Mechanisms of rapid vasodilation after a brief contraction in human skeletal muscle

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Crecelius AR, Kirby BS, Luckasen GJ, Larson DG, Dinenno FA. Mechanisms of rapid vasodilation after a brief contraction in human skeletal muscle. Am J Physiol Heart Circ Physiol 305: H29–H40, 2013. First published May 3, 2013; doi:10.1152/ajpheart.00298.2013.—A mono- phasic increase in skeletal muscle blood flow is observed after a brief single forearm contraction in humans, yet the underlying vascular signaling pathways remain largely undetermined. Evidence from experimental animals indicates an obligatory role of vasodilation via K+-mediated smooth muscle hyperpolarization, and human data suggest little to no independent role for nitric oxide (NO) or vasodilating prostaglandins (PGs). We tested the hypothesis that K+-mediated vascular hyperpolarization underlies the rapid vasodilation in humans and that combined inhibition of NO and PGs would have a minimal effect on this response. We measured forearm blood flow (Doppler ultrasound) and calculated vascular conductance 10 s before and for 30 s after a single 1-s dynamic forearm contraction at 10%, 20%, and 40% maximum voluntary contraction in 16 young adults. To inhibit K+-mediated vasodilation, BaCl2 and ouabain were infused intravenously to inhibit inwardly rectifying K+ channels and Na+(K+)-ATPase, respectively. Combined enzymatic inhibition of NO and PG synthesis occurred via Nω-monomethyl-L-arginine (Nω-NMMA; NO synthase) and ketorolac (cylooxygenase), respectively. In protocol 1 (n = 8), BaCl2 + ouabain reduced peak vasodilation (range: 30–45%, P < 0.05) and total postcontraction vasodilation (area under the curve, ∼55–75% from control) at all intensities. Contrary to our hypothesis, Nω-NMMA + ketorolac had a further impact (peak: ∼60% and area under the curve: ∼80% from control). In protocol 2 (n = 8), the order of inhibitors was reversed, and the findings were remarkably similar. We conclude that K+-mediated hyperpolarization and NO and PGs, in combination, significantly contribute to contraction-induced rapid vasodilation and that inhibition of these signaling pathways nearly abolishes this phenomenon in humans.

hyperemia; exercise; potassium

The regulation of skeletal muscle hyperemia during muscle contractions is complex and involves a variety of signals that control both the arteriovenous perfusion pressure gradient and arteriolar caliber (10, 13). In an attempt to isolate the local mechanisms underlying exercise hyperemia, early experiments used a single brief muscle contraction to allow for contraction-induced hyperemia without the continuous interruption of the blood flow response or further stimulus for hyperemia, as occurs with repeated contractions (16). In this regard, the single contraction model can serve as a tool to examine feedforward mechanisms of hyperemia that are largely independent of changes in tissue oxidative metabolism (48). The typical response is characterized by an intensity-dependent, rapid, monophasic increase in blood flow that occurs immediately (within one cardiac cycle) after contraction, achieves full magnitude in approximately five cardiac cycles, and then declines toward baseline. To date, the essential underlying mechanisms for this rapid hyperemia, and thus feedforward regulation of muscle blood flow, have yet to be determined in humans.

Given the rapid nature of single contraction-induced hyperemia, some investigators have suggested that this response is due to changes in the arteriovenous perfusion pressure gradient (45, 62, 65); however, several lines of evidence now clearly demonstrate that vasodilation is obligatory to observe this hyperemic phenomenon (33, 34, 64) and that a portion of this response is attributable to the mechanical compression of the vasculature that occurs during muscle contraction (14, 40). Over the last century, a multitude of vasodilating factors have been suggested to contribute to contraction-induced vasodilation, yet in humans, none have been found obligatory to observe a significant increase in muscle blood flow (12, 38).

Interestingly, using the hamster cremaster preparation, Armstrong and colleagues (3) demonstrated that K+ uptake, likely released during muscle contractions, evokes vascular smooth muscle cell hyperpolarization and the subsequent rapid vasodilation. In this study, K+-mediated vasodilation occurred through both inwardly rectifying K+ (Kir) channels and Na+(K+)-ATPase, similar to the mechanisms of K+-mediated vasodilation in humans (17, 21). Furthermore, these animal data substantiated early observations showing that changes in interstitial K+ concentration after brief muscle contraction have the appropriate magnitude and time course to have a significant involvement in the hyperemic response (26, 35, 39, 50, 53, 54) and that smooth muscle vascular hyperpolarization may be essential to observe rapid vasodilation (33). Whether K+-mediated vasodilation contributes to contraction-induced rapid vasodilation in humans is unknown.

In an attempt to understand the contributing vasoactive pathways to rapid vasodilation in human subjects, investigators have targeted ACh released from motor nerves as well as “traditional” substances synthesized by the vascular endothelium. In this context, ACh spillover from motor neurons is not obligatory to observe the vasodilator response, (5), and, furthermore, independent inhibition of nitric oxide (NO) (5) or vasodilating prostaglandin (PG) synthesis (63) does not impact rapid vasodilation. However, a considerable interplay is known...
to occur between NO and PGs during a variety of stimuli (46, 56, 61, 66), and thus blockade of these pathways in combination often reveals an unrecognized role (46, 61). To date, no studies have determined whether combined NO and PG inhibition reduces the rapid vasodilation in response to single contractions.

Given this information as background, we sought to determine the underlying signaling mechanisms of muscle contraction-induced rapid vasodilation in humans. We tested the hypothesis that K\(^+\)-stimulated vascular hyperpolarization underlies the vasodilation after a brief single contraction in humans and that, even in combination, there is little to no role for NO and PGs in this response.

METHODS

Subjects

With Institutional Review Board approval and after written informed consent, a total of 16 young healthy adults (13 men and 3 women; age: 23 ± 1 yr old, weight: 72.3 ± 2.4 kg, height: 176 ± 2 cm; body mass index: 23.4 ± 0.5 kg/m\(^2\); means ± SE) participated in the present study. All subjects were sedentary to moderately active, nonsmokers, nonobese, normotensive (resting blood pressure < 140/90 mmHg), and not taking any medications. Experiments were performed after an overnight fast and 24-h abstention from caffeine and exercise. Subjects were in the supine position with the experimental arm abducted to 90° and slightly elevated above the heart level upon a tilt-adjustable table. Female subjects were studied during the early follicular phase of their menstrual cycle or placebo phase of oral contraceptives to minimize any potential cardiovascular effects of sex-specific hormones. All experiments were performed according to the Declaration of Helsinki.

Arterial Catheterization, Arterial Blood Pressure, and Heart Rate

A 20-gauge, 7.6-cm catheter was placed in the brachial artery of the nondominant arm under aseptic conditions after local anesthesia (2% lidocaine) for the local administration of study drugs and blood sampling. The catheter was connected to a three-port connector as well as a pressure transducer for mean arterial pressure (MAP) measurement and continuously flushed at 3 ml/h with heparinized saline. The two side ports were used for drug infusions of vasoactive drugs (20, 42). Heart rate (HR) was determined using a three-lead ECG (Cardiocap/5, Datex-Ohmeda, Louisville, CO).

Forearm Blood Flow and Vascular Conductance

A 4-MHz pulsed Doppler probe (model 500M, Multigon Industries, Mt. Vernon, NY) was used to measure brachial artery mean blood velocity (MBV) with the probe securely fixed to the skin over the brachial arterial proximal to the catheter insertion site, as previously described by our laboratory (23, 40). The probe insonation angle relative to the skin was 45°. A linear 12-MHz echo Doppler ultrasound probe (GE Vingmed Ultrasound Vivid7, Horten, Norway) was placed in a holder securely fixed to the skin immediately proximal to the velocity probe to measure brachial artery diameter. Brachial artery diameter was measured in triplicate before any contractions under all experimental conditions, as we and others (11, 64) have shown that brachial diameter does not change in response to this stimulus. Forearm blood flow (FBF) was calculated as follows: FBF = MBV × π(brachial artery diameter/2)\(^2\) × 60, where FBF was measured in milliliters per minute, MBV was measured in centimeters per second, brachial diameter was measured in centimeters, and 60 was used to convert from milliliters per second to milliliters per minute. Forearm vascular conductance (FVC) was calculated as follows: FVC = (FBF/MAP) × 100, and was expressed as milliliters per minute per 100 mmHg.

Single Dynamic Forearm Contractions

Maximum voluntary contraction (MVC) was determined for the experimental arm as the average of three maximal squeezes of a handgrip dynamometer (Stoeling, Chicago, IL) that were within 3% of each other. Brief, dynamic forearm contractions were performed at 10%, 20%, and 40% of the subject’s MVC using a handgrip pulley system attached to weights corresponding to each workload. The weight was lifted 4–5 cm over the pulley for a single 1-s dynamic contraction, as previously described (11). These mild-to-moderate contraction intensities were chosen to limit the contribution of systemic hemodynamics to forearm vasodilator responses and to eliminate reflex increases in sympathetic nervous system activity and thus isolate the local effects of muscle contraction on vascular tone (11). At least 1.5 min of relaxation were given between each contraction to allow continuous measures of forearm hemodynamics postcontraction as well as ample time for hemodynamics to return to baseline values (11, 40, 64). Workload intensity was randomized and counterbalanced across subjects to eliminate any order effect, and trials were performed in triplicate to calculate an average response for each subject. Pilot studies in our laboratory have determined that MVC is not affected by any of the vasoactive substances, particularly BaCl\(_2\) and ouabain, administered in this study (n = 12, pre: 41 ± 2 kg vs. post: 40 ± 2 kg, P = 0.43).

Vasoactive Drug Infusions

All drug infusions occurred via the brachial artery catheter to create a local effect in the forearm. To inhibit K\(^+\)-mediated hyperpolarization and vasodilation, both ouabain octahydrate (no. 03125, Sigma, St. Louis, MO) and BaCl\(_2\) 10% (wt/vol) BDH-3238, EMD Chemicals, Gibbstown, NJ) were administered intra-arterially as previously described (17). Ouabain was infused at 2.7 mmol/min for 15 min as a loading dose to inhibit Na\(^+\)-K\(^+\)-ATPase, and BaCl\(_2\) was infused at 0.45 μmol·dl forearm volume\(^{-1}\) min\(^{-1}\) with a minimum dose of 4 μmol/min to a maximum dose of 5 μmol/min for 3 min as a loading dose to inhibit K\(_{IR}\) channels (9, 17, 21, 27, 37). This dose of BaCl\(_2\) has been adjusted to forearm volume compared with our previous study (17) to maximize efficacy while still remaining within doses safe for human administration and specific for K\(_{IR}\) channels (21, 36). Ouabain and BaCl\(_2\) were prepared in saline and confirmed sterile and free of fungus/endotoxin and particulate matter with a standard microbiology report (JCB-Analytical Research Labs, Wichita, KS) before use. To inhibit traditional endothelium-derived vasodilators that have not independently been shown to be playing a role in rapid vasodilation, N\(^\delta\)-monomethyl-L-arginine (L-NMMA; Bachem, Germany) was administered to inhibit NO synthase-mediated production of NO in combination with ketorolac (Hospital, Lake Forest, IL) to inhibit cyclooxygenase-mediated synthesis of PGs. Loading doses of L-NMMA and ketorolac were 25 mg (5 mg/min for 5 min) and 6 mg (600 μg/min for 10 min), respectively (17, 19). Depending on the protocol, maintenance doses of either BaCl\(_2\) (0.45 μmol·dl forearm volume\(^{-1}\) min\(^{-1}\)), ouabain (2.7 mmol/min), L-NMMA (1.25 mg/min), or ketorolac (150 μg/min) were infused for 3 min before set of single contractions to ensure continuous blockade (see Experimental Protocols below). Forearm volume used for the normalization for specific vasoactive drugs was determined from regional analysis of whole body dual-energy X-ray absorptiometry scans (QDR series software, Hologic, Bedford, MA). Three single contractions at the respective workload were performed at 15, 30, and 45 s of the 3-min loading infusion before each set of single contractions to facilitate drug delivery to the active tissue.
Signalings of immediate exercise hyperemia

Experimental Protocols

Two separate groups of eight subjects were studied, with the primary difference being the order in which pharmacological inhibitors were administered. The experimental timeline is shown in Fig. 1. To establish control contraction-induced rapid vasodilatory responses, subjects performed single brief forearm contractions in triplicate at 10%, 20%, or 40% MVC for 1 s with a minimum of 1.5 min of rest between contractions. Between contraction intensities, saline was infused for 3 min before the first contraction (Fig. 1).

Protocol 1. To address our primary hypothesis regarding K+-stimulated vascular hyperpolarization as the predominant signaling pathway involved in rapid vasodilation, in eight subjects (MVC: 52 ± 3 kg) after control responses, single contraction bouts were performed at each exercise intensity after the infusion of BaCl2 + ouabain. Next, in an attempt to further elucidate the underlying signaling of rapid vasodilation, we addressed endothelium-dependent vasodilators. The combined contribution of NO and PGs was assessed with the administration of L-NMMA + ketorolac, respectively, and single contractions were repeated.

Protocol 2. Given the unexpected findings from protocol 1 regarding the effect of combined inhibition of NO and PGs on rapid vasodilation (see RESULTS), in eight different subjects (MVC: 42 ± 4 kg), the order of inhibition was reversed so that after control responses were obtained, L-NMMA + ketorolac was infused to assess the combined contribution of NO and PGs, respectively, to contraction-induced rapid vasodilation. The third set of single contractions was performed after inhibition of K+-mediated vascular hyperpolarization (BaCl2 + ouabain) in the presence of combined NO and PG inhibition.

Control experiments. In a subset of subjects (n = 6), sodium nitroprusside (SNP; Nitropress, Hospira) was infused at 2 μg·100 ml forearm volume−1·min−1 for 5 min (41) in control (saline) conditions and after prior administration of all four antagonists (BaCl2 + ouabain + l-NMMA + ketorolac) as a negative control to confirm the intact capacity of the forearm resistance vasculature to vasodilate.

In a different subset of subjects (n = 4), before any pharmacological inhibition, phenylephrine (PE; Baxter, Irvine, CA) was infused at 6.25 ng·100 ml forearm volume−1·min−1 for 2 min to preconstrict the vasculature (42) before the performance of a bout of 40% MVC single contractions to determine the impact of reduced basal vascular tone per se on forearm rapid vasodilation. This dose of PE was selected to reduce basal FVC to a similar level (~20–30%) as we typically observed with the infusion of the antagonists used in the experimental protocols (17, 18).

Time-control experiments were not performed in this study as we have previously demonstrated that rapid vasodilation to a single contraction is repeatable over the course of an experiment of similar duration (~2–3 h) (43).

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A  GENERAL EXPERIMENTAL TIMELINE

<table>
<thead>
<tr>
<th>Trial 1: Control</th>
<th>GENERAL EXPERIMENTAL TIMELINE</th>
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<tr>
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<td>Saline</td>
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<tr>
<td></td>
<td>20% MVC</td>
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<td>40% MVC</td>
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<td>3 min</td>
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<tr>
<td>Trial 2: Dual Block</td>
<td>Ba/Ouab OR L-NMMA/Ket</td>
</tr>
<tr>
<td>3 min</td>
<td>10% MVC</td>
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<tr>
<td></td>
<td>Ba/Ouab OR L-NMMA/Ket</td>
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<td></td>
<td>20% MVC</td>
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</tr>
<tr>
<td>Trial 3: Quad Block</td>
<td>Ba/Ouab/ L-NMMA/Ket</td>
</tr>
<tr>
<td>3 min</td>
<td>10% MVC</td>
</tr>
<tr>
<td></td>
<td>Ba/Ouab/ L-NMMA/Ket</td>
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<tr>
<td></td>
<td>20% MVC</td>
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<tr>
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B  Single Contraction Bout for a Given % MVC

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<th>Contraction</th>
<th>Contraction</th>
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<td>Rest</td>
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<tr>
<td>3 min</td>
<td>1.5 min</td>
<td>1.5 min</td>
</tr>
<tr>
<td>Rest</td>
<td>Rest</td>
<td>Rest</td>
</tr>
<tr>
<td>3 min</td>
<td>1.5 min</td>
<td>1.5 min</td>
</tr>
</tbody>
</table>

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Fig. 1. Experimental timeline. A: each experimental protocol consisted of three trials of single dynamic forearm contractions at three different intensities [10%, 20%, 40% maximal voluntary contraction (MVC)], all performed in triplicate. In the first trial, saline was infused via a brachial artery catheter for 3 min before each set of contractions. In the second trial, depending on the experimental protocol, either BaCl2 + ouabain (Ba/Ouab; n = 8; protocol 1) or NO-monomethyl-l-arginine + ketorolac (l-NMMA/Ket; n = 8; protocol 2) was administered for 3 min before contractions. In the third trial, all subjects received all antagonists (Ba/Ouab/L-NMMA/Ket) before each set of contractions. B: for each intensity, three contractions were performed, each lasting ~1 s. Between each contraction, at least 1.5 min of rest was provided.
Data Acquisition and Analysis

Data were collected, stored on a computer at 250 Hz, and analyzed offline with signal-processing software (WinDaq, DATAQ Instruments, Akron, OH). Baseline FBF, FVC, MAP, and HR represent an average of the last 10 s of the resting time period before muscle contraction. The postcontraction data represent beat-by-beat analysis beginning with the first unimpeded cardiac cycle immediately after the release of the contraction for a total of 30 cardiac cycles (11, 40, 64). The data presented for SNP trials represent an average of the final 30 s of predrug and postdrug infusion. Percent changes in FVC were calculated as follows: \[
\frac{(FVC_{post} - FVC_{pre})}{FVC_{pre}} \times 100
\]
This tracks changes in blood vessel radius independent of the initial level of vascular tone and is therefore the most appropriate index of changes in vasomotor tone (6). The total contraction-induced vasodilator response [area under the curve (AUC)] was calculated as the sum of absolute FVC (in ml/min) after contraction for 30 cardiac cycles minus precontraction FVC.

Statistics

All values are reported as means ± SE. Baseline hemodynamics and total vasodilation (AUC) values for each intensity (10%, 20%, and 40% MVC) were assessed by one-way repeated-measures ANOVA for drug condition. We chose to analyze each intensity separately due to differences in the magnitude of these values and to limit our analysis to only the relevant comparisons. For the change in peak vasodilation, two-way (condition × intensity) repeated-measures ANOVA was used. Student-Newman-Keuls post hoc testing was performed when a significant F value was observed. Comparisons in the control protocols were made with paired Student’s t-tests. Significance was set at P < 0.05.

RESULTS

Protocol 1

Baseline hemodynamics for both experimental protocols are shown in Table 1. Figure 2 shows the dynamic absolute FVC (A, C, and E) and the vasodilatory response (%ΔFVC; B, D, and F) to single muscle contractions at 10%, 20%, and 40% MVC. These responses followed the typical temporal pattern of vasodilation, composed of an immediate rise in FVC in all trials that peaked in an intensity-dependent manner within approximately four to five cardiac cycles and then returned to baseline levels. It is these dynamic responses from which we calculate our main variables of interest (peak vasodilation and total vasodilation). We have not performed statistical analysis on these dynamic curves but present them in an effort to be comprehensive.

Inhibition of K⁺-mediated vasodilation via BaCl₂ + ouabain infusion reduced resting FVC (Table 1 and Fig. 2) and significantly reduced the magnitude of the peak vasodilatory response at all intensities (range: 30–45%, P < 0.05; Fig. 3). Similarly, total postcontraction vasodilation (AUC) was reduced from control after BaCl₂ + ouabain infusion for all intensities (10%: −74 ± 8%, 20%: −59 ± 10%, and 40%: −55 ± 4%, P < 0.05; Fig. 4).

Additional inhibition of NO and PGs via L-NMMA + ketorolac, respectively, tended to further reduce baseline FVC (P = 0.12; Table 1 and Fig. 2) and, contrary to our hypothesis, also attenuated the vasodilatory response after a single contraction (Fig. 2). The peak postcontraction vasodilatory response was further reduced by L-NMMA + ketorolac for 20% and 40% MVC (P < 0.05; Fig. 3) but only approached significance at 10% MVC (P = 0.065). Similarly, L-NMMA + ketorolac further reduced total postcontraction vasodilation from the BaCl₂ + ouabain condition at 20% and 40% (P < 0.05) but not at 10% (P = 0.2; Fig. 4). The presence of all inhibitors (BaCl₂ + ouabain + L-NMMA + ketorolac) reduced peak vasodilation by ~60% and the total vasodilatory response (AUC) by ~80% on average for the three contraction intensities, thus explaining nearly all of the rapid vasodilation in response to a single muscle contraction. In all conditions and exercise intensities, systemic hemodynamics did not change postcontraction.

Protocol 2

Given the findings of protocol 1 regarding an effect of combined inhibition of NO and PGs, we reversed the order of pharmacological inhibition in protocol 2 to address the combined role of these pathways without prior inhibition of K⁺-mediated vasodilation. As anticipated, combined inhibition of NO and PG synthesis via L-NMMA + ketorolac, respectively, significantly reduced baseline FBF and FVC (Table 1). Figure 5 shows dynamic absolute FVC (A, C, and E) and the vasodilatory response (%ΔFVC; B, D, and F) to single muscle contractions at 10%, 20%, and 40% MVC for protocol 2. Similar to protocol 1, these responses followed the typical temporal pattern of vasodilation observed, and prior combined inhibition of NO and PGs significantly reduced the magnitude of the peak vasodilatory response at all intensities (range: 27–34%, P < 0.05; Fig. 6). Total postcontraction vasodilation (AUC) was also reduced from preblockade after L-NMMA + ketorolac at all intensities (10%: −34 ± 14%, 20%: −25 ± 12%, 40%: −40 ± 7%, P < 0.05; Fig. 7).

Additional inhibition of K⁺-mediated vasodilation via BaCl₂ + ouabain further reduced baseline FVC (Table 1) and also attenuated the vasodilatory response after a single muscle contraction. In all conditions and exercise intensities, systemic hemodynamics did not change postcontraction.

Table 1. Baseline hemodynamics

<table>
<thead>
<tr>
<th>Protocol 1: BaCl₂ + ouabain first</th>
<th>Heart Rate, beats/min</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Forearm Blood Flow, ml/min</th>
<th>FVC, ml·min⁻¹·100 mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preblockade</td>
<td>56 ± 2</td>
<td>88 ± 2</td>
<td>30.5 ± 3.8</td>
<td>34.5 ± 4.0</td>
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<tr>
<td>BaCl₂ + ouabain</td>
<td>54 ± 2</td>
<td>92 ± 2*</td>
<td>22.6 ± 1.4*</td>
<td>24.4 ± 1.4*</td>
</tr>
<tr>
<td>BaCl₂ + ouabain + L-NMMA + ketorolac</td>
<td>53 ± 1</td>
<td>97 ± 1*†</td>
<td>20.2 ± 0.8*</td>
<td>19.4 ± 1.4*</td>
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</table>

<table>
<thead>
<tr>
<th>Protocol 2: L-NMMA + ketorolac first</th>
<th>Heart Rate, beats/min</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Forearm Blood Flow, ml/min</th>
<th>FVC, ml·min⁻¹·100 mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preblockade</td>
<td>55 ± 3</td>
<td>87 ± 3</td>
<td>21.1 ± 2.5</td>
<td>24.1 ± 2.8</td>
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<tr>
<td>L-NMMA + ketorolac</td>
<td>51 ± 2</td>
<td>88 ± 2</td>
<td>17.0 ± 1.3*</td>
<td>19.2 ± 1.5*</td>
</tr>
<tr>
<td>L-NMMA + ketorolac + BaCl₂ + ouabain</td>
<td>52 ± 3</td>
<td>91 ± 3*</td>
<td>14.1 ± 1.6*‡</td>
<td>15.5 ± 1.7*‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects/protocol. FVC, forearm vascular conductance; L-NMMA, Nω-monomethyl-L-arginine. *P < 0.05 vs. preblockade; †P < 0.05 vs. BaCl₂ + ouabain; ‡P < 0.05 vs. L-NMMA + ketorolac.

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Fig. 2. **Protocol 1**: effect of BaCl₂/ouabain and BaCl₂/ouabain + L-NMMA + ketorolac on dynamic vasodilator responses to single contractions. Absolute forearm vascular conductance (FVC; A, C, and E) and relative changes in FVC (%ΔFVC; B, D, and F) are shown for 10%, 20%, and 40% MVC single contractions, respectively. Across all intensities, prior infusion of BaCl₂/ouabain reduced the postcontraction vasodilatory response compared with the preblockade (saline) condition. Additional infusion of L-NMMA + ketorolac further inhibited these responses.
Infusion of BaCl2 significantly reduced peak postcontraction FVC (P < 0.05 vs. zero) at all contraction intensities. The addition of L-NMMA further inhibited this response at 20% and 40% MVC. *P < 0.05 vs. zero; †P < 0.05 vs. BaCl2 + ouabain.

Control Experiments

To confirm preserved vasodilator capacity after the administration of BaCl2 + ouabain + L-NMMA + ketorolac, SNP was administered preblockade and at the end of the experimental protocol in a subgroup of six subjects. SNP caused significant vasodilation that was unaffected by BaCl2 + ouabain + L-NMMA + ketorolac, despite a reduction in baseline FVC (Table 2).

As stated above and shown in Table 1, changes in baseline FVC were observed after administration of the experimental antagonists. To determine whether there was a direct effect of reduced baseline FVC per se on the vasodilator response to a single contraction, we preconstricted the forearm vasculature with PE (α1-adrenergic agonist) and had four subjects perform single contractions at 40% MVC and compared this with the control (preblockade) condition. As shown in Fig. 8A, we were successful in reducing baseline FVC to a similar extent as occurred in our experimental protocols (~30%). In contrast to what was observed in our experimental conditions (BaCl2 + ouabain, L-NMMA + ketorolac, and BaCl2 + ouabain + L-NMMA + ketorolac), preconstriction with PE did not reduce the dynamic vasodilator response to a single contraction (Fig. 8B), nor did it impact total postcontraction vasodilation (control: 1,585 ± 258 ml/100 mmHg vs. preconstriction: 1,497 ± 188 ml/100 mmHg, P = 0.7).

DISCUSSION

The purpose of the present study was to determine the primary vasodilator signaling pathways involved in the response to a single muscle contraction. Specifically, based on prior work, we were interested in the contribution of K⁺-stimulated vascular hyperpolarization and the combined contribution of NO and PGs. The primary novel findings of the present study support a significant role for K⁺-mediated vasodilation in all facets of the rapid vasodilator response. Inhibition of K⁺-mediated vascular hyperpolarization significantly reduced peak and total vasodilation in response to three increasing intensities of single muscle contractions. Additionally, and contrary to our original hypothesis, we demonstrated that combined inhibition of NO and PG synthesis significantly reduced peak and total vasodilation after a single contraction. When all signaling pathways were inhibited, peak and total vasodilatory responses were reduced remarkably by ~60 and ~80%, respectively, compared with control conditions, thus explaining the majority of the response. Our collective findings identify, for the first time, that inhibition of K⁺-mediated vascular hyperpolarization, along with NO and PG synthesis, nearly abolishes the rapid vasodilator response to a single muscle contraction and that these pathways largely explain this feedforward aspect of muscle blood flow regulation in humans.

Contraction-Induced Rapid Vasodilation and Contributing Signaling Pathways

A previous study (1) investigating muscle blood flow regulation in response to muscle contractions appreciated the rapid nature with which hyperemia occurs after even a brief contraction. Many different theories of what contributed to the rapid response were put forth, including contributions of a mechanical effect of the muscle pump to alter perfusion pressure (29,
Fig. 5. Protocol 2: effect of L-NMMA + ketorolac and L-NMMA + ketorolac + BaCl2 + ouabain on dynamic vasodilator responses to single contractions. Absolute FVC (A, C, and E) and relative changes in FVC (B, D, and F) are shown for 10%, 20%, and 40% MVC single contractions, respectively. Across all intensities, prior infusion of L-NMMA + ketorolac reduced the postcontraction vasodilatory response compared with the preblockade (saline) condition. Additional infusion of BaCl2 + ouabain further inhibited these responses.
30, 45), direct mechanically induced vasodilation via arteriole compression/distortion (14, 32, 40, 49), neurally mediated vasodilation (7, 8, 68), and metabolic vasodilation (31). Near the end of the 20th century, a key study by Tschakovsky and colleagues (65) eloquently demonstrated that mechanical effects of a contraction and resultant changes in perfusion pressure could not fully explain the hyperemic response and that vasodilation of the vasculature did in fact occur in response to a single contraction in humans. These findings were confirmed by studies (3, 33, 47, 67) in experimental animals where changes in arteriolar diameter were directly determined. Despite the acceptance of this immediate rapid vasodilation, studies in humans designed to understand the signaling mechanisms have yielded largely negative results. Typically performed in the forearm, human studies have shown little to no independent role for ACh (5), NO (5), or PGs (63) in the mediation of rapid vasodilation. To date, the most convincing evidence regarding the mechanism of rapid vasodilation comes from the subgroup experiment: forearm hemodynamics during SNP infusion.

Table 2. Subgroup experiment: forearm hemodynamics during SNP infusion

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline FVC</th>
<th>SNP (2 μg·dl−1 forearm volume−1·min FVC−1)</th>
<th>%ΔFVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preblockade</td>
<td>16.2 ± 2.4</td>
<td>138.5 ± 25.5</td>
<td>877 ± 276</td>
</tr>
<tr>
<td>t-NMMA + ketorolac + BaCl2 + ouabain</td>
<td>12.2 ± 1.4*</td>
<td>117.5 ± 12.6</td>
<td>912 ± 124</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. SNP, sodium nitroprusside. *P < 0.05 vs. preblockade.

...
from animal models and suggests that $K^+$ most likely from muscle released during contraction diffuses to vascular smooth muscle to activate both $K_{IR}$ channels and $Na^+-K^+-ATP$ase and cause hyperpolarization and subsequent rapid vasodilatation (3, 33). However, before the present study, this hypothesized mechanism of $K^+$-mediated vascular hyperpolarization had not been tested in humans.

Recently, we (17) demonstrated the ability to abolish exogenous $K^+$-mediated (intra-arterial KCl) vascular hyperpolarization and vasodilation with combined BaCl$_2$ to inhibit $K_{IR}$ channels and ouabain to inhibit $Na^+-K^+-ATP$ase. Thus, building on this established pharmacology and recent findings in experimental animals, we tested the hypothesis that $K^+$-stimulated vascular hyperpolarization contributes to contraction-induced rapid vasodilation in humans. In protocol 1, we demonstrated that combined blockade of $K^+$-mediated hyperpolarization significantly reduced both peak and total rapid vasodilatory responses (Figs. 2–4). In addition to the ~30–45% reduction in peak change in vascular conductance (Fig. 3), we observed an ~55–75% attenuation in total vasodilation after a single muscle contraction (Fig. 4). Taken together, the impact of inhibition of $K^+$-mediated hyperpolarization on total vasodilation occurring postcontraction was profound and was by far the largest in magnitude of any prior pharmacological inhibition of the rapid vasodilatory response in humans (5, 63).

In protocol 1, given prior observations in humans indicating little to no independent role for NO or PGs in single contraction-induced rapid vasodilatation (5, 63), we were somewhat surprised to observe a significant reduction in the response with combined inhibition of these substances (Figs. 3 and 4). Specifically, we demonstrated that combined inhibition of NO and PGs further reduced the peak dilatory response from the BaCl$_2$ + ouabain condition (~55–65% from control; Fig. 3) as well as total vasodilation (~80% from control; Fig. 4). These results contrast previous findings in response to brief muscle contractions when each substance was independently inhibited (5, 63), but, on the other hand, are consistent with what has been demonstrated during continuous steady-state exercise (51, 61). Based on the findings from protocol 1, we next sought to determine the combined role of NO and PGs in contraction-induced rapid vasodilation before inhibition of $K^+$-mediated vascular hyperpolarization.

In protocol 2, we reversed the order of drug infusions and found that combined inhibition of NO and PGs reduced the peak response by ~25–35%, whereas the total vasodilatory response was reduced by ~20–40%. These data are the first to clearly demonstrate a combined active role for these substances in contraction-induced rapid vasodilation in humans and is consistent with the previously observed increased role of NO/PGs when these pathways are inhibited in combination compared with inhibited independently (46, 61). When BaCl$_2$ + ouabain was infused after inhibition of NO and PGs, the peak response was further attenuated (~60–70% from control; Fig. 6) as was the total vasodilatory response (~80% from control; Fig. 7). Thus, in both protocols 1 and 2, the impact of all inhibitors on the rapid vasodilatory response was profound, and our data provide clear evidence for the combined importance of $K^+$-mediated vascular hyperpolarization and NO and PGs in mediating this response in healthy human subjects.

Potential Stimuli for Vasodilation After a Single Muscle Contraction

Evidence from the present study clearly supports a profound effect of $K^+$-mediated vasodilation and NO and PGs on contraction-induced rapid vasodilatation; however, the exact stimulus for the activation of these signaling pathways is unclear. Armstrong and colleagues (3) demonstrated that both BaCl$_2$ and ouabain can independently inhibit the arteriolar vasodilation observed in response to muscle contractions evoked via electrical stimulation and that these pathways were likely activated by $K^+$ efflux from skeletal muscle into the interstitial space; however, direct interstitial measurements $K^+$ were not made. In this model, they were able to pharmacologically inhibit voltage-gated $K^+$ channels to specifically address skeletal muscle $K^+$ as the stimulus to activate both $K_{IR}$ channels and Na$^+-K^+-ATP$ase. Unfortunately, in our human forearm model, we were not able to inhibit $K^+$ efflux from contracting muscle, and thus we are limited in the conclusions we are able to make regarding the source of $K^+$, as it could derive from skeletal muscle, as suggested by Armstrong and colleagues, or alternatively from small- and intermediate-conductance Ca$^{2+}$-activated $K^+$ channels on endothelial cells (28).

$K^+$ is an attractive candidate for the stimulus of rapid vasodilation as the time course is appropriate, it causes hyperpolarization via stimulation of both $K_{IR}$ and Na$^+-K^+-ATP$ase, and, if originating from contracting skeletal muscle, would serve as a feedforward mechanism that couples rapid vasomotor responses with muscle activation (9, 22, 53). Additionally, an animal study (9) has reported that $K^+$-mediated vasodilation is most often transient in nature, and this may in part contribute to the distinct temporal pattern of the response.

While $K^+$ is one candidate for stimulating both $K_{IR}$ channels and Na$^+-K^+-ATP$ase and supports our primary hypothesis regarding the mechanisms of rapid vasodilation, $K^+$ (specifically that from skeletal muscle) would not explain the unexpected combined involvement of NO and PGs that we observed (17). Recently, extracellular production of adenosine, resulting from the degradation of adenine nucleotides via ecto 5’-nucleotidase, has been shown to contribute to rapid vasodilation after electrically stimulated contraction of the hamster cremaster muscle (59). Previous evidence also suggests that adenosine is capable of stimulating NO and PG production in humans (52, 57). It is also possible that ACh, released at the neuromuscular junction, could diffuse to nearby capillaries and stimulate NO and PG production (25). However, data in humans using atropine to inhibit muscarinic receptors have shown little role for ACh in rapid vasodilatation (5), and, thus, this latter possibility is unlikely. Importantly, endothelial cell changes in intracellular Ca$^{2+}$ concentration may be directly sensitive to the mechanical compression/distortion of the vasculature or changes in shear stress resulting from muscle contraction and could stimulate NO and PG production (14, 32, 40, 44, 58). Endothelial cell Ca$^{2+}$ concentration changes can also activate Ca$^{2+}$-activated $K^+$ channels to stimulate both direct and $K^+$-mediated hyperpolarization and consequently could explain the observed effects of inhibition of $K^+$-mediated vasodilation via BaCl$_2$ + ouabain as well as the inhibition of combined NO and PG synthesis via l-NMMA + ketorolac.
Experimental Considerations

In several experimental conditions, alterations in baseline forearm vascular tone occurred as a result of the antagonists we administered. While we present our primary data as a relative change from baseline, as this appropriately tracks changes in arteriolar caliber from conditions of altered baseline blood flow (6), we felt it was necessary to more directly address whether increased basal vascular tone per se may impact the vasodilator response to a single contraction. In a subgroup of subjects, we preconstricted the forearm vasculature to a similar magnitude as observed in our experimental conditions with local infusions of the α1-adrenergic agonist PE. Despite starting from a reduced level of FVC, there was no impact of preconstriction on the vasodilator response after a brief contraction at 40% MVC (Fig. 8). Thus, although the mechanisms by which PE causes an increase in basal vascular tone may differ from those of our inhibitors, we do not believe our primary conclusions regarding K\textsuperscript{+}-mediated hyperpolarization and NO and PGs are simply due to a direct effect of the inhibitors on basal vascular tone.

Given the magnitude of the effect on vasodilation that we observed, it is reasonable to question whether vasodilator capacity per se was impaired in our subjects throughout the experimental trials. To address this potential concern, in a subgroup of subjects, we administered SNP, an endothelium-independent vasodilator, and demonstrated a preserved response after the administration of all of our antagonists (Table 2). This is consistent with recent findings from our laboratory (17) demonstrating that combined BaCl\textsubscript{2} + ouabain administration does not impair ACh-mediated vasodilation in humans. Thus, the present observations do not reflect a generalized impairment in the forearm vasculature to respond to vasodilator stimuli (i.e., our findings are specific to muscle contractions). While we have previously shown that BaCl\textsubscript{2} and ouabain inhibit KCl-mediated vasodilation (17) and a multitude of work supports that activation of K\textsubscript{IR} channels and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase leads to vascular hyperpolarization in vitro (9, 28, 55), we are inherently limited in our human in vivo model in that we are unable to directly determine cellular membrane potential and thus demonstrate vascular hyperpolarization, nor are we able to inhibit K\textsuperscript{+} release from contracting muscle. It should also be acknowledged that although we did not determine the efficacy of our inhibitors, we have clearly demonstrated efficacy of all drugs in various previous studies in the human forearm (17, 24, 61). It is possible that any potential intensity-dependent differences in the magnitude of the observed responses was due to incomplete blockade and the ability of high-intensity (i.e., 40% MVC) contractions to somewhat override our inhibitors. This is particularly true for BaCl\textsubscript{2}, which can be overridden by high concentrations of K\textsuperscript{+} (2, 37). Similarly, the remaining minimal vasodilation observed could be explained by a lack of complete blockade or, alternatively, other additional mechanisms that may contribute to rapid vasodilation in response to a brief muscle contraction.

Perspectives

As recognized in early studies and clearly appreciated in more recent investigations, the model of a single muscle contraction allows for the investigation of feedforward mechanisms of exercising blood flow regulation. Whereas much work has been done attempting to understand the underlying vasomotor signaling pathways of the metabolic feedback mechanisms of exercise hyperemia, less attention has been paid to the feedforward response (15). We (11, 43) have previously shown that rapid vasodilation is impaired in older healthy adults, and recent evidence suggests that obese individuals also demonstrate attenuated rapid vasodilation (4). The underlying mechanisms of this impairment in these populations are currently unknown but, based on the present findings, are most likely related to decreased K\textsuperscript{+}-mediated hyperpolarization and/or potentially attenuated NO and PG bioavailability (60).

Conclusions

Rapid vasodilation occurs after a brief skeletal muscle contraction, and, to date, the underlying vasomotor signaling pathways involved in this response have yet to be determined. In the present study, we demonstrated that K\textsuperscript{+}-stimulated vascular hyperpolarization and the subsequent vasodilation, as do NO and PGs, in combination. Collective blockade of these pathways nearly abolishes this phenomenon in humans, thus remarkably explaining the vast majority of rapid vasodilation. Future studies designed to determine whether vascular hyperpolarization via these pathways, independently and in tandem with NO and PGs, continues to regulate exercise hyperemia when muscle contractions are repeated in humans are warranted.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.R.C., B.S.K., and F.A.D. conception and design of manuscript; A.R.C., B.S.K., G.J.L., D.G.L., and F.A.D. performed experiments; A.R.C., B.S.K., and F.A.D. drafted manuscript; A.R.C., B.S.K., and F.A.D. edited and revised manuscript; A.R.C., B.S.K., G.J.L., D.G.L., and F.A.D. approved final version of manuscript.

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