Autonomic control of skeletal muscle vasodilation during exercise

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METHODS AND PROCEDURES

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.” Six mongrel dogs, weighing between 20 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; Genentech Pharmaceuticals, Irvine, CA). After intubation of the dogs with a cuffed 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, Kingsport-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 (17, 18). 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. ID, 0.015 in. OD, 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 (Angiocath, Deseret, Sandy, UT) was inserted retrogradely into the lumen of the carotid artery and attached to a solid-state pressure transducer (Viggo-Spectramed, Oxnard, CA). The flow probes were connected to a transit-time flowmeter (Transonic Systems). The dogs ran on a motorized treadmill at 6 miles/h (mph; 9.7 km/h) 0% grade, which represents a moderate workload. Six minutes into exercise, either a nonselective β-receptor agonist (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. Blood flow returned to baseline, and the relevant antagonist (1 mg propranolol, nonselective β-receptor antagonist, or 500 µg atropine, muscarinic-receptor antagonist) was infused at 10 min of exercise. The above doses were determined in pilot studies that showed that the agonist doses nearly doubled iliac blood flow and that the antagonist doses were sufficient to abolish the response to subsequent agonist infusion. At least 24 h separated each β-adrenergic-receptor or muscarinic-receptor blockade experiment.

Arterial blood pressure and right and left external iliac blood flow were simultaneously written to paper on a poly-
graph recorder (model 7, Grass Instruments, 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 (AD Instruments, Castle Hill, Australia). Data were analyzed off-line by using the MacLab software to calculate mean arterial pressure (MAP), heart rate (HR), iliac blood flow, and iliac vascular conductance (blood flow/MAP). Control measurements were averaged over 30 s before drug infusion. For each drug infusion, all variables were averaged over 1-s intervals, and the peak response was recorded. Where no response was obvious, the peak response was chosen over the same interval where it occurred with the initial agonist infusion.

Statistical analyses of the data were performed with a one-way repeated-measures analysis of variance for isoproterenol and acetylcholine infusions. 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. A paired t-test was used to examine hemodynamic changes before and after antagonist infusions. All descriptive statistics are presented as means ± SE.

RESULTS

Figure 1 is an original raw tracing from an individual dog exercising on the treadmill at 6 miles/h, 0% grade. There was an immediate increase in blood flow and conductance in experimental limb with intra-arterial bolus of 0.2 µg of isoproterenol. In contrast, bolus of 1 mg of propranolol did not alter blood flow or conductance. Subsequent infusion of 0.2 µg of isoproterenol demonstrated effectiveness of β-receptor blockade. None of the intra-arterial bolus infusions altered systemic blood pressure or blood flow in control limb.
Table 1. Hemodynamic values before and after antagonist infusion during exercise at 6 miles/h, 0% grade

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental</th>
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<tbody>
<tr>
<td>Atropine</td>
<td></td>
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<tr>
<td>Pre</td>
<td>MAP, mmHg 114 ± 2.8 HR, beats/min 162 ± 10</td>
<td>Blood Flow, ml/min 560 ± 70</td>
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<tr>
<td>Post</td>
<td>MAP, mmHg 113 ± 3.4 HR, beats/min 166 ± 11</td>
<td>Blood Flow, ml/min 553 ± 72</td>
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<tr>
<td>Propranolol</td>
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<tr>
<td>Pre</td>
<td>MAP, mmHg 114 ± 2.9 HR, beats/min 162 ± 15</td>
<td>Blood Flow, ml/min 609 ± 106</td>
</tr>
<tr>
<td>Post</td>
<td>MAP, mmHg 117 ± 3.5 HR, beats/min 159 ± 14</td>
<td>Blood Flow, ml/min 614 ± 109</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate. There were no statistically significant differences (P > 0.05) between any of the pre antagonist (Pre) variables and the post antagonist (Post) variables.

DISCUSSION

We observed no changes in canine hindlimb blood flow during dynamic exercise after blockade of β-adrenergic or muscarinic receptors. The lack of an effect indicates that neither β-adrenergic nor muscarinic receptors are involved in skeletal muscle hyperemia during moderate steady-state dynamic 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. Intravenous 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 HR or MAP. Administration of antagonists during, rather than before, exercise interrupts ongoing β-adrenergic-receptor or muscarinic-receptor activation. Coupled with continuous recordings of blood flow, this allows both control and experimental measurements during the same bout of exercise. Furthermore, because redundant mechanisms may be involved in the control of skeletal muscle blood flow, continuous recordings should allow the detection of any transient changes in blood flow, which would precede activation of compensatory mechanisms. Finally, infusion of antagonists during exercise ensures accessibility of receptors on blood vessels recruited during exercise.

β-Blockade. Previous studies examining the role of β-adrenergic-receptor-mediated vasodilation during exercise have come to conflicting conclusions. Several studies reported a reduction in blood flow to working skeletal muscle with β-adrenergic blockade (2, 11, 14, 15, 20), whereas others found no effect (12, 13). In the studies that found reductions in skeletal muscle blood flow during exercise with β-receptor blockade, there were also markedly lower HRs reported (2, 12, 13, 17, 20). Most certainly this was the result of blockade of β₂-receptors in the heart and could have produced corresponding decreases in cardiac output and MAP with reflex increases in sympathetic outflow. In fact, Lisander and Nilsson (16) showed that systemic propranolol administration increased total peripheral resistance via baroreceptor-mediated increases in sympathetic constriction rather than abolition of ongoing β₂-receptor-mediated vasodilation in the anesthetized cat. Furthermore, Smith and Warren (24) found greater reductions in skeletal muscle blood flow with a single oral dose of the selective β₂-receptor antagonists, metoprolol and atenolol, than with a single oral dose of the nonselective antagonist, propranolol. Thus systemic cardiovascular changes observed in previous studies have made it difficult to draw firm conclusions regard-
ing β2-receptor-mediated skeletal muscle vasodilatation during dynamic exercise.

In the present study the influence of β-adrenergic receptors on skeletal muscle hyperemia during steady-state dynamic exercise was examined by using small doses of a non-β-receptor antagonist to avoid confounding systemic cardiovascular changes. The nonselective β-adrenergic-receptor antagonist, propranolol, was chosen because of previously reported β1- and β2-receptor-mediated vasodilation in the skeletal muscle vasculature of dogs (25). Because of the small dose of propranolol and its localized effect, no β1-receptor-mediated reductions in HR were apparent, and any changes in blood flow would reflect ongoing β-adrenergic-receptor-mediated vasodilation alone. However, the results show no evidence of β-receptor-mediated vasodilation in the hindlimb vasculature of dynamically exercising dogs. Two previous studies employing intra-arterial infusions of propranolol in humans came to similar conclusions (12, 13). Hartling et al. (12) and Juhlin-Dannfelt and Astrom (13) found no β-receptor-mediated exercise hyperemia in skeletal muscle of the forearm or leg. Thus to date there is little support for a role of β-adrenergic receptors in the blood-flow response to steady-state exercise.

Fig. 3. Original tracing from 1 dog during steady-state exercise at 6 miles/h, 0% grade. There was an immediate increase in blood flow and conductance in experimental limb with intra-arterial bolus of 1 μg of acetylcholine. In contrast, bolus of 500 μg of atropine did not alter blood flow or conductance. Subsequent infusion of 1 μg of acetylcholine demonstrated effectiveness of muscarinic-receptor blockade. None of the intra-arterial bolus infusions altered systemic blood pressure or blood flow in control limb.

Fig. 4. Response to intra-arterial infusion of acetylcholine (ACh; 1 μg) and atropine (500 μg) in experimental limb during steady-state exercise. Values are means ± SE. *Acetylcholine elicited a significant increase (P < 0.001) in iliac conductance before, but not after, atropine. Atropine had no significant effect on iliac conductance.
We reasoned that activation of β-adrenergic receptors during exercise would be mediated by increased epinephrine release from the adrenal medulla or increased norepinephrine release from sympathetic nerve terminals. It is likely that there is only mild adrenal activation in dogs at the workload employed in this study (21). However, two lines of evidence suggest that there is an increase in efferent sympathetic nerve traffic to skeletal muscle during dynamic exercise. First, DiCarlo et al. (8) made direct measurements of postganglionic sympathetic nerve activity to the hindlimb (lumbar sympathetic trunk) in rats. They showed that there was an immediate and sustained increase in lumbar sympathetic nerve activity in response to dynamic exercise in rats. Second, our group (6) and O’Leary et al. (19) have recently demonstrated that there is substantial α₁-adrenergic-receptor restraint of skeletal muscle blood flow at mild, moderate, and severe workloads in dogs. We interpret these results to indicate that there is release of norepinephrine from sympathetic nerve terminals in the canine skeletal muscle vasculature across a wide range of exercise intensities. The data from the present study indicate that vascular β-receptors are not activated by the norepinephrine released from these nerve terminals.

Although β-adrenergic receptors do not play a role in skeletal muscle hyperemia during steady-state exercise, this study does not rule out a potential role for them at the onset of exercise. Indeed, Laughlin and Armstrong (15) reported no difference in rat hindlimb blood flow with propranolol at 5 min of exercise but did find significantly lower blood flow during the first 30 s of exercise. β-Adrenergic receptors also appear to play a role in postexercise hyperemia, as previously shown by several investigations (12, 24).

Muscarinic blockade. The existence of sympathetic cholinergic-mediated vasodilation in the vasculature of skeletal muscle has been demonstrated in a number of studies (4, 5, 7, 22). These sympathetic cholinergic dilator fibers have been shown to be activated by the defense reaction (7). Interestingly, the cardiovascular changes associated with exercise such as tachycardia, increases in MAP, vasoconstriction in the visceral organs, and vasodilation to skeletal muscle are also seen with electrical stimulation of regions of the brain eliciting the defense reaction (1, 7, 9, 10). However, the mechanisms for skeletal muscle vasodilation arising from the defense reaction and steady-state exercise appear to differ. In particular, skeletal muscle vasodilation evoked by the defense reaction can be blocked by atropine (4, 5, 7), whereas the results of the present study demonstrate that exercise hyperemia was unaffected by muscarinic-receptor blockade. Thus muscarinic receptors do not appear to be involved in the elevated blood flow to dynamically exercising skeletal muscle during steady-state exercise, although these results do not preclude involvement of muscarinic receptors at the onset of exercise.

Sanders and colleagues (22) were able to demonstrate sympathetic cholinergic-mediated vasodilation in conjunction with forearm exercise. However, this atropine-sensitive dilation was in the contralateral resting forearm. In contrast, several studies using systemic doses of atropine found little evidence to support the involvement of muscarinic receptors in exercise hyperemia. Bolme and Novotny (4) reported that elevations of canine iliac blood flow in anticipation of exercise, but not during exercise, were abolished with atropine. Similarly, systemic doses of atropine did not alter skeletal muscle blood flow during treadmill exercise in rats (3, 20). However, it must be noted that the existence of sympathetic cholinergic fibers in the rat is in doubt (5).

Recently it has been postulated that, besides sympathetic cholinergic fibers, another possible physiological source of acetylcholine release during exercise is the neuromuscular junction (23, 26). This is an attractive hypothesis, which 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. However, our results are not consistent with the hypothesis that acetylcholine spillover from the neuromuscular junction mediates skeletal muscle hyperemia during steady-state dynamic exercise.

In conclusion, the results from the present study show that, although functionally present, neither sympathetic β-adrenergic nor muscarinic receptors mediate skeletal muscle hyperemia during moderate steady-state dynamic exercise in dogs.

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