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Muscle contraction under capillaries in hamster muscle induces arteriolar dilatation via K_{ATP} channels and nitric oxide

Kenneth D. Cohen and Ingrid H. Sarelius

Department of Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, USA

We tested the hypothesis that adenosine and nitric oxide can be sensed by capillaries and are implicated in the remote arteriolar dilatation initiated by muscle contraction. We also explored a role for K_{ATP} channel activity in this response. Small bundles of muscle fibres underlying a group of capillaries in cremaster muscles of anaesthetized hamsters were electrically stimulated to contract for 2 min at each of 2, 4 and 8 Hz. Diameter changes were measured in the inflow arteriole to the group of capillaries after muscle contraction in the presence or absence of 10^{-6} M xanthine amine congener (XAC) to block A_1 and A_2 adenosine receptors, 10^{-4} or 10^{-3} M N^{ω}-nitro-L-arginine (LNNA) to block nitric oxide production, or 10^{-5} M glibenclamide to block K_{ATP} channel activity. Dilatations were unchanged with XAC (3.0 ± 0.5, 3.9 ± 0.7 and 6.1 ± 1.0 μm), but were significantly reduced with LNNA (to 1.8 ± 0.6, 3.5 ± 0.7 and 4.9 ± 0.7 μm) or glibenclamide (to 0.4 ± 0.3, 0.8 ± 0.7 and 1.9 ± 0.6 μm). Neither K_{ATP} channel activity nor nitric oxide was required for transmission or manifestation of the dilator response. Thus, muscle contraction can be sensed by capillaries and the signalling mechanism for the ensuing remote dilatation depends on K_{ATP} channel activity and on NO, but not adenosine. Local application of 10^{-4} M adenosine, 10^{-4} M sodium nitroprusside or 10^{-5} M pinacidil directly to capillaries initiated remote arteriolar dilatations. Thus, capillaries can respond directly to known mediators of metabolic vasodilatation, but these signalling pathways are not invariably implicated in the response to muscle contraction.

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Corresponding author I. H. Sarelius: Department of Pharmacology and Physiology, University of Rochester Medical Center, Box 711, Rochester, NY 14642, USA. Email: ingrid_sarelius@urmc.rochester.edu

Capillary recruitment is a significant early response to increased metabolism in skeletal muscle (Honig & Frierson, 1980; Honig *et al.* 1982; Klitzman *et al.* 1982; Hargreaves *et al.* 1990). The control of capillary recruitment and capillary blood flow redistribution resides in the terminal arteriolar region of the microcirculation (Klitzman *et al.* 1982; Lindbom & Arfors, 1984; Sarelius, 1986); in arterioles in this region of the microvasculature, dilatations have been observed in direct association with contracting muscle fibres (Gorczynski *et al.* 1978; Berg *et al.* 1997; Cohen *et al.* 2000) as well as in response to changes in vasoactive agents or conditions associated with muscle contraction (for example, increased adenosine (Proctor & Duling, 1982), release of NO (Hester *et al.* 1993; Lau *et al.* 1998, 2000) or decrease in PO_2 (Duling & Berne, 1970)). However, in order for flow to be increased in a network of capillaries via a metabolically linked dilatation of the upstream arteriole, there must be an appropriate matching between the metabolic needs of the region of tissue perfused by those capillaries, and the flow in the capillaries themselves. When muscle activity is increased, individual motor units are recruited to contract, with the numbers and types of motor units that are recruited being dependent on the output demanded of the whole muscle (for a review, see Burke, 1981). The individual muscle fibres comprising a single motor unit have common metabolic profiles, but are dispersed throughout the muscle (Burke, 1981), hence as motor units are recruited, different local metabolic signals will be generated at any given tissue site. Thus it is unlikely that the metabolic activity of fibres underlying a particular capillary network will be the same as that of the muscle fibres traversed by the controlling arteriole, suggesting that if metabolic needs are sensed and responded to solely at the level of the controlling arteriole, precise matching of capillary blood flow to metabolic needs will not necessarily be achieved. Effectively, this organization of control would require that the perfusion needs of a particular capillary bed would need to be predetermined upstream, where in fact the local metabolic signals may well be different.

Recently, we have demonstrated that muscle contraction under capillaries can generate a vasodilatatory signal that results in remote dilatation of the upstream arterioles (Berg *et al.* 1997; Cohen *et al.* 2000). This suggests a mechanism whereby the metabolic needs of a local region of tissue can be matched precisely to the capillary blood flow of that region. Implicit in this conclusion is that the

capillaries themselves must be able to sense and respond to metabolic signals generated by contracting muscle fibres. Indeed, it has been established that remote vasomotor responses can be produced in arterioles when neurotransmitters are applied to capillaries (Dietrich, 1989; Dietrich & Tyml, 1992; McGahren *et al.* 1998; Sarelius *et al.* 2000), indicating that capillaries have the ability to sense these agents and initiate signals that result in the appropriate response remotely in arterioles. For example, application of noradrenaline to capillaries can produce remote arteriolar vasoconstriction (Dietrich, 1989; Dietrich & Tyml, 1992), whereas acetylcholine application to capillaries results in upstream arteriolar dilatation (Sarelius *et al.* 2000). We therefore hypothesized that the remote arteriolar vasodilatation initiated by muscle contraction under capillaries would be mediated by vasodilator products of muscle contraction that are known to dilate arterioles directly. Both adenosine (Proctor & Duling, 1982; Proctor, 1984) and NO (Hester *et al.* 1993; Lau *et al.* 1998, 2000) are known to contribute to arteriolar dilatation during contraction of cremaster muscle (which is our model system), hence we asked whether these agents could, in addition, be sensed at the capillary level to initiate the remote vasodilatatory signal generated by skeletal muscle contraction. Because both adenosine receptor activation (Jackson, 1993; Kuo & Chancellor, 1995; Danialou *et al.* 1997; Bryan & Marshall, 1999) and NO dilator capacity (Murphy & Brayden, 1995; Tare *et al.* 2000) have been linked to ATP-sensitive K^+ (K_{ATP}) channel function, and because K_{ATP} channels have been identified as important in arteriolar dilatations during muscle contraction (Saito *et al.* 1996; Murrant & Sarelius, 2000*b*; Hammer *et al.* 2001), we also sought to determine whether activity of these channels could be linked to the vasodilatatory signal initiated in capillaries by the contraction of muscle fibres.

METHODS

Preparation and muscle fibre stimulation

All procedures were approved by the Animal Care and Use Committee of the University of Rochester and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, USA).

Male adult Golden Hamsters (HSD Han : Aura, 100–150 g) were anaesthetized with pentobarbital sodium (70 mg kg^{-1}, I.P.). Supplemental anaesthesia was maintained throughout the experiments via a left femoral venous catheter; depth of anaesthesia was assessed by monitoring reflex withdrawal to a toe pinch. Body temperature was maintained at 37 ± 0.5 °C by convective heating. In most animals, blood pressure was monitored (left femoral arterial catheter). The right cremaster muscle was prepared and viewed as described elsewhere (Baez, 1973; Sweeney & Sarelius, 1989; Cohen *et al.* 2000). The prepared muscle was continuously superfused with warmed (34 °C) bicarbonate-buffered physiological salt solution containing (in mM): NaCl, 131.9; KCl, 4.7; $CaCl_2$, 2.0; $MgSO_4$, 1.2; $NaHCO_3$, 30.0; equilibrated with 5 % CO_2–95 % N_2 to maintain pH at 7.4 ± 0.05 and PO_2 < 10 mmHg. After surgery, the cremaster preparation was allowed to stabilize for 30–60 min. The microcirculation was visualized with a Leitz Laborlux microscope equipped with a 25 × long working distance objective (Leitz, NA 0.35), and displayed via a video camera (RCA SIT E1103 or Dage MTI 72S) on a Hitachi monitor. Final magnification from tissue to monitor was × 1400–2800. Before the start of data collection, viability of the preparation was assessed by confirming in three randomly selected arterioles that there was a brisk vasodilatation to 10^{-4} M local topical adenosine and constriction to transient superfusate equilibration with 10 % oxygen. The small number of preparations that did not display these responses were discarded. At the completion of all experimental protocols, animals were administered a lethal I.V. dose of sodium pentobarbital.

Key aspects of the terminal arteriolar and capillary architecture in this tissue have been described elsewhere (Sweeney & Sarelius, 1989; Berg & Sarelius, 1995) and are schematized in Fig. 1. Briefly,

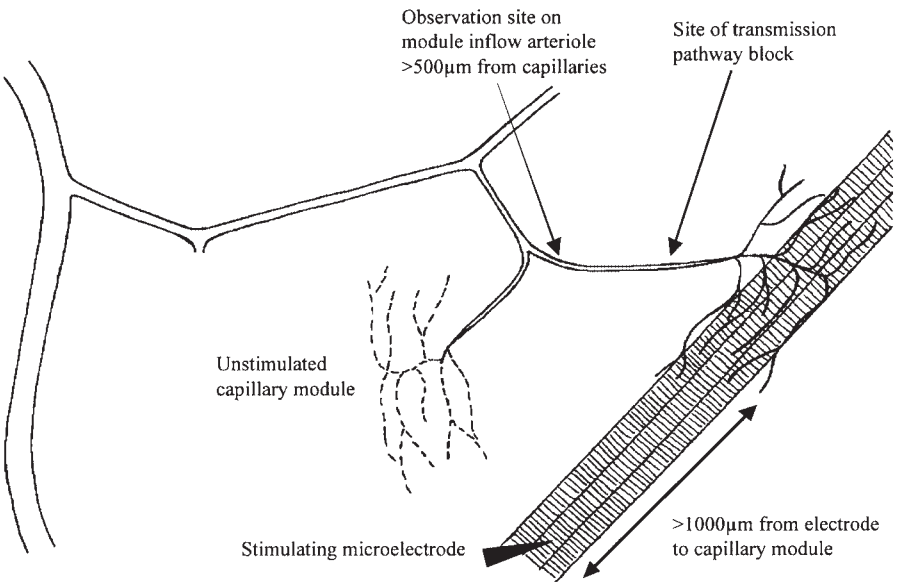

Figure 1. Schematic (not to scale) to show the principal features of the experimental site in cremaster muscle of anaesthetized hamsters

capillaries in this muscle occur in groups (modules) arising from an arteriole (the module inflow arteriole), which is the terminal vessel of the arteriolar tree. Usually, 3–8 capillary modules arise from a common arteriolar branch (Berg & Sarelius, 1995), which in turn branches from a larger vessel that runs approximately transversely across the muscle fibres. Capillary modules in a clear central region of the prepared tissue were identified by their vascular geometry as well as their inability to dilate to locally applied adenosine. Selection criteria included clarity and suitability of the site for electrode or micropipette placement but never included functional criteria such as the number of capillaries in the module or the blood flow rate: many suitable sites are identifiable in each cremaster preparation. Microelectrodes were used to contract small bundles of muscle fibres (usually 4–5 fibres) underlying the selected capillary module, as described previously (Berg *et al.* 1997; Cohen *et al.* 2000). Microelectrodes consisted of a 25 μm diameter platinum/iridium wire enclosed in a glass micropipette and connected to the silver wire in a microelectrode holder (WPI, Inc., Sarasota, FL, USA). The pipette tip was insulated with silicone (Dow Corning) so that only ~2–5 μm of the wire was exposed. Electrodes were placed on the selected muscle fibre bundle $\geqslant$ 1000 μm from the selected capillary module; the ground electrode was a silver wire placed around the rim of the pedestal supporting the tissue. Muscle contraction for 2 min was induced by square wave pulses of 0.4 ms duration and 5–30 V at each of 2, 4 and 8 Hz. A recovery period of 3 min was allowed before proceeding to 2 min contraction at the next highest frequency.

General experimental protocols

In all contraction experiments, muscle fibre bundles that ran underneath the selected capillary module were stimulated to contract for 2 min at each frequency, and the vasodilator response was observed at the proximal end of the inflow arteriole to that module. Contracting muscle fibres were always $\geqslant$ 500 μm away from the observation site, thus ruling out diffusion of vasoactive substances from muscle fibres as a primary determinant of the arteriolar response. In previous work, we have also ruled out the contribution of neural or flow-dependent mechanisms to the arteriolar response (Berg *et al.* 1997; Cohen *et al.* 2000).

To characterize the initiating mechanism(s) for the remote dilatation of the module inflow arteriole, we first completed the muscle contraction protocol under control conditions before repeating the sequence of 2 min muscle contractions in the presence of selected test substances. The test substances (see below) were added either to the superfusate solution bathing the cremaster muscle or, in some experiments, were applied directly to a local region of the microvasculature using a micropipette as previously described (Frame & Sarelius, 1995; Cohen *et al.* 2000). Briefly, micropipettes with tip diameter ~10 μm were filled with the test solution and placed close to (~15–25 μm), and directed towards, the selected arteriolar region or the selected capillaries. Flow out of the micropipette was achieved by raising pressure in the water manometer to which it was connected. A tracer of fluorescein isothiocyanate dextran (40 kDa, 10^{-4} M) was added to each pipette solution so that brief epifluorescence could be used to confirm that there was flow out of the micropipette, and to verify the flow direction of the micropipette contents. It has been confirmed that this tracer concentration is not vasoactive (Frame & Sarelius, 1995). Care was taken to ensure that convective flow of pipette contents was directed only over the selected region of interest, and away from other components of the microvasculature that were being studied. Furthermore, local application sites were chosen so that they were always $\geqslant$500 μm away from the site at which responses were being monitored. Thus, responses of the module inflow arteriole could not be attributed to either convection or diffusion of the micropipette contents between the application and observation sites.

Materials

Xanthine amine congener (XAC, 10^{-6} M) was added to the superfusate to block A_1 and A_2 adenosine receptors (Fredholm *et al.* 1994). We used the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine (LNNA, 10^{-4} or 10^{-3} M) and the NO donor sodium nitroprusside (SNP, 10^{-4} M) to test for involvement of NO, and we explored K_{ATP} channel activity by using the K_{ATP} channel antagonist glibenclamide (10^{-5} M) and channel opener pinacidil (10^{-5} M). For both routes of application (in the superfusate or locally using a micropipette), XAC and LNNA were applied for 10 min and glibenclamide for 40 min before re-testing the arteriolar response. These exposure times were determined in preliminary experiments in which we identified the concentration of antagonist required to block at least 80 % of the vasodilatation to each agonist. In experiments using micropipettes to produce direct local application of adenosine, SNP or pinacidil to capillaries, we used 2 min exposures to mimic the 2 min muscle contraction periods.

Adenosine, SNP and LNNA were made as 10^{-2} M stock solutions in distilled water and stored at 4 °C. On experimental days, they were diluted to 10^{-3} or 10^{-4} M with control superfusate. XAC was frozen (−70 °C) as 10^{-4} M aliquots in 0.1 N NaOH and diluted with control superfusate to 10^{-6} M. Glibenclamide and pinacidil were made as stock solutions with DMSO and diluted to 10^{-5} M with control superfusate. We (Cohen *et al.* 2000) and others (Jackson, 1993) have shown that this concentration of DMSO in the working superfusate (0.04 %) has no effect on vessel reactivity. All chemicals were purchased from Sigma Chemical Co.

Data collection and statistical analyses

Arteriolar responses were videotaped (SonyVO9600 or Panasonic AG6300) and analysed off-line, using a videotaped stage micrometer for reference. We measured the average (resting) diameter in the 15–30 s before the start of each contraction frequency. For each contraction frequency, diameters were measured in the 10 s immediately following cessation of the 2 min muscle contraction, because tissue movement during muscle contraction often precluded measurements during the contraction period. Maximal arteriolar diameter was measured at the end of the experiment after 5 min exposure to 10^{-4} M adenosine added to the superfusate.

Results were expressed either as diameter change from baseline ($D_{test} - D_{baseline}$), or, where resting arteriolar diameter was significantly changed by the test condition (e.g. in some experiments with glibenclamide in the superfusate), we also expressed the diameter change as a percentage of the dilator capacity, where this latter was calculated as ($D_{max} - D_{baseline}$). There were no differences in the conclusions drawn from the statistical analyses when data were expressed in either form. All data are reported as mean ± standard error with n representing the number of arterioles in each data set. In most experiments, one arteriole was observed per animal: occasionally in experiments using local drug application, two arterioles were observed per animal. Group means were compared using Student's paired t test (Snedecor & Cochran, 1967), with differences judged significant at $P \leqslant 0.05$.

RESULTS

The mean maximal diameter of arterioles used in this study was 18.3 ±3.1 μm, with a range of 9.2–26.6 μm.

Role of A_1 and A_2 adenosine receptors in remote dilatations

To test whether adenosine contributed to the signal sensed by the capillaries during muscle contraction, 10^{-6} M xanthine amine congener (XAC) was added to the superfusate to block A_1 and A_2 adenosine receptors. This addition of 10^{-6} M XAC to the superfusate did not affect the remote dilatation of the module inflow arteriole in response to muscle contraction (Fig. 2). Because addition of XAC did not inhibit the dilator response, we verified the efficacy of the XAC by measuring the arteriolar diameter change in response to 10^{-4} M adenosine locally applied to a capillary module for 2 min in the absence or presence of XAC. XAC significantly decreased adenosine-induced remote dilatations from 4.5 ± 0.9 μm to 1.1 ± 0.4 μm ($n = 9$), indicating not only that XAC in the superfusate was able to produce significant antagonism of adenosine receptors, but also that capillaries have the capacity to respond to adenosine if it is present. Thus A_1 and A_2 receptor activation appears not to be involved in initiation of the remote dilatations induced in the module inflow arteriole by muscle contraction, and a role for adenosine in the contraction-dependent stimulation of capillaries to initiate remote vasodilator signals was not explored further.

Contibution of NO to the remote dilatation

To explore a role for NO in contributing to the remote dilatation, the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine (LNNA, 10^{-4} or 10^{-3} M) was added to the superfusate and the arteriolar response to muscle contraction was observed. Baseline diameters were not significantly changed in the presence of LNNA (12.2 ± 1.0 μm with LNNA *versus* 10.5 ± 1.0 μm in controls, $n = 9$). Figure 3*A* shows that dilatation of the module inflow arteriole during remote muscle contraction was significantly attenuated during addition of LNNA to the superfusate (10^{-4} M, $n = 5$, 10^{-3} M, $n = 4$; there was no difference between data sets so the data were pooled). Control dilatations of 2.6 ± 0.6, 5.5 ± 0.8 and 7.1 ± 0.7 μm at 2, 4 and 8 Hz were significantly reduced to 1.8 ± 0.6, 3.5 ± 0.7 and 4.9 ± 0.7 μm with LNNA, a significant overall reduction to 30.9 ± 5.7 % of the dilator capacity.

However, a substance added to the superfusate has the potential to affect the arteriolar dilatation via a number of different scenarios, by affecting any or all of (i) the initiation of the dilator signal at the capillaries in the vicinity of the contracting muscle fibres; (ii) the transmission of the dilator signal along the vessel wall; (iii) the ability of the arteriole to dilate at the observation site. Thus if addition of a test substance to the superfusate modifies the

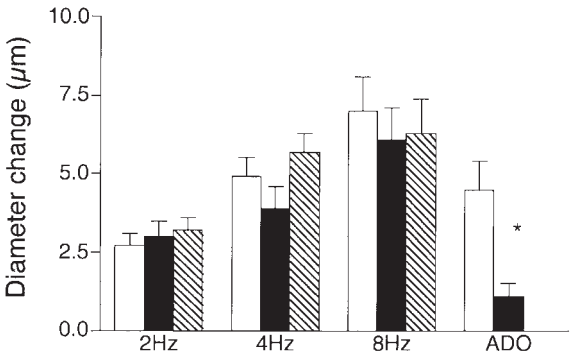

Figure 2. Diameter changes of module inflow arterioles ($n = 7$) in the absence (control, open bars; recovery, hatched bars) or presence (filled bars) of the adenosine A_1 and A_2 receptor antagonist xanthine amine congener (XAC, 10^{-6} M), in response to 2 min of remote muscle contraction under capillaries at 2, 4 and 8 Hz

Also shown are responses of module inflow arterioles when adenosine (ADO, 10^{-4} M) was applied topically to capillary modules ($n = 9$), in the absence (open bar) or presence (filled bar) of XAC. Bars are means ± S.E.M. All changes during muscle contraction are significantly different from rest, but XAC is not different from controls at any stimulation frequency.
* Significantly different from the control for that condition.

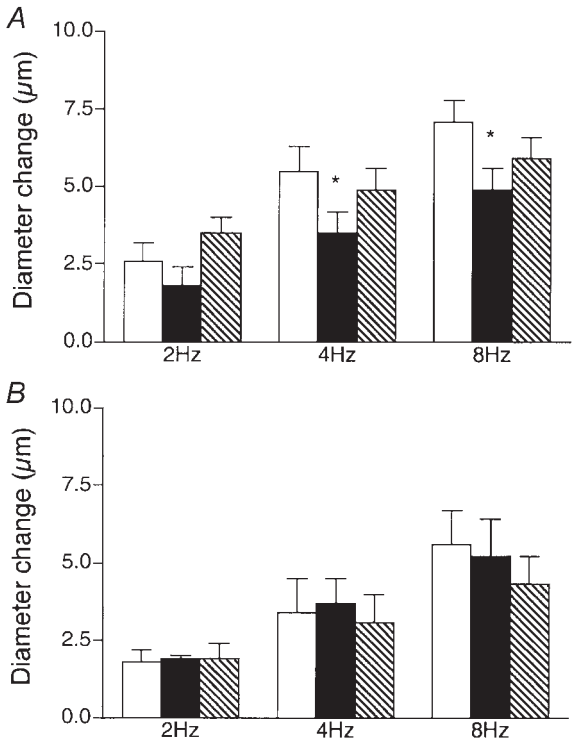

Figure 3. Diameter changes of module inflow arterioles in the absence (control, open bars; recovery, hatched bars) or presence (filled bars) of the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine (LNNA, 10^{-4} or 10^{-3} M), in response to 2 min of remote muscle contraction under capillaries at 2, 4 and 8 Hz

A, addition of LNNA to the superfusate, ($n = 9$); *B*, local application of LNNA to the observation site via micropipette ($n = 5$). Bars are means ± S.E.M. * Significantly different from the control for that condition.

remote arteriolar dilatation, it is necessary to undertake additional experiments to determine whether the response could be attributed either to inhibition of transmission of the dilator signal along the vessel wall, or to direct inhibition of the arteriolar dilator machinery at the observation site. In a previous study (Cohen *et al.* 2000) we showed that local micropipette-application of LNNA to the transmission pathway between the capillaries and the upstream observation site did not affect the remote contraction-induced arteriolar dilatation, indicating that NO is not involved in transmission of this dilator signal along the blood vessel wall. To determine whether LNNA directly affected the ability of the module inflow arteriole to dilate, we completed separate experiments in which LNNA was directly micropipette-applied to the observation site during the muscle contraction protocol. LNNA had no significant effect on this arteriolar response (Fig. 3*B*), thus we conclude that NO is also not required for manifestation of the arteriolar dilatation. In previous work (Frame & Sarelius, 1995; Cohen, 2000) we have confirmed the efficacy of LNNA delivery from micropipettes. Taken together, these findings indicate that as NO is neither required for manifestation of the arteriolar dilatation itself, nor is it required for transmission of the dilator signal along the vessel wall, its site of action must be at the capillaries, where the dilator signal is initiated.

To confirm that capillaries have the ability to respond directly to NO, we used a micropipette to apply the NO donor sodium nitroprusside (SNP, 10^{-4} M) directly to module capillaries for 2 min (to match the 2 min period of muscle stimulation) and monitored the diameter of the upstream arteriole. There was an increase in diameter of 5.0 ± 1.4 μm ($n = 6$) in upstream arterioles in response to SNP applied locally across the capillary module.

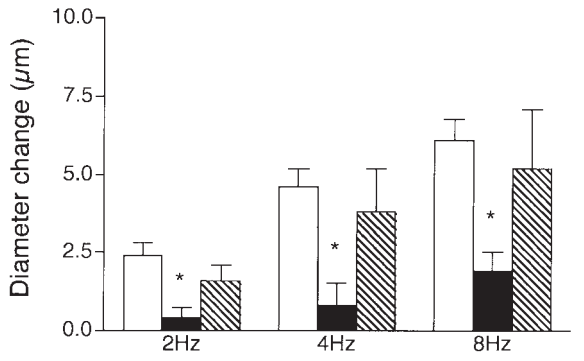

Figure 4. Diameter changes of module inflow arterioles ($n = 7$) in the absence (control, open bars; recovery, hatched bars) or presence (filled bars) of the K_{ATP} channel antagonist glibenclamide (10^{-5} M), in response to 2 min of remote muscle contraction under capillaries at 2, 4 and 8 Hz

Bars are means ± S.E.M. * Significantly different from the control for that condition.

K_{ATP} channel involvement in remote arteriolar dilatation

To determine if remote contraction-induced dilatations in the module inflow arteriole were dependent on K_{ATP} channel activity, the K_{ATP} channel antagonist glibenclamide (10^{-5} M) was added to the superfusate. As shown in Fig. 4, this resulted in significant attenuation of the contraction-induced remote dilatation of the module inflow arteriole. Control dilatations of 2.4 ± 0.4, 4.6 ± 0.6 and 6.1 ± 0.7 μm at 2, 4 and 8 Hz respectively were significantly reduced to 0.4 ± 0.3, 0.8 ± 0.7 and 1.9 ± 0.6 μm in the presence of glibenclamide ($n = 7$). As outlined above for NO, significant attenuation of the remote dilatation under these conditions could reflect inhibition of K_{ATP} channel activity at the capillaries, or in the transmission pathway along the blood vessel wall, or at the site of arteriolar response. Hence, in separate sets of experiments, we used a micropipette to locally apply 10^{-5} M glibenclamide to either the transmission pathway or the observation site, as described earlier for NO. When glibenclamide was applied via a micropipette directly to the transmission pathway (Fig. 5*A*), the remote dilatation of the module inflow arteriole was unaffected, indicating that the inhibition of dilatation observed when glibenclamide

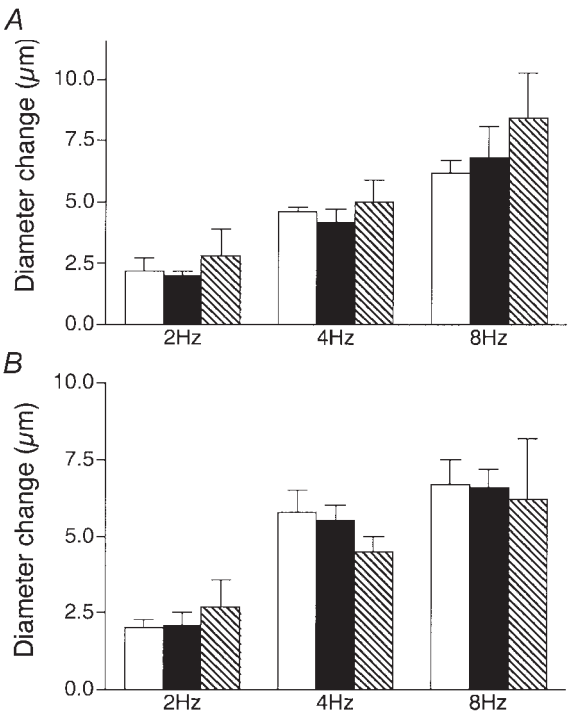

Figure 5. Diameter changes of module inflow arterioles in response to 2 min of remote muscle contraction under capillaries at 2, 4 and 8 Hz, in the absence (control, open bars; recovery, hatched bars) or presence (filled bars) of the K_{ATP} channel antagonist glibenclamide (10^{-5} M), locally applied via micropipette to the transmission pathway (*A*, $n = 5$) or the upstream observation site (*B*, $n = 5$)

Bars are means ± S.E.M. Responses in the presence of glibenclamide are not different from controls.

was added to the superfusate was not due to block of the transmission of the dilator signal from its origination at the capillaries to the site of arteriolar response. Similarly, micropipette-application of glibenclamide directly to the observation site in the module inflow arteriole did not block the dilator response (Fig. 5*B*). We confirmed that glibenclamide was indeed able to reverse a local pinacidil-induced dilatation (Fig. 6), verifying the efficacy of the glibenclamide. To confirm that capillaries can respond to a K_{ATP} channel opener by producing a remote arteriolar dilatation, we micropipette-applied the K_{ATP} channel activator pinacidil (10^{-5} M) directly to module capillaries for 2 min while module inflow arteriolar diameter was monitored. This application of pinacidil produced a remote dilator response of 3.4 ± 0.8 μm ($n = 5$).

Is the response to NO linked to K_{ATP} channel activity? There is evidence that NO can stimulate K_{ATP} channel activity (Murphy & Brayden, 1995; Tare *et al.* 2000), hence an obvious question for us to ask is whether the observed contribution of NO to the remote dilatation was via a K_{ATP} channel-dependent mechanism. To test this we measured remote arteriolar dilatations with both glibenclamide and LNNA added to the superfusate (Fig. 7). This combination significantly attenuated the response to muscle stimulation from 16.6 ± 2.6, 42.7 ± 3.6 and 60.7 ± 10.8 % of the dilator capacity at 2, 4 and 8 Hz to 5.8 ± 2.7, 16.3 ± 4.2 and 24.3 ± 4.1 % respectively in the presence of both glibenclamide and LNNA ($n = 9$). This attenuation was not different from that with glibenclamide alone (to 4.0 ± 3.4, 5.4 ± 7.7 and 17.2 ± 4.1 % respectively at 2, 4 and 8 Hz, $n = 7$), but was a greater attenuation than with LNNA alone (to 19.8 ± 9.6, 31.1 ± 12.1 and 41.7 ± 6.2 %, $n = 9$), implying that the response to LNNA could be accounted for by an effect on K_{ATP} channel activity.

To further explore the possibility of a mechanistic link between NO and K_{ATP} channel activity, we micropipette-applied 10^{-4} M SNP directly to capillaries for 2 min as described earlier, and measured the ensuing remote arteriolar dilatation in the absence or presence of 10^{-5} M glibenclamide in the superfusate. There was no significant difference in the response between the two conditions (69 ± 9 % of the dilator capacity with SNP alone *versus* 63 ± 9 % for SNP plus glibenclamide), representing diameter changes of 5.0 ± 0.9 and 5.9 ± 0.9 μm respectively ($n = 10$).

Taken together, these results suggest that in this system K_{ATP} channel activity may be affected by NO, but in a complex way that is beyond the scope of the present study to resolve.

DISCUSSION

The principal finding from this study is that skeletal muscle contraction activates vasodilator pathways that can be sensed by capillaries, which then initiate signals that are transmitted upstream to remote arteriolar sites, causing them to dilate. Further, capillaries are differently responsive to different putative metabolic dilators, as illustrated by the finding that while both adenosine and NO can be sensed by capillaries and cause initiation of a remote vasodilator response, only NO, and not adenosine, is implicated in the remote dilatation induced by muscle contraction.

We conclude that K_{ATP} channel activity is a major component of the response initiated by muscle contraction because glibenclamide applied to the tissue via the superfusion solution substantially inhibited the remote dilatation, while glibenclamide applied either directly to

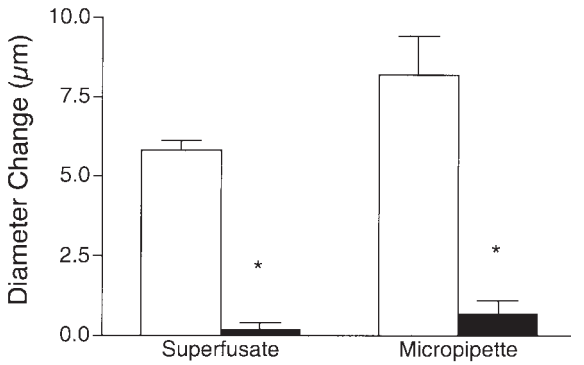

Figure 6. Diameter changes of module inflow arterioles induced by local micropipette application of the K_{ATP} channel opener pinacidil (10^{-5} M, open bars) are reversed by addition of glibenclamide (10^{-5} M, filled bars) added either to the superfusate ($n = 6$) or locally via micropipette ($n = 4$)

Bars are means ± S.E.M. * Significantly different from the control for that condition.

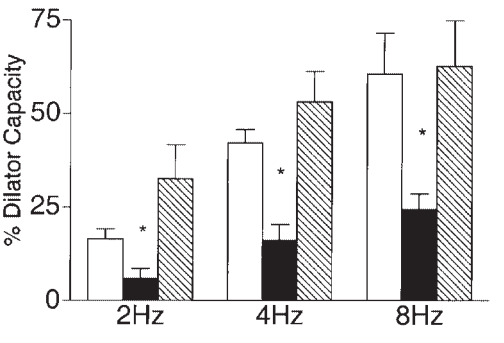

Figure 7. Diameter changes of module inflow arterioles ($n = 9$) in the absence (control, open bars; recovery, hatched bars) or presence (filled bars) of the K_{ATP} channel antagonist glibenclamide (10^{-5} M) together with the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine (LNNA, 10^{-4} M), in response to 2 min of remote muscle contraction under capillaries at 2, 4 and 8 Hz

Bars are means ± S.E.M. * Significantly different from the control for that condition.

the transmission pathway for the remote response or directly to the upstream observation site had no effect on the remote dilatation. This indicates that the transmission pathway and dilator machinery for the response were intact when glibenclamide was present in the superfusate, and therefore indicates that inhibition must have occurred at the capillaries, i.e. at the site of initiation of the response. The force developed during contraction of isolated cremaster muscles is the same in the presence or absence of 10^{-5} M glibenclamide (Murrant & Sarelius, 2002), thus we conclude that K_{ATP} channel activity is necessary for the initiation of the remote dilatation. Because glibenclamide was added to the superfusate and therefore applied to the entire tissue, we are not able to identify whether the relevant location for this K_{ATP} channel activity is on the skeletal muscle fibres or on the capillaries themselves. We confirmed that a remote arteriolar dilatation can be initiated in the region of the capillaries by locally applying the K_{ATP} channel opener pinacidi using a micropipette. We were not able to test whether glibenclamide applied locally to the capillaries could inhibit the upstream dilatation produced by muscle contraction because this required close apposition of the micropipette tip and the contracting muscle fibres, usually resulting in broken pipette tips. Thus K_{ATP} channels on the capillary endothelial cells (Brayden, 1990), the skeletal muscle fibres (Renaud *et al.* 1996) or possibly intracellularly on mitochondrial membranes (Garlid *et al.* 1996) remain as candidates for involvement in this response.

Our study also indicates that NO is involved in initiation of the remote dilatation. We found an overall reduction in the remote arteriolar dilatation in the presence of LNNA, and were able to localize this inhibition to the site of initiation of the dilator response, i.e. the capillaries themselves. Force development during contraction of isolated cremaster muscle is not affected by LNNA (Cohen, 2000; Murrant & Sarelius, 2002), hence this reduced arteriolar dilatation cannot be attributed to reduced metabolic workload. NO has been implicated in vasodilator responses to muscle contraction (Murrant & Sarelius, 2002), and it is known that NO of skeletal muscle origin can play a role in functional hyperaemia (Lau *et al.* 1998, 2000). In the present study we demonstrated that direct application of the NO donor SNP to the capillary region could initiate a remote dilatation, suggesting that these vessels do indeed have the capacity to respond to NO such as could be released by contracting muscle fibres. We could not demonstrate a significant effect of glibenclamide on the remote dilatation produced by SNP. However, we found that the substantial glibenclamide-dependent attenuation of the muscle contraction-dependent remote response was not different when LNNA was present with the glibenclamide, suggesting that the NO-dependent signalling pathway is not independent of a pathway involving K_{ATP} channels, and implying that NO does, in some way, influence K_{ATP} channel activity in this system. Clearly, however, it seems unlikely that all of the response attributable to K_{ATP} channel activity can be accounted for by a direct action of NO on these channels.

Our data do not exclude the possibility that NO could be a signalling intermediate generated at the capillaries themselves (in response to some other as yet unidentified signal from contracting muscle fibres) and subsequently involved in initiation of the remote dilator signal. We have shown elsewhere that NO is not involved in the transmission of the remote response to the module inflow arteriole (Cohen *et al.* 2000), hence we conclude that NO released by skeletal muscle and sensed by capillaries, or NO generated by the capillaries, must contribute to initiation of the dilator signal that is transmitted upstream.

Adenosine has been implicated in local arteriolar dilatations produced by muscle contraction (Proctor & Duling, 1982; Proctor, 1984; Murrant & Sarelius, 2002), and is implicated in the production of remote dilatations when those dilatations are initiated at the arteriolar level (Murrant & Sarelius, 2002). The present study shows, in contrast, that although capillaries do indeed have the ability to respond to adenosine by producing a remote dilatation, adenosine is not involved in stimulation of capillaries by muscle contraction to produce a remote dilatation, indicating that different signalling pathways are involved in the contraction-dependent remote response.

It has been established that while transmission of the remote dilator signal initiated at the capillary level can involve gap junctions (Berg *et al.* 1997; Cohen *et al.* 2000), transmission of the remote dilator signal initiated in arterioles does not (Murrant & Sarelius, 2000*a*), again indicating that the remote response pathways initiated by muscle contraction are complex and must involve more than one mechanism. We speculate that a component of the remote response initiated by muscle contraction might involve another transmission route along the blood vessel wall, such as the local paracrine route mediated by cellular calcium waves that has been described for endothelium and other cell types (Charles, 1998; Sauer *et al.* 2000; Ohata *et al.* 2001). Although we have shown that local muscle fibre contraction in the region of capillaries or arterioles does not induce measurable whole cell calcium changes in endothelial cells (Murrant *et al.* 2000; Sarelius *et al.* 2000), this does not rule out involvement of phenomena such as local calcium oscillations or waves as contributors to the signalling pathway for remote dilator mechanisms. Clearly, studies addressing such possibilities are beyond the scope of the present work.

Capillary recruitment and capillary blood flow redistribution in an exercising skeletal muscle involve integration of many vasodilatatory mechanisms, both centrally and locally mediated, that together contribute to the whole organ response. In the present study, we have

taken advantage of the spatial relationships between the microvasculature and skeletal muscle fibres in cremaster muscle to identify a direct role for capillaries in local sensing of, and response to, acute changes in skeletal muscle contractile activity. Clearly, the relative importance of this response in an intact exercising muscle will depend on the balance of many inputs, including this one.

In summary, we have shown that muscle contraction underneath capillaries can be sensed by the capillaries themselves, which initate remote dilatations in upstream arterioles. These dilatations are dependent on K_{ATP} channel activity and on NO, although it is not yet clear how the response to NO is related to K_{ATP} channel activity. Adenosine, while implicated in other local dilator responses, and which can indeed be sensed by capillaries, is not involved in the capillary-initiated remote dilatation to muscle contraction. Thus, not all established mediators of functional hyperaemia contribute to this flow increase by directly signalling capillaries to initiate remote dilatations. Although aspects of these signalling pathways remain to be described, it is clear that initiation of upstream arteriolar dilatations can control capillary recruitment and contributes to functional hyperaemia by locally increasing blood flow to those capillaries that are directly associated with contracting skeletal muscle fibres.

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