Rapid vasodilation in response to a brief tetanic muscle contraction

JAY S. NAIK, ZORAN VALIC, JOHN B. BUCKWALTER, AND PHILIP S. CLIFFORD
Departments of Anesthesiology and Physiology, Veterans Affairs Medical Center
and Medical College of Wisconsin, Milwaukee, Wisconsin 53295

Naik, Jay S., Zoran Valic, John B. Buckwalter, and Philip S. Clifford. Rapid vasodilation in response to a brief tetanic muscle contraction. *J. Appl. Physiol.* 87(5): 1741–1746, 1999.—To test the hypothesis that vasodilation occurs because of the release of a vasoactive substance after a brief muscle contraction and to determine whether acetylcholine spillover from the motor nerve is involved in contraction-induced hyperemia, tetanic muscle contractions were produced by sciatic nerve stimulation in anesthetized dogs ($n = 16$), instrumented with flow probes on both external iliac arteries. A 1-s stimulation of the sciatic nerve at 1.5, 3, and 10 times motor threshold increased blood flow above baseline ($P < 0.01$) for 20, 25, and 30 s, respectively. Blood flow was significantly greater 1 s after the contraction ended for 3 and $10 \times$ motor threshold ($P < 0.01$) and did not peak until 6–7 s after the contraction. The elevations in blood flow to a 1-s stimulation of the sciatic nerve and a 30-s train of stimulations were abolished by neuromuscular blockade (vecuronium). The delayed peak blood flow response and the prolonged hyperemia suggest that a vasoactive substance is rapidly released from the contracting skeletal muscle and can affect blood flow with removal of the mechanical constraint imposed by the contraction. In addition, acetylcholine spillover from the motor nerve is not responsible for the increase in blood flow in response to muscle contraction.

blood flow; skeletal muscle; exercise; acetylcholine; muscle pump; dog

IT IS WELL KNOWN that blood flow increases to active skeletal muscle to supply additional oxygen to meet its metabolic demands. The amount of blood flow to a vascular bed is proportional to the pressure gradient across that bed and the caliber of the vessel (i.e., vascular conductance). There is a rapid rise in blood flow to the active skeletal muscle with the onset of dynamic exercise (5, 21, 23). The principal mechanism(s) that effect this rapid increase in blood flow, especially during the first few seconds of exercise, have remained elusive. Several potential mechanisms for the rapid hyperemic response to exercise have been hypothesized. These include neurally mediated vasodilation (2, 4, 5, 8, 21), the muscle pump (10, 11, 15, 20, 23, 24, 27), metabolic vasodilation (12–14, 26), and acetylcholine (ACh) spillover (9, 28).

A neural mechanism for skeletal muscle vasodilation is particularly appealing because of the rapidity of the increase in skeletal muscle blood flow at the onset of exercise. Furthermore, both sympathetic cholinergic and β-adrenergic vasodilation have been proposed as potential mediators of this response. However, two classic studies showed that surgical sympathectomy failed to alter exercise-induced blood flow responses in dogs and humans (6, 8). More recent experiments from our laboratory using pharmacological approaches have provided additional evidence that the autonomic nervous system does not play a role in the initial hyperemic response to exercise (5, 21).

The concept of the muscle pump has long been advanced as a rapid, localized mechanism by which blood flow could increase to active skeletal muscle (10, 11, 20). The muscle pump theory of skeletal muscle hyperemia suggests that contraction of skeletal muscle increases flow to its own vascular bed by squeezing blood out of venules removing the hydrostatic component of venous pressure, thereby increasing the arteriovenous pressure gradient driving flow (7, 20). Whether the muscle pump has the capability to function independent of vasodilation to alter muscle blood flow, hence acting as the sole mediator of the initial hyperemia at the onset of exercise, is controversial.

In the late 19th century, Gaskell (12) proposed that vasodilation occurred in exercising skeletal muscle because of the release of metabolites from the muscle fibers. Since that time, a number of chemical substances have been proposed as potential mediators of metabolic vasodilation in active skeletal muscle (14, 16, 26). However, studies employing in vitro techniques have suggested that metabolic vasodilation is too slow to play a role in the initial active hyperemia. These studies have shown that vasodilation has an onset latency of 5–20 s, taking 30–60 s to reach steady state levels (13, 18). However, in vivo studies demonstrate that changes in vessel caliber can occur rapidly, contributing to the initial blood flow response to exercise (6, 25, 27).

Recently, Welsh and Segal (28) demonstrated that ACh released from the motor end plate could concomitantly bind sarcolemmal nicotinic receptors, causing muscle contraction and abluminal muscarinic receptors, causing vasodilation. In addition, neuromuscular junctions have been shown to be within 89.8–111.1 µm from arterioles and within 11.9–14.5 µm from capillaries in hamster cremaster muscle (19). Because of the temporal relationship between the blood flow response to exercise and the muscle contraction itself, ACh spillover from the motor nerve terminal seems a logical candidate for mediating the blood flow response to exercise.

To test the hypothesis that vasodilation can occur because of release of vasoactive substances after a brief skeletal muscle contraction, we examined the time

course and duration of the blood flow response to a brief, tetanic contraction. We reasoned that a peak in blood flow immediately after the contraction would indicate that the muscle pump was the primary mechanism for the hyperemia. On the other hand, if the peak was delayed and the hyperemia prolonged, this would suggest contraction-induced release of a vasoactive substance. To test the hypothesis that ACh released from the motor nerve initiates the hyperemic response to exercise, the blood flow response to motor nerve stimulation was examined under both normal and neuromuscular blockade conditions. The rationale for these experiments was that neuromuscular blockade would abolish muscle contraction, but that the release of ACh at the motor end plate would be identical under the two conditions.

METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the American Physiological Society's *Guiding Principles in the Care and Use of Animals.* Sixteen mixed-breed dogs (9–20 kg) were studied. Anesthesia was induced with 100 mg/kg α-chloralose and 500 mg/kg urethan and was maintained with a constant infusion of 20 mg·kg^{-1}·h^{-1} α-chloralose and 100 mg·kg^{-1}·h^{-1} urethan. The animals were intubated with a cuffed endotracheal tube and ventilated with room air by using a mechanical ventilator (Harvard Apparatus, Dover, MA) set to a tidal volume of 15 ml/kg. End-tidal CO_2 measured with an infrared analyzer (Ohmeda, Miami, FL) was adjusted to 35–40 Torr by alterations in respiratory frequency. Just before initiation of the experimental protocol, arterial blood samples were taken for the measurement of arterial Po_2, Pco_2, and pH (model ABL-30, Radiometer, Copenhagen, Denmark). If necessary, metabolic acidosis was corrected with intravenous infusion of sodium bicarbonate.

Arterial blood pressure was measured by using an 18-gauge intravascular catheter inserted retrogradely into the lumen of the carotid artery and attached to a solid-state pressure transducer (Ohmeda). Hindlimb blood flow was measured by using transit-time ultrasound flow probes (Transonic Systems, Ithaca, NY) placed around the external iliac arteries ~1–2 cm below the terminal aorta. Air within the probe's measurement window was displaced with the use of an acoustic couplant (model 1181, Nalco) as per manufacturer's instructions. Arterial blood pressure and blood flow signals were recorded continuously and stored on microcomputer (Apple G3 Power PC) by using a MacLab data-acquisition system sampling at 100 Hz (AD Instruments, Castle Hill, Australia). Data were analyzed off-line by using MacLab software for the calculation of arterial blood pressure, heart rate, and hindlimb blood flow.

The dogs were placed in the prone position in a stereotaxic apparatus (Stoelting, Wood Dale, IL). The torso was suspended through caudal tension applied via a hip-pin clamp. With the dog in the stereotaxic frame, the hindlimb was positioned below heart level. To prevent movement during contraction, the hindlimb was secured, thus producing an isometric contraction. Contraction of the left hindlimb muscles was produced by electrical stimulation of the sciatic nerve. The sciatic nerve was exposed unilaterally and cut centrally to avoid stimulus conduction along afferent fibers. Two stimulating electrodes consisting of 0.20-mm-diameter Teflon-coated stainless steel wires (A-M Systems, Everett, WA) were

inserted and wrapped tightly around the nerve. The minimum current required to elicit a visible contraction was defined as the motor threshold (MT). Two separate protocols were used.

Protocol I (n = 8). The sciatic nerve was stimulated (20 Hz, 1 ms) for 1 s at 1.5, 3, and 10 × MT in random order to produce brief tetanic contractions. Each contraction intensity was performed in triplicate, and the data were averaged over 1-s intervals. An interval of 5 min was allowed between each contraction.

Protocol II (n = 8). The sciatic nerve was stimulated (30 Hz, 1 ms) for 1 s at 10 × MT. In addition, to mimic dynamic exercise, a 30-s train of 1-s tetanic contractions (50% duty cycle) was performed at 10 × MT. Electrical stimulation of the sciatic nerve was performed under both control and neuromuscular blockade conditions (i.e., in the absence of muscle contraction). Neuromuscular block was achieved with 0.1 mg/kg vecuronium bromide administered intravenously. Because vecuronium abolished the blood flow response, a single trial was performed for both conditions and a 1-s "peak" flow value was taken during neuromuscular block at the same time point that it occurred during control conditions. Five minutes were allowed after administration of vecuronium before sciatic stimulation was repeated.

Statistical analysis. To examine the response to muscle contraction in *protocol I*, blood pressure, heart rate, and hindlimb blood flow were analyzed by using two-way (time × intensity) repeated-measures analyses of variance. A second two-way repeated-measures analysis of variance was performed on the data at 5-s intervals to determine the duration of the blood flow response to muscle contraction in *protocol I*. To examine the response to muscle contraction in *protocol II*, blood pressure, heart rate, and hindlimb blood flow were analyzed by using a three-way (condition × type of contraction × time) repeated-measures analysis of variance. Where significant *F*-ratios were found a Tukey's post hoc test was performed. Data are presented as means ± SE. The level of statistical significance was set at $P < 0.01$.

RESULTS

Protocol I. Figure 1 depicts raw tracings of the typical hemodynamic responses observed to muscle contraction from an individual animal for the 1.5, 3, and 10 × MT contraction intensities. It can be seen that there were intensity-dependent increases in hindlimb blood flow and that there were no changes in blood pressure in response to muscle contraction. In addition, there were no changes in blood flow in the contralateral limb in response to sciatic stimulation. Careful inspection of the blood flow recordings shows that blood flow during the contraction period was reduced, with an immediate rise on cessation of the contraction (i.e., once the mechanical constraint is removed). Peak blood flow was observed several seconds after contraction ceased.

Figure 2 is a composite of the blood flow responses to the three contraction intensities averaged over 1-s intervals in all eight dogs. Peak blood flow occurred 6 ± 1, 7 ± 1, and 7 ± 1 s for 1.5, 3, and 10 × MT, respectively, after the contraction ended and was significantly greater for 10 × MT than both 1.5 and 3 × MT ($P < 0.01$). Hindlimb blood flow remained significantly elevated for 20, 25, and 30 s for 1.5, 3, and 10 × MT, respectively.
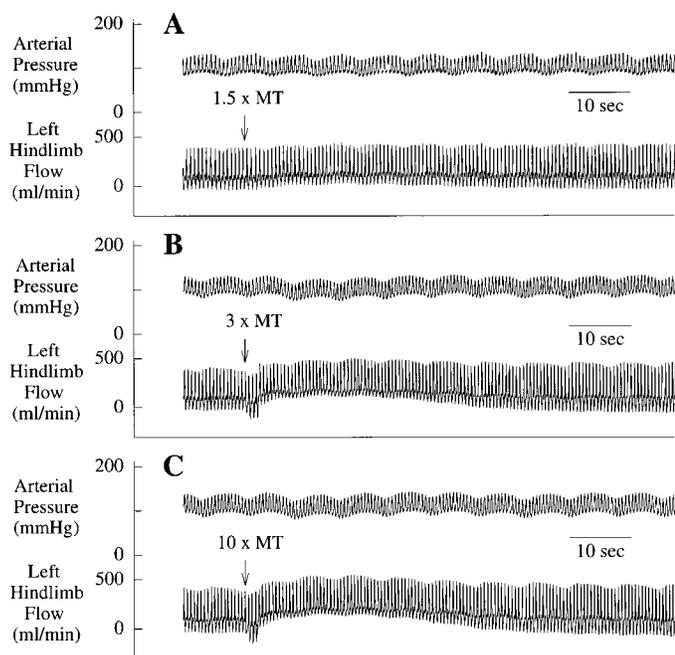
Fig. 1. Original tracings from an individual dog of blood pressure and hindlimb blood flow response to a 1-s tetanic muscle contraction at $1.5 \times$ motor threshold (MT; A), $3 \times$ MT (B), and $10 \times$ MT (C). Arrows, beginning of sciatic stimulation. Note intensity-dependent increases in iliac blood flow and that there were no changes in blood pressure in response to muscle contraction.

A summary of the mean blood flow responses to muscle contraction is presented in Fig. 3. Baseline blood flows before the initiation of the contraction were not different across the contraction intensities. During the contraction period, blood flow was reduced from baseline in a contraction intensity-dependent manner. The reduction in blood flow during contraction was statistically significant ($P < 0.01$) for the 3 and 10 $\times$ MT contraction intensities. One second after the contraction ended, blood flow was significantly elevated above baseline for the 3 and 10 $\times$ MT contractions ($P < 0.01$).
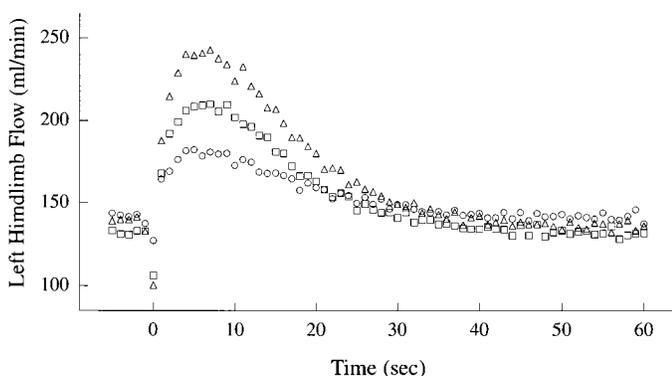

Fig. 2. Hindlimb blood flow response to muscle contraction at 3 stimulation intensities, averaged at 1-s intervals ($n = 8$ dogs). For clarity of presentation, SE bars are not included. ○, $1.5 \times$ MT; □, $3 \times$ MT; △, $10 \times$ MT. Note that blood flow was elevated above baseline 1 s after removal of the mechanical constraint imposed by the contracting muscle and that this elevation in blood flow persisted for at least 20 s.
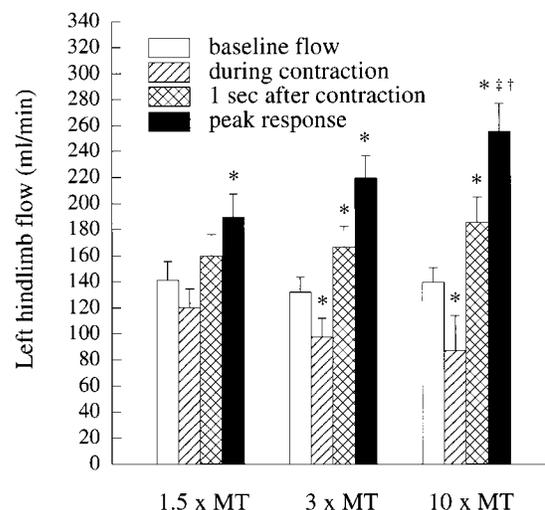

Fig. 3. Summary of blood flow response to muscle contraction for 3 stimulation intensities. Note that blood flow is reduced from baseline during contraction and is elevated above baseline 1 s after contraction. Values are means ± SE. * Significantly different from baseline, $P < 0.01$. ‡ Significantly different from $1.5 \times$ MT, $P < 0.01$. † Significantly different from $3 \times$ MT, $P < 0.01$.

There were no alterations in heart rate or blood pressure over the time periods analyzed (Table 1). Muscle contractions did not alter blood flow in the contralateral limb (73 ± 13 to 74 ± 12 ml/min, 75 ± 13 to 70 ± 13 ml/min, and 70 ± 12 to 63 ± 12 ml/min, for $1.5 \times$ MT, $3 \times$ MT, and $10 \times$ MT, respectively).

Protocol II. A raw tracing from an individual animal of the hindlimb blood flow responses under both control and neuromuscular block conditions is presented in Fig. 4. The blood flow response to a single tetanic contraction is shown in Fig 4A. Under control conditions, blood flow decreased during the contraction period, rose immediately on cessation of the muscle contraction, and remained elevated for ~25 s. Under neuromuscular block, blood flow did not respond to stimulation of the sciatic nerve (Fig. 4B).

Figure 5 summarizes the blood flow responses to sciatic stimulation in eight dogs under both control and neuromuscular block conditions. In response to muscle contraction, blood flow under control conditions rose significantly above baseline for both the single tetanic contraction and the contraction train (168 ± 35 to 306 ± 72 ml/min and 165 ± 32 to 408 ± 92 ml/min for the single and contraction train, respectively). The blood flow response to the train of contractions was significantly greater than that of the single tetanic contraction ($P < 0.01$). Sciatic stimulation after administration of vecuronium did not produce muscle contraction. Under neuromuscular block, blood flow after sciatic stimulation was not altered from baseline blood flow (172 ± 30 to 169 ± 30 ml/min and 168 ± 30 to 164 ± 29 ml/min for the single and 30-s train stimulation protocols, respectively; $P > 0.01$). Sciatic stimulation had no effect on either heart rate or blood pressure (Table 2). Blood flow in the contralateral limb was not altered by sciatic stimulation (98 ± 18 to 95 ± 17 ml/min and 93 ± 18 to 93 ± 19 ml/min under control and neuromuscular block conditions, respectively).

Table 1. *Heart rate and blood pressure responses to muscle contraction in protocol I*

Contraction Intensity	Precontraction		During Contraction		1 s After Contraction		Peak	
	Heart rate, beats/min	Blood pressure, mmHg	Heart rate, beats/min	Blood pressure, mmHg	Heart rate, beats/min	Blood pressure, mmHg	Heart rate, beats/min	Blood pressure, mmHg
$1.5 \times$ MT	167 ± 13	112 ± 6	168 ± 13	112 ± 6	167 ± 13	112 ± 5	168 ± 13	114 ± 5
$3 \times$ MT	175 ± 13	111 ± 6	176 ± 13	112 ± 6	176 ± 13	111 ± 6	179 ± 13	112 ± 6
$10 \times$ MT	168 ± 15	115 ± 6	167 ± 15	116 ± 5	167 ± 15	117 ± 6	171 ± 15	117 ± 6

Values are means $\pm$ SE. MT, motor threshold. There were no statistically significant differences among any of the time periods ($P > 0.01$).

DISCUSSION

For this study, an anesthetized animal model was employed to investigate two hypotheses of exercise hyperemia. The data show that hindlimb blood flow was significantly elevated within 1 s after cessation of a brief, tetanic muscle contraction and that this elevation in blood flow persisted for at least 20 s beyond the end of contraction. These results provide evidence that a vasoactive substance(s) is rapidly released during contraction and can contribute to the blood flow response immediately on removal of the mechanical constraint imposed by the contracting muscle. In addition, in the absence of muscle contraction, sciatic stimulation did not cause any alteration in blood flow, providing convincing evidence that ACh released from the motor nerve terminal is not responsible for initiating the elevation in blood flow in response to muscle contraction.

At the onset of exercise there is a rapid ($\sim$1- to 2-s) increase in blood flow to active skeletal muscle (5, 21, 23). However, the mechanism(s) responsible for this increase in blood flow are not completely understood. Both the muscle pump and ACh spillover have been advanced as possible mechanisms for exercise hyperemia because of their potential ability to cause a rapid, localized increase in blood flow to active skeletal muscle. However, the results of the present study suggest that the muscle pump cannot be solely responsible for the hyperemic response to a brief muscle contraction and that ACh spillover from the motor nerve terminal does not play an important role in mediating this response.

Fig. 4. Original tracing from an individual dog of blood pressure and iliac blood flow response to sciatic nerve stimulation in presence (*A*) and absence of muscle contraction (*B*). Note that under neuromuscular blockade, there was no alteration in iliac blood flow in response to sciatic nerve stimulation. Arrows, beginning of sciatic stimulation.

Although at least a portion of the initial ($\leq$1-s) rise in blood flow may be attributable to the muscle pump, the time course (rapid rise followed by a slow decline) of the blood flow curves in the present study followed the pattern expected if a vasoactive substance(s) were released from the contracting muscle and subsequently washed out by the elevated blood flow. The magnitude of the arteriovenous pressure gradient and any increase in blood flow mediated by the muscle pump should be greatest immediately after the contraction. In the present study, blood flow was elevated above baseline 1 s after the contraction ended and increased progressively until reaching a peak 6–7 s after the contraction ended. Previous investigations have reported a similar time course, with peak blood flow occurring 4–5 s after a single contraction (3, 27). The progressive increase in blood flow after a single contraction is a compelling argument against the muscle pump as the sole mediator of the observed hyperemia and is consistent with alterations in vessel caliber via release of a vasoactive substance.

Further evidence for the release of a vasoactive substance(s) during contraction is the duration of the blood flow response. Venous refilling rapidly eliminates the widened arteriovenous pressure gradient produced by the muscle pump. Indeed, Folkow et al. (10) showed that venous pressure returns to baseline levels within 1 s after muscle contraction. Thus, although the muscle pump may contribute to the hyperemic response in the first second postcontraction, any elevation in blood flow beyond this point must be due to an increase in vessel caliber (i.e., vasodilation). In the present study, the hyperemic response to a 1-s muscle contraction persisted for 20–30 s and the duration of the response was related to the intensity of contraction. Similarly, forearm blood flow remained elevated for 15–20 s after a 0.3-s voluntary contraction (6) and for >30 s after a voluntary 1 s contraction at ~20% of maximal voluntary contraction (Fig. 2 in Ref. 27). Both the rapid increase in blood flow and prolonged duration of hyperemia after a single, brief contraction suggest that a dilator substance is released from contracting skeletal muscle. Direct evidence in support of this postulate comes from the demonstration that venous effluent collected after a 0.15-s contraction of a dog gastrocnemius muscle produced vasodilation when infused into the hindlimb of a separate resting animal (1).

Theoretical calculation of the venous pressures required to achieve the observed blood flow increases suggests that the muscle pump alone cannot entirely account for this response. For the muscle pump to cause
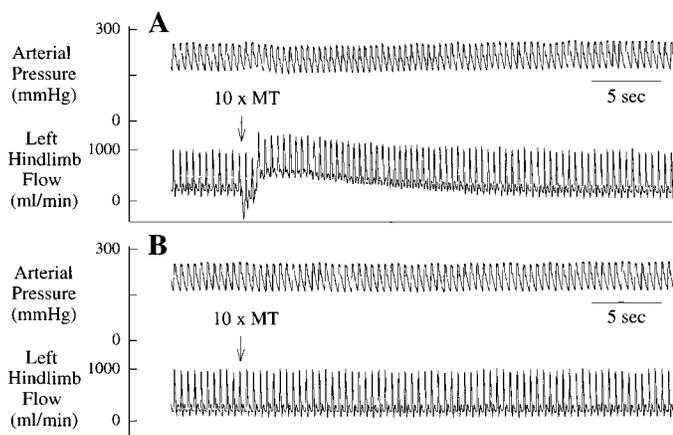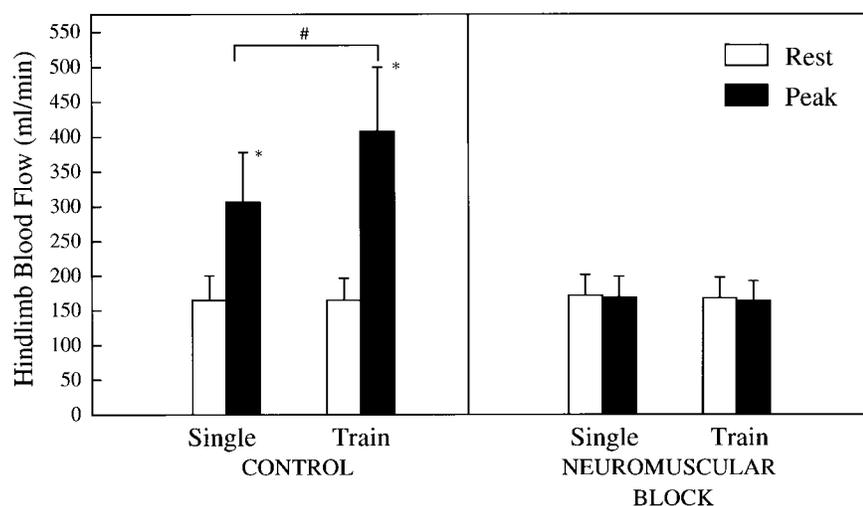
Fig. 5. Summary of the blood flow response to sciatic nerve stimulation under both normal and neuromuscular blockade conditions. Under normal conditions, blood flow rose above baseline for both types of muscle contraction ($P < 0.01$). Under neuromuscular blockade, blood flow response to sciatic stimulation was completely abolished. Values are means ± SE. Single, individual 1-s sciatic nerve stimulation (30 Hz, 1 ms, 10 × MT); train, 30-s train of sciatic nerve stimulations (30 Hz, 1 ms, 10 × MT). *Significantly different from rest, $P < 0.01$. #Significantly different from each other, $P < 0.01$.

an elevation in blood flow independent of changes in vessel caliber, venous pressure must be lowered via the expulsion of blood out of the venous vasculature by the contracting skeletal muscle. In the classic study by Pollack and Wood (Fig. 4 in Ref. 20), it was demonstrated that venous pressure measured at the ankle during human upright locomotion increased with the first step, thereafter falling by ~65 mmHg during the next four to five steps (20). More recently, Tschakovsky et al. (27) demonstrated that rhythmic mechanical compression of the forearm via inflation of a pneumatic cuff could produce an elevation in skeletal muscle blood flow. Furthermore, Laughlin (15) proposed that the sudden relaxation of the contracting muscle rapidly pulls veins apart, producing a negative venous pressure, increasing the pressure gradient even further. Our calculations suggest that the venous pressures that would have to be achieved for the muscle pump to account for the immediate increase in blood flow would be more negative than any values previously reported (22). If we take the average resting hindlimb blood flow from the present study (140 ml/min) and assume an arteriovenous pressure difference of 90 mmHg, the conductance at rest would have been 1.55 ml·min^{-1}·mmHg^{-1}. To achieve a peak blood flow of 250 ml/min (as in the 10 × MT condition in *protocol I*) with no vasodilation (i.e., the same conductance), venous pressure would have to average −51 mmHg. Under such conditions, it seems likely that veins would collapse, restricting blood flow.

A recent hypothesis has suggested that ACh released from the motor nerve terminal initiating muscle contraction could also be responsible for vasodilation by binding to abluminal muscarinic receptors (28). Welsh and Segal (28) showed that the blood flow response to contraction of hamster retractor muscle superfused with *d*-tubocurarine caused alterations in vessel diameter that were atropine and eserine sensitive. A possible explanation of the difference between their results and those of the present study is differences in the methods used to produce muscle contraction. Welsh and Segal used field stimulation, whereas direct stimulation of the motor nerve was used in the present study. The use of field stimulation exposed not only the muscle but also the vessel under observation to an electrical field, which may have affected the function of the vessel itself, perhaps through stimulation of endothelial cells. The present study examined the blood flow response to sciatic stimulation in an intact animal model, allowing a more direct test of the functional role ACh spillover plays in exercise hyperemia. Dyke et al. (9) demonstrated that the blood flow response to attempted voluntary handgrip exercise in humans was nearly abolished after neuromuscular blockade, suggesting that ACh spillover has a minimal role in mediating the blood flow response to contraction in humans. However, there could be some question about whether efferent signals were actually being conducted down the motor nerve during attempted contractions. Unlike the study of Dyke et al., the present study employed direct

Table 2. *Heart rate and blood pressure responses to muscle contraction in protocol II*

	Control				Vecuronium			
	Precontraction		Peak		Precontraction		Peak	
	Heart rate, beats/min	Blood pressure, mmHg	Heart rate, beats/min	Blood pressure, mmHg	Heart rate, beats/min	Blood pressure, mmHg	Heart rate, beats/min	Blood pressure, mmHg
Single	146 ± 14	177 ± 8	150 ± 14	176 ± 7	144 ± 14	185 ± 7	142 ± 13	185 ± 7
Train	134 ± 15	184 ± 7	155 ± 13	181 ± 7	141 ± 13	185 ± 7	139 ± 13	187 ± 6

Values are means ± SE. Single, individual 1-s sciatic nerve stimulation (30 Hz, 1 ms, 10×MT); train, 30-s train of sciatic nerve stimulations (30 Hz, 1 ms, 10×MT). There was no statistically significant effect of contraction on heart rate or blood pressure for either condition ($P > 0.01$).

electrical stimulation of the motor nerve ensuring the same release of ACh under both conditions.

In summary, although the present study cannot discount the possibility that the muscle pump is responsible for a portion of the initial hyperemic response to a 1-s muscle contraction, our results suggest that a vasoactive substance is rapidly released during contraction and can contribute to the blood flow response immediately on removal of the mechanical constraint imposed by the muscle contraction. In addition, the data clearly demonstrate that ACh spillover does not play an important role in the hyperemic response to muscle contraction.

REFERENCES

1. **Anrep, G. V., and E. Von Saalfeld.** The blood flow through the skeletal muscle in relation to its contraction. *J. Physiol. (Lond.)* 85: 375–399, 1935.

2. **Armstrong, R. B., and M. H. Laughlin.** Atropine: no effect on exercise muscle hyperemia in conscious rats. *J. Appl. Physiol.* 61: 679–682, 1986.

3. **Brock, R. W., M. E. Tschakovsky, J. K. Shoemaker, J. R. Halliwill, M. J. Joyner, and R. L. Hughson.** Effects of acetylcholine and nitric oxide on forearm blood flow at rest and after a single muscle contraction. *J. Appl. Physiol.* 85: 2249–2254, 1998.

4. **Buckwalter, J. B., P. J. Mueller, and P. S. Clifford.** Autonomic control of skeletal muscle vasodilation during exercise. *J. Appl. Physiol.* 83: 2037–2042, 1997.

5. **Buckwalter, J. B., S. B. Ruble, P. J. Mueller, and P. S. Clifford.** Skeletal muscle vasodilation at the onset of exercise. *J. Appl. Physiol.* 85: 1649–1654, 1998.

6. **Corcondilas, A., G. T. Koroxenidis, and J. T. Shepherd.** Effect of brief contraction of forearm muscles on forearm blood flow. *J. Appl. Physiol.* 19: 142–146, 1964.

7. **Crader, S. E., and M. H. Laughlin.** Skeletal muscle venous outflow is phasic and correlated with muscle tension development (Abstract). *Med. Sci. Sports Exerc.* 28: S175, 1988.

8. **Donald, D. E., D. J. Rowlands, and D. A. Ferguson.** Similarity of blood flow in the normal and sympathectomized dog hindlimb during graded exercise. *Circ. Res.* 26: 185–99, 1970.

9. **Dyke, C. K., N. M. Dietz, R. L. Lennon, D. O. Warner, and M. J. Joyner.** Forearm blood flow responses to handgripping after local neuromuscular blockade. *J. Appl. Physiol.* 84: 754–758, 1998.

10. **Folkow, B., P. Gaskell, and B. A. Waaler.** Blood flow through limb muscles during heavy rhythmic exercise. *Acta Physiol. Scand.* 80: 61–72, 1970.

11. **Folkow, B., U. Haglund, M. Jodal, and O. Lundgren.** Blood flow in the calf muscle of man during heavy rhythmic exercise. *Acta Physiol. Scand.* 81: 157–163, 1971.

12. **Gaskell, W. H.** On the tonicity of the heart and blood vessels. *J. Physiol. (Lond.)* 3: 48–75, 1880.

13. **Gorczynski, R. J., B. Klitzman, and B. R. Duling.** Interrelations between contracting striated muscle and precapillary microvessels. *Am. J. Physiol.* 235 (*Heart Circ. Physiol.* 4): H494–H504, 1978.

14. **Haddy, F. J., and J. B. Scott.** Metabolic factors in peripheral circulatory regulation. *Federation Proc.* 34: 2006–2011, 1975.

15. **Laughlin, M. H.** Skeletal muscle blood flow capacity: role of muscle pump in exercise hyperemia. *Am. J. Physiol.* 253 (*Heart Circ. Physiol.* 22): H993–H1004, 1987.

16. **Laughlin, M. H., R. J. Korthuis, D. J. Dunker, and R. J. Bache.** Control of blood flow to cardiac and skeletal muscle during exercise. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*. Bethesda, MD: Am. Physiol. Soc., 1996, sect. 12, chapt. 16, p. 705–769.

17. **Magder, S.** Venous mechanics of contracting gastrocnemius muscle and the muscle pump. *J. Appl. Physiol.* 79: 1930–1935, 1995.

18. **Marshall, J. M., and H. C. Tandon.** Direct observations of muscle arterioles and venules following contraction of skeletal muscle fibers in the rat. *J. Physiol. (Lond.)* 350: 447–459, 1984.

19. **Pierzga, J. M., and S. S. Segal.** Spatial relationship between neuromuscular junctions and microvessels in hamster cremaster muscle. *Microvasc. Res.* 48: 50–67, 1994.

20. **Pollack, A. A., and E. H. Wood.** Venous pressure in the saphenous vein at the ankle in man during exercise and changes in posture. *J. Appl. Physiol.* 1: 649: 662, 1949.

21. **Ruble, S. B., J. B. Buckwalter, and P. S. Clifford.** Autonomic influence on skeletal muscle blood flow at the onset of dynamic exercise (Abstract). *FASEB J.* 12: A691, 1998.

22. **Sejersted, O. M., A. R. Hargens, K. R. Kardel, P. Blom, O. Jensen, and L. Hermansen.** Intramuscular fluid pressure during isometric contraction of human skeletal muscle. *J. Appl. Physiol.* 56: 287–295, 1984.

23. **Sheriff, D. D., L. B. Rowell, and A. M. Scher.** Is rapid rise in vascular conductance at onset of dynamic exercise due to muscle pump? *Am. J. Physiol.* 265 (*Heart Circ. Physiol.* 34): H1227–H1234, 1993.

24. **Sheriff, D. D., and R. Van Bibber.** Flow-generating capacity of the isolated skeletal muscle pump. *Am. J. Physiol.* 274 (*Heart Circ. Physiol.* 43): H1502–H1508, 1998.

25. **Shoemaker, J. K., M. E. Tschakovsky, and R. L. Hughson.** Vasodilation contributes to the rapid hyperemia with rhythmic contractions in humans. *Can. J. Physiol. Pharmacol.* 76: 1–10, 1998.

26. **Sparks, H. V., Jr., and F. L. Belloni.** The peripheral circulation: local regulation. *Annu. Rev. Physiol.* 40: 67–92, 1978.

27. **Tschakovsky, M. E., J. K. Shoemaker, and R. L. Hughson.** Vasodilation and muscle pump contribution to immediate exercise hyperemia. *Am. J. Physiol.* 271 (*Heart Circ. Physiol.* 40): H1697–H1701, 1996.

28. **Welsh, D. G., and S. S. Segal.** Coactivation of resistance vessels and muscle fibers with acetylcholine release from motor nerves. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H156–H163, 1997.