innervation, somatic neuromuscular junctions are found closest to capillaries and small arterioles (25), and a similar distribution has been confirmed in retractor muscle (G. Gonzalez-Lomas, JWGE VanTeeffelen, and SS Segal, unpublished observations). Moreover, this anatomical relationship is consistent with ROV being observed primarily in the more abundant distal (2A and 3A) versus proximal (1A and FA) branches of the resistance network.
In addition to its unique neuromuscular junction distribution, the retractor muscle is a thin, strap-like muscle (31) and is reflected away from the hamster for intravital studies. This contrasts with intact forearm or locomotor muscles that contract within anatomically defined "compartments," whereby intramuscular pressure can increase during a contraction to levels that occlude arterial inflow while expelling venous effluent (1). Thus compressive forces during contraction are small or absent under the conditions of our experiments, as well as in spinotrapezius (20) and cremaster (9, 22) preparations (i.e., exteriorized thin muscles). Nevertheless, these preparations have independently demonstrated rapid "active" vasodilation of distal arterioles, and the present experiments are the first to document how ROV is related to active tension produced by contracting muscle fibers. It should also be recognized that control experiments in which quiescent muscle fibers were manipulated to simulate the mechanical displacement of arterioles have failed to evoke a vasomotor response (9).

Supramaximal stimulation voltage ensured that all fibers in the retractor muscle were activated during a tetanic contraction. Nevertheless, ROV typically did not ascend into the proximal 1A or FA, even when the duration of a contraction was extended to 1 s. From the consistency of ascending vasodilation observed during repetitive contractions of the retractor muscle (15, 32, 43, 45), we suggest that a "threshold" level of contractile activity is required to initiate dilation of proximal (1A) arterioles and FA, which is not attained with a single contraction or during repetitive contractions once the muscle has fatigued (15). Indeed, the magnitude of ROV in 2A and 3A branches observed here was smaller than that reported for functional dilation of these branches in response to 1 min of rhythmic contractions (15, 43, 45). In contrast to ROV in the current study, latency times for the onset of vasodilation of ≥5 s were typically found in these earlier studies of the retractor muscle, as well as in the cremaster muscle (9). This difference may be explained by higher stimulation frequency (100 Hz) used in the present study and our finding that the dynamics of ROV are most accurately resolved while the muscle remains stationary following a single contraction.

Dilation of distal arterioles increases capillary perfusion (16, 38) and, thereby, the functional surface area for exchange between the blood and skeletal muscle fibers (30). Thus the manifestation of ROV in distal arterioles promotes oxygen delivery to active muscle fibers with minimal delay upon the initiation of contractile activity. Because of its small mass (70–100 mg), the contracting retractor muscle imposes negligible stress on systemic cardiovascular homeostasis (15, 43, 45). However, with a sufficiently large mass of active muscle, sustained vasomotor tone in the proximal vessels during rapid dilation of distal vasculature will limit the drop in systemic arterial blood pressure and ensure adequate perfusion of other dependent vascular beds. As contractile activity increases, central (e.g., sympathetic) cardiovascular control mechanisms are activated to maintain arterial blood pressure (28, 39). Vasodilation can then ascend into proximal resistance vessels of active muscle to provide greater total blood flow and thereby match oxygen delivery with metabolic demand (30).

The concentration of atropine used here effectively prevents vasodilation to the direct application of acetylcholine (45) yet did not completely eliminate ROV. This finding implies the contribution of at least one other signaling mechanism by which blood flow is increased rapidly in response to muscle contraction. A likely candidate is extracellular potassium ion concentration, which increases transiently in response to muscle contraction (23). Indeed, the elevation of extracellular potassium ion concentration can rapidly increase to levels that activate inward rectifier K⁺ channels as well as the Na⁺/K⁺-ATPase in arterioles, thereby promoting hyperpolarization and vasodilation (5). Although we did not investigate these signaling pathways in the present experiments, future studies aimed at defining the nature and regulation of ion channel expression in arteriolar smooth muscle and endothelium, as well as how respective cell layers are functionally coupled, should provide new and mechanistic insight to the regulation of muscle blood flow (14, 30).

Delayed Onset of Vasodilation

A secondary delayed increase in V_{rbc} was observed consistently in FA and was accompanied by a second component of vasodilation throughout the resistance network, confirming observations of biphasic arteriolar dilation after single contractions of hamster cremaster muscle fibers (9, 22). As observed for the rapid (P1) responses, the magnitude of P2 for both V_{rbc} and diameter increased with contraction duration (Fig. 2). Moreover, the time to attain P2 decreased as the duration of contraction increased (see RESULTS). These dynamics are consistent with the longstanding theory of metabolic vasodilation (10, 11). Thus, in response to muscle contraction, vasoactive metabolites released from contracting fibers accumulate in the interstitium. With an increase in contraction duration, both an earlier onset and greater magnitude of vasodilation would be predicted based on the greater production of metabolites. Indeed, such behavior has been confirmed in earlier studies of arterioles in skeletal muscle (9, 20).

In contrast to P1 being effectively constrained to distal arterioles, the delayed component of vasodilation consistently encompassed proximal 1A and FA as well, indicating that the signal underlying the delayed component was of sufficient strength to promote ascending vasodilation. However, the magnitude of P2 dilation in 1A and FA also remained substantially less than that observed in response to repetitive contractions (15, 43, 45). Conduction (e.g., of hyperpolarization) from cell to cell along the endothelium has been proposed as a key signaling pathway underlying ascending vasodilation of FA in the retractor muscle (32). Alternatively, in accord with evidence from other model systems, the delayed ascending vasodilation in FA could reflect a flow-induced response (17, 26). Thus, with no change in FA diameter, the transient 1.5- to 2-fold increase in V_{rbc} invoked by ROV in distal vessels resulted in proportional increases in wall shear stress in FA immediately after contraction. However, flow-induced vasodilation has been excluded as the mechanism for ascending vasodilation in FA of the hamster retractor muscle (32). In the rat cremaster muscle, an increase in arteriolar blood flow (and luminal shear stress) following occlusion of a parallel segment resulted in vasodilation after delays of up to ~15 s (17), which is consistent with the time required to attain P2 in the present experiments. Nevertheless, mechanisms other than flow-induced vasodilation have been proposed to explain responses of proximal segments during occlusion of a distal arteriolar branch (21). Because the present study was focused on the...
initial, rapid vasodilator response, the mechanism(s) underlying the delayed (P2) components of $V_{TBC}$ and diameter increases were not explored in further detail.

In conclusion, the present study demonstrates that, in the hamster retractor muscle, a brief tetanic contraction rapidly initiates an increase in blood flow through proximal FAs that is attributable to the nearly instantaneous (<1 s) dilation of arterioles downstream. Although ascertained by direct observations using intravital microscopy, this behavior is consistent with indirect studies performed in humans, where rapid changes in blood flow through the brachial artery have been interpreted to reflect vasomotor responses occurring within dependent forearm muscles. We suggest that the rapid onset of vasodilation can be initiated through activation of muscarinic receptors and provides a mechanism for promoting oxygen delivery with minimal delay in response to activation of skeletal muscle fibers by somatic motor nerves.

GRANTS

This research was supported by the National Heart, Lung, and Blood Institute Grant RO1-HL-56786.

REFERENCES


