Elevation in resting blood flow attenuates exercise hyperemia

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Valic, Zoran, Jay S. Naik, Stephen B. Ruble, John B. Buckwalter, and Philip S. Clifford. Elevation in resting blood flow attenuates exercise hyperemia. J Appl Physiol 93: 134–140, 2002. First published March 15, 2002; 10.1152/japplphysiol.00421.2001.—These experiments tested the hypothesis that elevating muscle blood flow before exercise would wash out vasoactive substances produced by muscle contraction and reduce the magnitude of exercise hyperemia and/or delay the response. In chronically instrumented dogs (n = 7), hindlimb blood flow was measured with chronically implanted flow probes during mild treadmill exercise. In an anesthetized preparation (n = 8), arterial and venous blood flows of a single hindlimb were obtained during 1-s tetanic contractions evoked by electrical stimulation of the cut sciatic nerve. Elevation of blood flow by intra-arterial infusion of adenosine attenuated the increase in flow during exercise and tetanic contraction by 48 and 47%, respectively. No delay was observed in the latency to peak flow. The attenuated hyperemic response to exercise or contraction is best explained by washout of vasoactive substance(s) produced by contracting muscle, but the residual response suggests that a metabolic mediator may not be the sole explanation for exercise hyperemia.

skeletal muscle; vasodilation; tetanic contractions; muscle pump; dog

AT THE ONSET OF DYNAMIC EXERCISE, blood flow to active skeletal muscle increases rapidly to meet muscle metabolic demand. The mechanism responsible for matching arterial oxygen delivery to metabolic demand in exercising muscle is an enigma. One explanation that dates back over a century (6) postulates that there is a metabolic mediator, but thus far no studies have been able to identify the substance(s) responsible for the increase in blood flow during exercise. We have argued that the time course of the blood flow response to a single contraction is consistent with the metabolic hypothesis (21). Metabolic regulation of vascular tone is a feedback control system in which alterations in metabolite concentration are directly related to vessel caliber. The prolonged increase in blood flow with a peak response developing several seconds after the end of contraction is the pattern expected by the release and subsequent washout of a vasoactive substance from contracting muscle. Such a pattern of response to a single contraction has been observed in other investigations (2, 26).

If accumulation of vasoactive substances is entirely responsible for vasodilation in active muscle, we reasoned that increasing the blood flow pharmacologically to the same level elicited by exercise would wash out the vasoactive substances and abolish the exercise hyperemia. To that end, we tested the hypothesis that elevating muscle blood flow before exercise would reduce the magnitude of exercise hyperemia and/or delay the response.

METHODS

Two animal models were employed: conscious, chronically instrumented dogs exercising on a treadmill and anesthetized dogs in which muscle contractions were elicited by electrical stimulation of the sciatic nerve. 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.

Series 1: dynamic exercise in conscious dogs. Seven mixed-breed dogs (19.2 ± 0.2 kg), selected for their willingness to run on a motorized treadmill, were surgically instrumented for measurement of arterial blood pressure, monitoring hindlimb blood flow, and intra-arterial drug infusion. For all surgical procedures, anesthesia was induced with thiopental sodium (25 mg/kg) and maintained through mechanical ventilation with 1.5% halothane and 98.5% oxygen. Antibiotics (1 g of cefazolin for 10 days) and analgesic drugs (0.3 mg of buprenorphine as needed) were given postoperatively. Carotid arteries were exteriorized and placed in skin tubes in the neck to allow repeated percutaneous cannulation for measurement of arterial blood pressure (20, 22). Flow probes were placed around the external iliac arteries to measure hindlimb blood flow. The leads with skin button connectors were located between the scapulae. Finally, a heparinized catheter (0.045 in. OD, 0.015 in. ID) was implanted chronically through a side branch of the femoral artery for drug infusion. The catheter was tunnelled to the back of the dog, flushed daily with saline, and filled with a heparin lock (100 IU heparin/ml in 50% dextrose solution) to maintain patency.

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Dogs were given at least 2 days to recover from the catheter surgery before any experiments were performed. All experiments were performed in a laboratory in which the temperature was maintained below 20°C. An intravascular catheter (20 gauge) was inserted retrogradely into the lumen of the carotid artery and attached to a solid-state pressure transducer (Ohmeda, Madison, WI). After the flow probes were connected to a transit-time flowmeter (Transonic Systems, Ithaca, NY) and calibrated, dogs ran on a motorized treadmill at 3 miles/h (4.8 km/h) and 0% grade under both normal and elevated blood flow conditions. Both conditions were performed in duplicate on separate days. The elevated-flow condition, achieved by continuous intra-arterial infusion of adenosine, was always performed after the control condition. While the animal rested on the treadmill, adenosine (3 mg/ml) was infused into the femoral artery catheter by using an infusion pump (Harvard Apparatus, Dover, MA). The infusion rate of adenosine was adjusted in each dog to approximate the peak blood flow observed during exercise under control conditions. Once a stable flow was achieved, the treadmill was turned on and the infusion continued throughout the exercise bout. On a separate day, maximal blood flow and conductance were determined by exercising the dogs at the highest intensity that could be achieved.

Series 2: tetanic contractions in anesthetized dogs. Eight mongrel dogs (14.3 ± 0.7 kg) were used in this series of experiments. Anesthesia was induced with bolus intravenous infusion of 100 mg/kg α-chloralose and 500 mg/kg urethane into the antecubital vein and was maintained with continuous intravenous infusion of 20 mg·kg⁻¹·h⁻¹ α-chloralose and 100 mg·kg⁻¹·h⁻¹ urethane. Animals were intubated and ventilated with room air by using a mechanical ventilator (Harvard Apparatus, Dover, MA). Tidal volume was set to 15 ml/kg and end-tidal PCO₂, measured with an infrared analyzer (Ohmeda, Miami, FL), was kept in a range between 35 and 40 Torr by adjusting respiratory frequency. After the initial surgical procedures were finished, arterial blood samples were taken for measurement of arterial Po₂, Pco₂, and pH (model ABL-30, Radiometer, Copenhagen, Denmark). Metabolic acidosis was corrected with slow intravenous infusion of sodium bicarbonate. Body temperature of the dogs was regulated via a heating pad (Gaymar, Orchard Park, NY). The following surgical procedures were performed. After dissection of the left carotid artery, an intravascular catheter (18 gauge) was inserted retrogradely into the lumen and attached to a solid-state pressure transducer (Ohmeda) placed at the level of the dog’s heart for measurement of systemic arterial blood pressure. An external iliac artery and the ipsilateral external iliac vein were exposed through an abdomino-inguinal incision. Hindlimb blood flow was measured with transit-time ultrasound flow probes (Transonic Systems) placed around both the external iliac artery and vein. An acoustic couplant (model 1181, Nalco) was used to displace air within the probe’s measurement window. To reduce collateral blood flow into and out of the hindlimb, the internal iliac artery and vein were ligated. For intra-arterial administration of drugs, a catheter was introduced into a side branch of the external iliac artery. The dog’s head was then positioned in a stereotaxic apparatus (Stoelting, Wood Dale, IL) and the torso was extended by caudal tension applied via a hip pin clamp to place it in a normal upright posture. A brief tetanic contraction of the hindlimb muscles was evoked by electrical stimulation (1 s at 30 Hz) of the distal end of the left sciatic nerve, which had been previously dissected and cut to avoid centrally conducted impulses along afferent nerve fibers. Two stimulating electrodes consisting of 0.20-mm-diameter Teflon-coated stainless steel wires (A-M Systems, Everett, WA) were inserted into the nerve and then wrapped tightly around it. To prevent movement of the contracting hindlimb, which could potentially move the position of the ultrasound flow probe, the leg was secured at the ankle, thus producing an isometric contraction. The minimal current required to elicit an observable contraction was defined as the motor threshold, and all contractions were performed at 10 times this intensity, which is a supramaximal stimulus. Duplicate contractions were performed for each of the three experimental conditions employed: 1) normal arterial inflow into hindlimb, 2) doubled arterial inflow with intra-arterial infusion of adenosine, and 3) doubled arterial inflow with intra-arterial infusion of acetycholine. The mean of the two responses for each dog was used for statistical analysis. Adenosine (3 mg/ml) or acetycholine (1 μg/ml) was administered by continuous infusion (Harvard Apparatus), and acetycholine infusion was always done last because of adherence of this chemical to the catheter.

Data analysis. Systemic arterial blood pressure and blood flow signals were recorded continuously by using a MacLab data-acquisition system (AD Instruments, Castle Hill, Australia) sampling at 100 Hz and stored on microcomputer (Apple G3 Power PC). In series 1, data were analyzed off-line for the calculation of 1-s averages of arterial blood pressure, heart rate, conductance, and hindlimb blood flow. In series 2, ensemble averages were used for calculation of arterial blood pressure, heart rate, and hindlimb blood flows. The volume of blood expelled from the muscle during contraction was calculated by integrating the area under the venous flow curve from the beginning of contraction to the point where flow returned to baseline.

Statistical analysis. To examine the hemodynamic response to exercise under both normal and elevated blood flow conditions in series 1, a two-way ANOVA (condition × time) was performed on each of the following dependent variables: blood flow, conductance, heart rate, and blood pressure. In series 2, a two-way (time × drug) repeated-measures ANOVA was used to examine the effect of contraction and drug administration on heart rate and blood pressure. One-way repeated-measures ANOVA was employed to examine the effect of contraction on the difference in postcontraction hyperemia as well as the volume of blood expelled from venous system. Where significant F-variance ratios were found, a Tukey’s post hoc test was performed. Data are expressed as means ± SE. The level of statistical significance was set at P < 0.05.

RESULTS

Figure 1 is a raw tracing from an individual dog of the blood flow and blood pressure response to exercise under both the normal and elevated blood flow conditions. In both control and experimental limbs, there were immediate and rapid increases in blood flow at the start of exercise under control conditions. Intra-arterial infusion of adenosine elevated flow in the experimental limb to the level observed during control exercise and attenuated the increase in blood flow in that limb at the onset of exercise.

Figure 2 is a composite of the blood flow response to exercise in all seven dogs. Elevating resting blood flow to the level of the peak hyperemic response under control conditions reduced the initial blood flow response to exercise in the experimental limb, whereas
baseline and exercise blood flows in the control limb were unaffected. The exercise-induced increase in experimental limb blood flow was significantly less (P < 0.01) during adenosine infusion, whereas the response in the control limb was not significantly changed (Fig. 3). The time to peak blood flow was identical under the two conditions (11.0 ± 0.9 s for control and 11.0 ± 1.2 s during adenosine infusion). A summary of the hemodynamic data is presented in Table 1. It should be noted that blood pressure and heart rate were unaffected by adenosine infusion at baseline or during exercise. Importantly, the peak blood flow reached during the adenosine trial was lower for all seven dogs than the peak blood flow achieved during a separate bout of maximal exercise (985 ± 68 vs. 1,213 ± 71 ml/min, P < 0.01).

Figure 4 is a raw tracing of the typical response to a 1-s tetanic contraction under normal conditions (A), elevated flow with adenosine (B), and elevated flow with acetylcholine (C). It can be seen that under all three conditions drug infusion or contraction elicited no change in blood pressure and heart rate. Very rapid ejection of the blood is the most prominent characteristic of venous blood flow tracings. Three phases are obvious in the arterial blood flow tracing: initial reduction in flow during contraction, rapid increase on the release of contraction, and slow return to baseline flows. The time course of the blood flow response can be more easily seen in Fig. 5, in which the venous and arterial blood flows are averaged continuously for all dogs under each of the three experimental conditions. As expected, venous and arterial flows were increased similarly, regardless of which drug was used. Under control conditions, the latency to the peak flow was 4 ± 1 s and flow remained elevated for 28 ± 1 s. After flows were increased with adenosine or acetylcholine, the
fundamental shape of the curve was changed so that arterial and venous flows were increased immediately after the release of contraction, and there were no further increases.

Arterial blood flow responses to muscle contraction are summarized in Fig. 6. Under control conditions, a 1-s tetanic contraction elicited an arterial hyperemic response of 130 ± 11 ml/min. Doubling baseline flow with adenosine or acetylcholine attenuated hyperemia to 69 ± 11 and 49 ± 13 ml/min, respectively. Similar reductions were seen in venous blood flow responses (Table 2). The volume of blood expelled during contraction was not significantly different among the three conditions (Fig. 7).

DISCUSSION

Our findings demonstrate that artificially elevating hindlimb blood flow attenuated, but did not abolish, the hyperemic response to exercise or tetanic contraction. Attenuation of the hyperemic response to exercise or contraction is best explained by washout of vasoactive substance(s) produced by contracting muscle. Although the data indicate that metabolic vasodilation makes a considerable contribution to the initial blood flow response, the fact that the response was not abolished suggests that a metabolic mediator is not the sole explanation for exercise hyperemia.

Despite over a century of investigation, the evidence for metabolic vasodilation is indirect, arising from the tight coupling between oxygen consumption and muscle blood flow (1) and the fact that infusion of putative metabolic mediators evokes vasodilation. Since the first demonstration by Gaskell (6) that infused lactate produced vasodilation, numerous chemical substances

Table 1. Heart rate, blood pressure, arterial blood flow, and conductance responses to exercise and drug infusion (series 1)

<table>
<thead>
<tr>
<th>Time</th>
<th>Heart Rate, beats/min</th>
<th>Blood Pressure, mmHg</th>
<th>Arterial Blood Flow, ml/min</th>
<th>Conductance, ml · min⁻¹ · mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control limb</td>
<td>Exp limb</td>
<td>Control limb</td>
</tr>
<tr>
<td>Baseline</td>
<td>115 ± 9</td>
<td>115 ± 4</td>
<td>156 ± 22</td>
<td>1.36 ± 0.16</td>
</tr>
<tr>
<td>Peak</td>
<td>216 ± 11</td>
<td>111 ± 5</td>
<td>656 ± 55</td>
<td>5.82 ± 0.55</td>
</tr>
<tr>
<td>Adenosine infusion</td>
<td>134 ± 10</td>
<td>117 ± 3</td>
<td>156 ± 36</td>
<td>1.42 ± 0.26</td>
</tr>
<tr>
<td>Baseline</td>
<td>224 ± 24</td>
<td>114 ± 5</td>
<td>601 ± 53</td>
<td>5.88 ± 0.35</td>
</tr>
</tbody>
</table>

Values are means ± SE. Exp, experimental.
have been proposed to mediate the hyperemic response to exercise (17). The evidence is strongest for the involvement of potassium ions (10, 11), although it is unlikely that increases in interstitial potassium can totally account for the vasodilation (12). A previous study from this laboratory (21) revealed that the time course of the blood flow response to a single contraction displayed a delayed peak and prolonged increase as would be expected after release of a vasoactive substance. We reasoned that, if accumulation of vasoactive substances were entirely responsible for vasodilation in active muscle, increasing the blood flow pharmacologically would prevent the development of hyperemia by washing out the vasoactive substances. This approach is reminiscent of the study by Patterson and Shepherd (23), who infused various vasodilators intraarterially in human volunteers during 2 min of forearm contractions but, because of the limitations of plethysmographic blood flow measurements, were unable to measure blood flow during contractions. They found that vasodilator infusion failed to alter the postexercise "blood flow debt" or hasten its repayment and suggested the possibility of a metabolite whose removal was insensitive to flow. In contrast, in series 1, we measured blood flow at the onset of dynamic exercise and observed a reduction in the hyperemic response. In addition, hyperemia after a single 1-s contraction was reduced in series 2. We attribute the attenuated hyperemia in these series of experiments to washout of substances.

Table 2. Heart rate, blood pressure, and arterial and venous blood flow responses to muscle contraction and drug infusion (series 2)

<table>
<thead>
<tr>
<th>Time</th>
<th>Heart Rate, beats/min</th>
<th>Blood Pressure, mmHg</th>
<th>Arterial Blood Flow, ml/min</th>
<th>Venous Blood Flow, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Baseline 145 ± 8</td>
<td>130 ± 6</td>
<td>230 ± 33</td>
<td>241 ± 28</td>
</tr>
<tr>
<td></td>
<td>Peak 140 ± 9</td>
<td>127 ± 6</td>
<td>359 ± 42</td>
<td>347 ± 34</td>
</tr>
<tr>
<td>Adenosine infusion</td>
<td>Baseline 158 ± 10</td>
<td>131 ± 5</td>
<td>455 ± 60</td>
<td>456 ± 54</td>
</tr>
<tr>
<td></td>
<td>Peak 151 ± 10</td>
<td>130 ± 6</td>
<td>524 ± 63</td>
<td>499 ± 53</td>
</tr>
<tr>
<td>Acetylcholine infusion</td>
<td>Baseline 150 ± 11</td>
<td>130 ± 5</td>
<td>459 ± 66</td>
<td>456 ± 61</td>
</tr>
<tr>
<td></td>
<td>Peak 147 ± 11</td>
<td>129 ± 5</td>
<td>508 ± 63</td>
<td>496 ± 51</td>
</tr>
</tbody>
</table>

Values are means ± SE. Peak venous blood flows represent the peak postcontraction value. *Significantly different from baseline (P < 0.01). †Significantly different from control (P < 0.01).

Fig. 5. Averaged venous (top) and arterial (bottom) blood flow responses of 8 dogs to 1-s tetanic contraction under the 3 different experimental conditions: control (thick lines) and adenosine or acetylcholine infusion (thin lines). Attenuation of the contraction-induced hyperemia is clearly visible in the arterial blood flow tracings.

Fig. 6. Arterial blood flow response to muscle contraction. Shown are the changes from baseline to peak blood flow under the 3 experimental conditions. There was a reduced arterial inflow after blood flow was elevated with adenosine (ADO; hatched bar) or acetylcholine (ACh; solid bar). *Significantly different from control (P < 0.01).

Fig. 7. Total blood volume expelled during a 1-s tetanic muscular contraction, calculated as area under the venous flow curve. There were no significant differences in the volume expelled under the 3 conditions (P = 0.29).
vasoactive substance(s) produced by the contracting muscles.

There are two alternative explanations that could be considered to account for our observations. First, the augmented oxygen delivery associated with the artificially elevated blood flow may reduce the formation of metabolites related to the supply of oxygen. This is an unlikely explanation because aerobic metabolic processes are too slow to account for the rapid (< 1 s) blood flow response. In contracting single skeletal muscle fibers, there was a 13-s delay from the onset of contractions before intracellular PO2 began to decrease (9). Furthermore, hyperoxia (13) and polycythemia (5) do not alter skeletal muscle blood flow during exercise. A second explanation is that the dose-response curve for the endogenous vasodilator agent is nonlinear, and pharmacological vasodilation shifts the vascular smooth muscle to a flatter part of the curve. This possibility cannot be excluded, but we would point out that results were similar in both series of experiments despite that peak flows encountered during vasodilator infusion averaged 985 ml/min in series 1 and just over 500 ml/min in series 2. Thus the two series of experiments were performed over different portions of the vascular smooth muscle dose-response curve.

A major critique of the metabolic theory comes from in vitro studies of skeletal muscle arterioles that show that the latency to dilation after exposure to putative metabolic mediators is too slow to account for the rapid vasodilation (7, 27). The present results also raise questions about the ability of the metabolic theory to fully account for the initial increases in blood flow at the onset of contractions. After flow was artificially elevated to a level that should have been adequate to support the increased metabolic needs of the muscles, exercise and brief tetanic contraction elicited additional increments in blood flow. It is conceivable that the distribution of increased flow within the muscle did not match the regions of increased metabolite production and that the additional increment in blood flow is due to accumulation of metabolites in these regions. It is equally plausible that there is a nonmetabolic mechanism involved in the initial increases in blood flow at the onset of contractions.

One such nonmetabolic mechanism is the muscle pump, which has been proposed as a rapid, local mechanism by which active skeletal muscle can regulate its own blood supply (3, 4, 15). It is believed that muscle contraction empties the venous circulation that lowers venous pressure during relaxation and increases the pressure gradient across the muscle vascular bed. Unfortunately, because of the presence of venous valves, it has been technically impossible to measure pressure in the venules in skeletal muscle, so investigators have been forced to make inferences about the muscle pump from indirect data. It is unlikely that the muscle pump is solely responsible for the increase in blood flow at the onset of exercise because theoretical calculations suggest that achieving observed magnitudes of blood flow would require negative pressures so great that the veins would collapse (21, 25). Nevertheless, the fact that limb position affects the magnitude of the increase in blood flow to muscle contractions supports the muscle pump theory (4, 18, 24, 26). Accordingly, it has been proposed that vasodilation and the muscle pump are together responsible for the rapid hyperemia observed in exercising humans and animals (18, 24, 26). In the present study, the exercise-induced increment in blood flow after elevation of resting blood flow could be attributed to the muscle pump. In fact, it might be expected that the muscle pump would be more effective in a dilated vascular bed. The failure of vasodilators to increase the volume of blood expelled during supramaximal muscle contraction in series 2 does not support this notion. All considered, the present data seem consistent with the concept of blood flow to exercising skeletal muscle consisting of a metabolic vasodilation component and a muscle pump component.

The use of adenosine as the vasodilator in the experimental protocols may raise a methodological concern because adenosine is a putative mediator of metabolic vasodilation. Adenosine was employed here because of its potency and rapid degradation that would minimize systemic effects. Blood flow to exercising skeletal muscle is unchanged after administration of the adenosine-receptor antagonist aminophylline (14) or the adenosine-transport inhibitor diprydiamole (16). In a novel set of experiments, Hester et al. (8) showed that contraction-induced hyperemia was unchanged after desensitization of the vasculature to adenosine. Thus, despite long-standing interest in adenosine as a mediator of metabolic vasodilation, there is strong evidence against its involvement in exercise hyperemia. It is also noteworthy that series 2 experiments were repeated with essentially identical results by using acetycholine as the vasodilator.

There are several strengths to the experimental paradigms employed in these experiments. Local infusions of vasodilator drugs elevate blood flow to one hindlimb without confounding systemic effects such as changes in heart rate or blood pressure. Continuous measurement of iliac blood flow permits examination of the time course of blood flow changes to the hindlimb. Although resting iliac blood flow perfuses skin and bone in addition to skeletal muscle, all of the increase in iliac blood flow goes to exercising skeletal muscle (19). The study of conscious, instrumented animals allows assessment of cardiovascular function without the confounding effects of anesthetics and permits physiological patterns of motor recruitment during dynamic exercise. In acute animal studies, a detailed analysis can be performed on the response to a single contraction, which may provide insight into the hemodynamic events at the onset of exercise.

The results of the present study show that elevating hindlimb blood flow by intra-arterial infusion of vasodilators attenuated the hyperemic response to exercise or tetanic contraction. The attenuated hyperemic response to exercise or contraction is best explained by washout of vasoactive substance(s) produced by contracting muscle, suggesting that metabolic vasodilation makes a considerable contribution to the initial
blood flow response. The residual response suggests that a metabolic mediator may not be the sole explanation for exercise hyperemia.

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