Skeletal muscle vasodilation at the onset of exercise

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Buckwalter, John B., Stephen B. Ruble, Patrick J. Mueller, and Philip S. Clifford. Skeletal muscle vasodilation at the onset of exercise. J. Appl. Physiol. 85(4): 1649–1654,1998.—The purpose of this study was to determine whether β-adrenergic or muscarinic receptors are involved in skeletal muscle vasodilation at the onset of exercise. Mongrel dogs (n = 7) were instrumented with flow probes on both external iliac arteries and a catheter in one femoral artery. Propranolol (1 mg), atropine (500 µg), both drugs, or saline was infused intra-arterially immediately before treadmill exercise at 3 miles/h, 0% grade. Immediate and rapid increases in iliac blood flow occurred with initiation of exercise under all conditions. Peak blood flows were not significantly different among conditions (682 ± 35, 646 ± 49, 637 ± 68, and 705 ± 50 ml/min, respectively). Although the doses of antagonists employed had no effect on heart rate or systemic blood pressure, they were adequate to abolish agonist-induced increases in iliac blood flow. Because neither propranolol nor atropine affected iliac blood flow, we conclude that activation of β-adrenergic and muscarinic receptors is not essential for the rapid vasodilation in active skeletal muscle at the onset of exercise in dogs.

VASODILATION IN ACTIVE SKELETAL MUSCLES during exercise reflects the transition from the low oxygen demands at rest to the high oxygen demands associated with exercise. The mechanism by which vasodilation occurs in active skeletal muscles is poorly understood, but a neural mechanism is particularly appealing because of the rapidity of the increase in skeletal muscle blood flow at the onset of exercise. Although β-adrenergic receptors and muscarinic receptors are present in the arterial vasculature of skeletal muscle (6, 17, 22, 48), there is limited evidence suggesting that sympathetic β-adrenergic (17, 23, 26, 36) and sympathetic cholinergic (40, 44) receptors may play a role in skeletal muscle hyperemia during exercise. In addition, it has been postulated that acetylcholine spillover from the neuromuscular junctions of exercising skeletal muscles may provide a source of acetylcholine for muscarinic-mediated vasodilation during exercise (41, 49). A recent study by Buckwalter et al. (8) concluded that neither β-adrenergic receptors nor muscarinic receptors are essential in maintaining vasodilation during steady-state exercise. However, the design of that study precluded examination of skeletal muscle blood flow at the onset of exercise. It has been suggested that the mechanism mediating the initial skeletal muscle hyperemia at the onset of exercise may be different from that which sustains blood flow during steady-state exercise (16).

The purpose of the present study was to examine the role of β-adrenergic- and muscarinic-mediated vasodilation in active skeletal muscles at the onset of exercise. We used a unique experimental approach that allowed manipulation of blood flow to one hindlimb without affecting systemic hemodynamics or blood flow in the contralateral limb in conscious exercising dogs. We hypothesized that β-adrenergic and muscarinic receptors play an essential role in skeletal muscle vasodilation at the onset of exercise.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” Seven mongrel dogs, weighing between 16 and 24 kg, were selected for their willingness to run on a motorized treadmill and were instrumented in a series of sterile surgical procedures. Anesthesia was induced with thiopental sodium (15–30 mg/kg; Gensia Pharmaceuticals, Irvine, CA). After intubation with auffed endotracheal tube, a surgical level of anesthesia was maintained through mechanical ventilation with 1.5% halothane (Halocarbon Laboratories, River Edge, NJ) and 98.5% oxygen. Antibiotics (cefazolin sodium, Apothecon, Princeton, NJ) and analgesic drugs (buprenorphine hydrochloride, 0.3 mg; Reckitt and Colman, Kingson-upon-Hull, UK) were given postoperatively. During the first surgical procedure, the carotid arteries were placed in skin tubes in the neck so that they could be cannulated percutaneously to measure arterial blood pressure (31, 33). In the second surgery, all dogs were instrumented with flow probes (4- or 6-mm ultrasonic transit-time flow probes, Transonic Systems, Ithaca, NY) around the external iliac arteries to measure hindlimb blood flow. The cables were tunneled under the skin to the back, and the dogs were given 2 wk to recover from flow probe implantation. In the final surgery, a heparinized catheter (0.045 in. OD, 0.015 in. ID, Data Science International, St. Paul, MN) was implanted chronically through a side branch of the femoral artery for drug infusion. The catheter was tunneled to the back of the dog. The catheter was flushed daily with saline and filled with a heparin lock (100 IU heparin/ml in 50% dextrose solution) to maintain patency. The dogs were given at least 2 days to recover from the final surgery before any experiments were performed.

All experiments were performed in a laboratory in which the temperature was maintained below 20°C. A 20-gauge Teflon catheter (Insyte, Becton Dickinson, Sandy, UT) was inserted retrogradely into the lumen of the carotid artery and attached to a solid-state pressure transducer (Ohmeda, Madison, WI). The flow probes were connected to a transit-time flowmeter (Transonic Systems). Immediately before the start of exercise, 1 mg propranolol (nonselective β-adrenergic-
receptor antagonist), 500 µg of atropine, (muscarnic antagonist), both drugs, or a saline (as a control) was infused intra-arterially into one hindlimb. The dogs then ran on a motorized treadmill at 3 miles/h (mph; 4.8 km/h), 0% grade, which represents a mild workload. Between 1 and 2 min of exercise, either a nonselective β-adrenergic-receptor antagonist (0.2 µg isoproterenol, Abbott Laboratories, Chicago, IL) or a muscarinic-receptor agonist (1 µg acetylcholine, Sigma Chemical, St. Louis, MO) was infused intra-arterially to test the effectiveness of receptor blockade. These doses of the receptor agonists and antagonists have been previously used in this laboratory (8). At least 24 h separated each experiment, all experiments were performed in duplicate, and data were averaged for each dog.

Arterial blood pressure and right and left external iliac blood flow were simultaneously written to paper on a polygraph recorder (model 7, Grass, Warwick, RI) and stored on both a videocassette data recorder (model D, Vetter, Rebersburg, PA) and computer (Apple 8500 Power PC) by using a MacLab system at 100 Hz (ADInstruments, Castle Hill, Australia). Data were analyzed off-line by using the MacLab software to calculate mean arterial pressure, heart rate, and iliac blood flow. Blood flow was averaged over 10 s of rest preceding the initiation of exercise and over 1-s intervals for the first 30 s of exercise. The peak blood flow attained and the time to peak blood flow at the onset of exercise were also analyzed for each condition. Hemodynamic variables were averaged over 15 s before agonist infusion and over 1-s intervals after infusion. The peak response was recorded, and, where no response was obvious, the peak response was chosen over the same interval where it occurred during the control (no antagonist) experiment.

Statistical analyses of the data were performed with a two-way repeated-measures (drug x time) analysis of variance for the blood flow averages at 5-s intervals over the first 30 s of exercise. One-way repeated-measures analyses of variance were used to examine the peak blood flow response at the onset of exercise, time to peak blood flow, blood pressure, and heart rate. A paired t-test was used to examine the magnitude of agonist-induced increases in blood flow. An α level of 0.05 was used to establish statistical significance. Where significant F-ratios were found, a Tukey’s post hoc test was performed. All descriptive statistics are presented as means ± SE.

RESULTS

Table 1 provides heart rate and blood pressure data during exercise with the four different receptor-blockade conditions. There were no statistically significant differences in heart rate or blood pressure among any of the conditions in these experiments.

Figure 1A is an original tracing from an individual dog beginning exercise on the treadmill at 3 mph, 0% grade, after pretreatment with saline. There were immediate increases in blood flow in the experimental and control hindlimbs that exceeded the eventual steady-state values. Between 1 and 2 min of exercise, an intra-arterial bolus of 0.2 µg of isoproterenol was given and caused an immediate increase in blood flow in the experimental hindlimb with no change in heart rate, blood pressure, or blood flow in the contralateral limb. Mean data from seven dogs revealed a statistically significant increase in experimental hindlimb blood flow with isoproterenol (465 ± 22 to 923 ± 40 ml/min; P < 0.0001).

Table 1. Hemodynamic values during exercise at 3 mph, 0% grade, after intra-arterial antagonist infusion

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>Peak Blood Flow, ml/min</th>
<th>Time to Peak Blood Flow, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>119 ± 3.7</td>
<td>147 ± 7.8</td>
<td>705 ± 50.3</td>
<td>14.1 ± 1.9</td>
</tr>
<tr>
<td>Atropine</td>
<td>112 ± 4.0</td>
<td>138 ± 6.3</td>
<td>646 ± 48.5</td>
<td>14.1 ± 0.7</td>
</tr>
<tr>
<td>Propranolol</td>
<td>119 ± 2.8</td>
<td>144 ± 6.7</td>
<td>682 ± 34.7</td>
<td>12.0 ± 1.3</td>
</tr>
<tr>
<td>Atropine + propranolol</td>
<td>123 ± 2.6</td>
<td>135 ± 7.9</td>
<td>637 ± 67.7</td>
<td>13.1 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate. There were no statistically significant differences (P > 0.05) among any of the 4 antagonist conditions.
control hindlimbs that exceeded the eventual steady-state values. Between 1 and 2 min of exercise, an intra-arterial bolus of 1 µg acetylcholine was given and caused an immediate increase in blood flow in the experimental hindlimb with no change in heart rate, blood pressure, or blood flow in the contralateral limb. For the group, blood flow in the experimental hindlimb increased with acetylcholine from 491 ± 25 to 914 ± 49 ml/min (P = 0.0002). Figure 2B is an original tracing from the same dog beginning exercise after pretreatment with atropine. As before, there were immediate increases in blood flow in both hindlimbs. The intrarterial bolus of 1 µg of acetylcholine between 1 and 2 min of exercise did not affect experimental limb blood flow. Mean blood flows for the 7 dogs were 555 ± 27 ml/min before infusion of acetylcholine and 554 ± 30 ml/min postinfusion (P > 0.05).

Figure 3 presents the iliac blood flow response in the experimental hindlimb over the first 30 s of exercise for the four different conditions. As can be readily appreciated from inspecting Fig. 3, there was a significant effect of time on blood flow (P < 0.0001). However, statistical analysis of blood flow data revealed no statistically significant differences among any of the drug conditions (P > 0.05), and there was no significant interaction between time and drug condition (P > 0.05). Analysis of the peak blood flow response and the time to peak blood flow among the four conditions yielded similar results (Table 1). There were no significant differences in peak blood flow responses or times to peak blood flow among any of the drug conditions (P > 0.05).

**DISCUSSION**

We observed no changes in the time course or the magnitude of the blood flow response in the canine hindlimb at the onset of mild exercise after blockade of β-adrenergic or muscarinic receptors. The lack of an effect indicates that neither β-adrenergic nor muscarinic receptors are required for the immediate skeletal muscle hyperemia at the onset of mild exercise in the dog.

The experimental design in this study provides several distinct advantages over previous attempts to examine the role of β-adrenergic or muscarinic receptors during exercise. 1) Intra-arterial infusion of small doses of receptor antagonists creates a functionally isolated hindlimb in which localized blockade is produced in the experimental limb without confounding systemic changes in heart rate, mean arterial pressure, or blood flow in the control hindlimb. 2) Continuous recordings of blood flow with transit-time flow probes, as contrasted with discrete measurements with radioactive microspheres, allow more thorough characteriza-
tion of the time course of blood flow changes. 3) We employed a mild intensity of exercise because we reasoned that this would minimize the metabolic contribution to vasodilation and thus provide the optimal conditions to unmask a neural component. The time course of changes in blood flow shows an overshoot at this workload (Fig. 3). Substantial overshoot has been shown in previous studies at mild exercise intensities (25, 37) and has been attributed to neurally mediated vasodilation (4).

Differential reactivity of arterioles to intravascular vs. extravascular administration of agonists has been demonstrated (19, 28, 29), leading to the suggestion that there is a barrier to diffusion of water-soluble molecules from the lumen to smooth muscle of arterioles (29). Because both propranolol and atropine are lipophilic molecules, we assume that both intraluminal and extraluminal receptors are blocked by intrarterial infusions of these receptor antagonists. In addition, we have previously shown that intrarterial infusion of the α1-adrenergic-receptor antagonist prazosin interrupts tonic sympathetic nerve activity in the hindlimb, causing vasodilation (7). This indirect evidence suggests that the receptor antagonists in this study would bind the receptors accessible to norepinephrine released from sympathetic nerve terminals in the canine skeletal muscle vasculature. However, it must be acknowledged that the accessibility of smooth muscle β-adrenergic and muscarinic receptors with intrarterial infusion of propranolol and atropine is a potential limitation of this study.

β-adrenergic-receptor blockade. Previous studies examining the role of β-adrenergic-mediated vasodilation during exercise have come to conflicting conclusions with several studies reporting a reduction in blood flow to working skeletal muscles (2, 17, 23, 26, 36), whereas others found no effect (8, 18, 22). In the studies that found reductions in skeletal muscle blood flow during exercise with systemic nonselective β-blockade, there were also markedly lower heart rates (2, 17, 23, 26, 36). The reductions in skeletal muscle blood flow were most likely the result of reduced cardiac output and arterial pressure due to β2-blockade in the heart rather than to peripheral β1-blockade. In support of this, systemic propranolol administration in anesthetized cats increased total peripheral resistance via baroreceptor-mediated increases in sympathetic constriction rather than via abolition of tonic β2-mediated vasodilation (30). In contrast, the studies that showed no effect of β-adrenergic blockade on skeletal muscle blood flow employed localized administration of the antagonist with no systemic effects (8, 18, 22).

Only one of the previous studies examined the effect of β-blockade on the transient changes in blood flow at the onset of exercise. Laughlin and Armstrong (26) found reduced muscle blood flow in all the rat thigh muscle samples at 30 s of exercise with propranolol pretreatment and statistically significant reductions in blood flow in 8 of 32 muscles sampled. These differences were observed at mild, but not heavy, exercise intensities. The present study is the first to quantitatively assess the rapid increase in blood flow to working skeletal muscles throughout the first 30 s of dynamic exercise. The influence of β-adrenergic receptors was examined by using small doses of a nonselective β-antagonist to avoid confounding systemic cardiovascular changes. The nonselective β-adrenergic antagonist propranolol was chosen because of previously reported β1- and β2-mediated vasodilation in the skeletal muscle vasculature of dogs (48). Because of the small dose of propranolol and its localized effect, no β1-mediated reductions in heart rate were apparent and any changes in blood flow would reflect β-adrenergic-mediated vasodilation alone. However, the results provide no evidence of β-mediated vasodilation at the onset of exercise in the hindlimb vasculature of dynamically exercising dogs. Thus we find little support for a role of β-adrenergic receptors in the rapid hyperemia at the onset of exercise.

We reasoned that activation of β-adrenergic receptors at the onset of exercise would be mediated by increased norepinephrine release from sympathetic nerve terminals. The evidence suggests that there is an increase in efferent sympathetic nerve traffic to skeletal muscle during dynamic exercise. Direct measurements of postganglionic sympathetic nerve activity to the hindlimb (lumbar sympathetic trunk) in rats and cats showed that there was an immediate and sustained increase in lumbar sympathetic nerve activity in response to dynamic exercise (11, 20). Furthermore, our group (7) and others (21, 35, 47) have demonstrated that there is substantial sympathetic restraint of skeletal muscle blood flow at mild, moderate, and heavy exercise intensities. We interpret these results to indicate that there is release of norepinephrine from sympathetic nerve terminals in the canine skeletal muscle vasculature. The data from the present study suggest that vascular β-adrenergic receptors are not activated by the norepinephrine released from these nerve terminals at the onset of exercise.

Muscarinic-receptor blockade. The existence of sympathetic cholinergic-mediated vasodilation in the skeletal muscle vasculature has been demonstrated in a number of studies (5, 6, 9, 40). Sympathetic cholinergic dilator fibers have been shown to be activated by the defense reaction (9). Interestingly, the cardiovascular changes associated with initiation of exercise, such as tachycardia, increases in blood pressure, vasconstriction in visceral organs, and vasodilation to skeletal muscle, are remarkably similar to those seen with electrical stimulation of regions of the brain eliciting the defense reaction (1, 9, 10, 13). Besides sympathetic cholinergic fibers, another potential physiological mechanism for muscarinic-receptor activation during exercise is spillover of acetylcholine from the neuromuscular junction (41, 49). This is an attractive hypothesis that would provide a link between muscular contraction and blood flow because motor nerve activity and skeletal muscle blood flow both increase at the onset of exercise and are augmented in an intensity-dependent manner. We hypothesized that activation of muscarinic receptors is involved in the elevated blood flow to
The potential role of nitric oxide release during contractions is not consistent with the hypothesis that rhythmic muscle contractions help alter systemic vascular conductance or hindlimb conductance (39) argue against a neural mechanism. However, data from the present study as well as data demonstrating the failure of total autonomic blockade (42) or hindlimb hyperemia (16, 27). The rapidity of the increase in skeletal muscle blood flow at the onset of exercise suggests a neural component. Particularly strong is that, unlike in previous investigations (3, 5, 36), atropine was given in a small, localized dose. This allowed for interpretation of the data without confounding increases in heart rate due to blockade of cardiac muscarinic receptors. However, we find no evidence for muscarinic-receptor-mediated vasodilation in the hindlimb of the dog at the onset of exercise.

The possibility of muscarinic-receptor-mediated vasodilation compensating for the removal of β-adrenergic receptor-mediated vasodilation during selected blockade and vice versa was tested by intra-arterial infusion of both receptor antagonists in the same trial. Combined β-adrenergic- and muscarinic-receptor blockade did not affect the blood flow response at the onset of exercise. This suggests that β-adrenergic receptors and muscarinic receptors do not function as reciprocal, redundant vasodilator mechanisms at the onset of exercise.

Potential mechanisms. The physiological mechanism responsible for skeletal muscle exercise hyperemia has been a topic of investigation for over 100 years, since Gaskell’s proposal (14) that vasodilation occurred in contracting muscle due to the release of metabolites by the muscle fibers. Although skeletal muscle blood flow and metabolic activity increase in an exercise intensity-dependent manner, a mechanistic link between these two events has not been established. A number of vasodilators such as adenosine, potassium, hypoxia, osmolarity, and ATP have received attention over the years without definitive support as being essential for skeletal muscle hyperemia (16, 27). The rapidity of the increase in skeletal muscle blood flow at the onset of exercise and the immediate decrease in blood flow at the cessation of exercise suggest a neural component. However, data from the present study as well as data demonstrating the failure of total autonomic blockade to alter systemic vascular conductance (42) or hindlimb conductance (39) argue against a neural mechanism. There is evidence in support of the muscle pump hypothesis, i.e., that rhythmic muscle contractions help propel blood through the muscles (24, 42, 46). However, the finding that increased venous filling did not change blood flow during contractions is not consistent with this hypothesis (32, 43). Recently, there has been interest in the potential role of nitric oxide release due to increased shear stress (38) or release from hemoglobin (45). The experimental results show that nitric oxide synthase inhibition has, at most, a modest effect on exercising blood flow in dogs (34) and humans (12, 15, 44, 50). After more than 100 years of research, the physiological mechanism responsible for skeletal muscle exercise hyperemia remains elusive.

In conclusion, the results from the present study show that there is a population of β-adrenergic and muscarinic receptors that are functionally present and accessible to intraarterial infusions of agonists and antagonists. However, these receptors do not mediate the rapid skeletal muscle hyperemia at the onset of dynamic exercise in dogs.

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