Exercise training enhances adrenergic constriction and dilation in the rat spinotrapezius muscle

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Lash, Julia M. Exercise training enhances adrenergic constriction and dilation in the rat spinotrapezius muscle. J. Appl. Physiol. 85(1): 168–174, 1998.—Treadmill training increases functional vasodilation in the rat spinotrapezius muscle, although there is no acute increase in blood flow and no increase in oxidative capacity. To assess concurrent changes in vascular reactivity, we measured arterial diameters in the spinotrapezius muscle of sedentary (Sed) and treadmill-trained (Tr) rats during iontophoretic application of norepinephrine, epinephrine (Epi), and H+ (HCl) and during superfusion with adenosine. Terminal-feed arteries and first-order arterioles in Tr rats constricted more than those in Sed rats at the higher current doses of norepinephrine and Epi. In contrast, at low-current doses of Epi, first- and second-order arterioles dilated in Tr but not in Sed rats. The vascular responses to HCl were highly variable, but second-order arterioles of Tr rats constricted more than those of Sed rats at intermediate-current doses. There were no significant differences between Sed and Tr rats in the vascular responses to adenosine. Both adrenergic vasodilation and vasoconstriction were enhanced in the spinotrapezius muscle of Tr rats, and enhanced adrenergic vasodilation may contribute to increased functional vasodilation. These observations further demonstrate vascular adaptations in “nontrained” skeletal muscle tissues.

AEROBIC EXERCISE TRAINING results in an increase in blood flow to active skeletal muscles during both submaximal and maximal exercise (1, 16, 18). This increase in blood flow may be due to either functional or anatomic adaptations to training. The minimal vascular resistance of skeletal muscle tissues has been shown to be lower in trained than in sedentary animals and humans (5, 23, 24). These results suggest that anatomic increases in resistance vessel size or number may contribute to the increased flow capacity observed in aerobically trained skeletal muscle. In fact, an increase in the passive diameters of large (1A) and intermediate-sized (2A) arterioles, as well as an increase in the numerical density of small arterioles, has been reported in the spinotrapezius muscle of treadmill-trained rats (10). In addition, the relative dilation of arterioles during low-to-moderate intensity contractions of the spinotrapezius muscle is greater in trained than in sedentary animals (8, 10, 11). These results suggest that functional vascular regulation favors vasodilation in trained animals, possibly independent of changes in anatomic dimensions. Such changes in vascular reactivity could be accomplished by either increased responses to dilator agents or decreased responses to constrictor agents; the precise vascular regulatory adaptations to aerobic exercise training are relatively unknown.

Lash and Bohlen (11) recently reported that treadmill training increases the dilation of rat spinotrapezius muscle arterial vessels in response to sodium nitroprusside and acetylcholine, which are endothelium-independent and -dependent responses, respectively. However, these adaptations were expressed differently among the three orders, or sizes, of vessels studied, with endothelial cell adaptations most evident in the arteriolar vessels and vascular smooth muscle cell adaptations most evident in the larger terminal-feed artery. In addition, the magnitudes of these adaptations were maintained or increased between the 8th and 16th wk of training. However, the training-related increase in functional dilation that was evident after 8 wk of training was no longer present after 16 wk of training. The authors concluded that the endothelial and vascular smooth muscle cell adaptations observed in that study could not fully account for the increased functional dilation consistently apparent in the rat spinotrapezius muscle after 8 wk of treadmill training.

It seems reasonable to hypothesize that the effects of aerobic training on the vascular responses to muscle contractions are mediated by vasoactive compounds that are present in greater or lesser amounts during the training bouts and/or during isolated muscle contractions. This hypothesis is based on the premise that the vascular adaptations are due to up- or down-regulation of vasoactive receptors or conditioning/extinction of vascular response pathways. On the basis of this perspective, the present study sought to determine whether the vascular responses to norepinephrine (NE), epinephrine (Epi), H+, or adenosine are altered as a result of aerobic exercise training. Circulating blood concentrations of NE and Epi are increased during exercise as a result of increased sympathetic neural activity, whereas local concentrations of H+ and adenosine are increased during acute muscle contractions due to increases in metabolism. The arterial vessels evaluated in this study included the terminal-feed artery and 1A and 2A arterioles of the rat spinotrapezius muscle. This preparation was chosen because previous results indicated that functional dilation is increased in these vessels as a result of 8 wk of treadmill training (8, 10, 11).

METHODS

Animals. All procedures were approved by the Animal Care and Use Committee of Indiana University. Male Sprague-Dawley rats (4–5 wk old) were received in shipments of eight each from Harlan Laboratories (Indianapolis, IN). Animals...
were exposed to a 12:12-h light-dark cycle and received food and water ad libitum. Three to four days after arrival, animals were randomly assigned to sedentary (Sed) or trained (Tr) groups, and animals in the Tr group began exercising daily on a rodent treadmill (Columbus Instruments, Columbus, OH). Initial exercise intensity was 15 mL/min, 0° incline, for 30 min; exercise intensity was increased over the next 5 wk and then maintained at an intensity of 30 mL/min, 1.5° incline, for 90 min. Experiments were performed during the 9th or 10th wk of training, at 12–14 wk of age. This training regimen has previously been shown to result in a 40% increase in plantaris citrate synthase activity and to enhance functional vasodilation and hyperemia in the rat spinotrapezius muscle (8, 10, 11). Animals in Sed and Tr groups were housed together, and experiments were performed at comparable ages.

Surgical preparation. Animals were prepared for experimental observations at least 24 h after their last training session to minimize the residual effects of acute exercise. Anesthesia was induced with thiopental sodium (initial dose 100 mg/kg, supplemental dose 10 mg sc as needed; Pentothal, Abbott Laboratories, North Chicago, IL), and body temperature was maintained near 37°C by placing the animal on a flow-through heating pad. Atropine was administered to minimize respiratory secretions (0.10–0.15 mg sc; American Reagent Laboratory, Shirley, NY). The trachea was intubated (PE-240) to ensure a patent airway, and the left femoral artery was cannulated (PE-50) for subsequent blood pressure monitoring.

The right spinotrapezius muscle was prepared for experimental observation as previously described (9, 10). Care was taken to maintain in situ length and width, and the rostral region of the muscle was carefully isolated to allow observation of the terminal feed artery as it entered the muscle tissue (8). During surgical preparation, drying of the tissue was prevented by intermittent superfusion with a heated physiological saline solution (8). During experimental observations, the tissue was perfused with a flow-through heating pad. Atropine was administered to minimize respiratory secretions (0.10–0.15 mg sc; American Reagent Laboratory, Shirley, NY). The trachea was intubated (PE-240) to ensure a patent airway, and the left femoral artery was cannulated (PE-50) for subsequent blood pressure monitoring.

Incremental increases in current dose were applied in sequence, and diameter responses were measured after 90–120 s to allow for development of the steady-state response. Pipette drug concentrations and application currents were as follows: 10^{-7} M NE at 5, 10, 20, 40, 80, and 160 nA; 10^{-6} M Epi at 25, 50, 75, 100, and 200 nA; and 10^{-5} M HCl at 10, 25, 50, 75, 100, and 200 nA. These parameters were determined in preliminary experiments and were chosen to elicit the widest possible range of responses for the three orders of vessels studied. All drugs were obtained from Sigma Chemical (St. Louis, MO).

Preliminary attempts to use iontophoretic application of adenosine were unsuccessful, as diameter responses at attainable currents were highly variable. Therefore, vascular responses to adenosine were determined during superfusion of the tissue with increasing concentrations of the drug (10^{-6}–10^{-4} M). A minimum of 5 min of superfusion with each concentration was allowed to ensure attainment of a steady-state response.

Citrate synthase activity. At the end of the experimental procedures, the right spinotrapezius and plantaris muscles were removed and stored at −20°C until enzymatic analyses were performed. Homogenized solutions (10% spinotrapezius; 5% plantaris) of each muscle were prepared in 0.1 M of Tris buffer containing 0.1% Triton X-100, and citrate synthase activity was determined by using the method described by Sane (25). Samples were analyzed at 30°C in duplicate or triplicate by using a Spectronic 401 spectrophotometer (Milton Roy). The average coefficients of variation for repeated samples were 2.2% for the plantaris muscle and 3.2% for the spinotrapezius muscle. Citrate synthase activity was expressed as micromoles per minute per gram of muscle tissue.

Data analysis. Measurements of luminal vessel diameters were determined from the digitized images by using the image-analysis software previously described. The system was calibrated by using the image of a stage micrometer.
marked with 10- and 100-µm increments. Dimension calibrations were performed in both the x and y directions to account for any spatial distortion inherent to the system. Results are presented as means ± SE, and statistical analyses were performed with the use of the CSS:Statistica software package (StatSoft, Tulsa, OK). Descriptive characteristics were compared between Sed and Tr rats by using an unpaired t-test and an α-level of 0.05. Vessel diameter data were analyzed by using planned orthogonal comparisons within each vessel order and between Sed and Tr rats at like contraction frequencies or drug doses. Least significant difference analysis was performed at an α-level of 0.05. Control diameters were compared based on a two-way factorial design (group × vessel order). Vessel diameter responses to muscle contractions and drug application were further analyzed on the basis of a repeated-measures factorial design (muscle contractions: group × vessel order × frequency of contraction; drug applications: group × dose). A higher order comparison between vessel orders was not performed for drug application, because each vessel order was examined independently in time. On the basis of the analyses of variance, main effects and interaction effects were noted when the F value was significant at P < 0.10.

RESULTS

Physical characteristics. As presented in Table 1, body weights tended to be lower in aerobically trained Tr animals than in Sed animals (1-tailed, P = 0.064), whereas mean arterial blood pressures were similar between groups (P = 0.35). As in previous studies that used the same training protocol, citrate synthase activity was significantly elevated in the plantaris, but not spinotrapezius, muscle of Tr compared with Sed rats (Table 1). Arteriolar diameters in the resting spinotrapezius muscle were similar between groups (Table 2) and were relatively consistent over time (P > 0.1). Subsequent diameter responses are presented below relative to the immediately preceding control diameter (resting and no drug condition; %control).

Vascular responses to muscle contractions. The relative dilatatory responses of feed, 1A, and 2A vessels during 4- and 8-Hz contractions are presented in Fig. 1. A significant group effect was evident (F = 7.69, P = 0.008), and analyses of planned comparisons indicated that 1A and 2A arterioles of Tr rats dilated more than those of Sed rats in response to 4-Hz contractions and that terminal-feed arterioles of Tr rats dilated more than those of Sed rats in response to 8-Hz contractions. These results are consistent with previous observations of increased functional dilation in the spinotrapezius muscle of Tr rats (8, 10, 11).

Table 1. Descriptive characteristics of sedentary and trained rats

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<thead>
<tr>
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<th>Sedentary</th>
<th>Trained</th>
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<tr>
<td>Body weight, g</td>
<td>377 ± 10</td>
<td>357 ± 7</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>116 ± 4</td>
<td>111 ± 3</td>
</tr>
<tr>
<td>Citrate synthase activity, µmol·min⁻¹·g⁻¹</td>
<td>37.4 ± 1.6</td>
<td>43.9 ± 3.0*</td>
</tr>
<tr>
<td>Plantaris</td>
<td>16.5 ± 1.2</td>
<td>16.4 ± 1.2</td>
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<tr>
<td>Spinotrapezius</td>
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Values are means ± SE; n = 10 sedentary and 9 trained rats. *P < 0.05, trained vs. sedentary.

Vascular responses to NE. The vascular responses to incremental iontophoretic doses of NE are presented in Fig. 2. The terminal-feed arteries of Tr rats constricted more than those of Sed rats at moderate through high iontophoretic doses of NE (40 nA, P = 0.008; 80 nA, P = 0.0047; 160 nA, P = 0.0189). The 1A arterioles of Tr rats also constricted more than those of Sed rats at the lower doses of NE (20 nA, P = 0.010; 160 nA, P = 0.019). No between-group differences were evident in the 2A arteriole responses to NE. A marginally significant overall group (Sed vs. Tr) main effect was also evident for the terminal-feed arteries (F = 3.35, P = 0.09). These results indicate that the NE reactivity of larger spinotrapezius muscle arterial vessels is enhanced by exercise training.

Vascular responses to Epi. The vascular responses to incremental iontophoretic doses of Epi are presented in Fig. 3. As with NE, the terminal-feed arterioles of Tr rats tended to constrict more than those of Sed rats at the higher current doses of Epi. The 1A arterioles also constricted more in Tr than in Sed rats at the 75-nA dose of Epi. In contrast, the 1A and 2A arterioles of Tr rats dilated in response to the lower doses of Epi, whereas those of Sed rats tended to constrict. Analysis of planned comparisons indicated that the 1A arterioles of Tr rats were larger than those of Sed rats at the 25-nA dose (P = 0.012) and smaller than those of Sed rats at the 75-nA dose (P = 0.011). The 2A arterioles of Tr rats were larger than those of Sed rats at the 25-nA dose (P = 0.012) and smaller than those of Sed rats at the 75-nA dose (P = 0.011). The 2A arterioles of Tr rats were larger than those of Sed rats at the 25-nA dose (P = 0.012) and smaller than those of Sed rats at the 75-nA dose (P = 0.011). The 2A arterioles of Tr rats were larger than those of Sed rats at the 25-nA dose (P = 0.012) and smaller than those of Sed rats at the 75-nA dose (P = 0.011).
Tr rats were also significantly larger than those of Sed rats at the 50-nA dose ($P < 0.0013$). The group main effect was not statistically significant for any vessel order, but a significant interaction effect (group $\times$ dose) was evident in the 1A arterioles ($F = 3.67, P < 0.011$). These results parallel those of NE in that $a$-mediated constriction in response to the higher doses of Epi is enhanced in the larger arterial vessels of Tr compared with Sed rats. In addition, an enhancement of adrenergic $\beta$-mediated vasodilation is found in the smaller arterioles of Tr relative to Sed rats. The significant interaction effect evident in the 1A arterioles appears to reflect adaptations of both the adrenergic vasoconstrictor and vasodilator mechanisms.

Vascular responses to HCl. The vascular responses to incremental iontophoretic doses of HCl are presented in Fig. 4. Analyses of the planned comparisons and main effects revealed no group differences between Sed and Tr rats in the vascular responses to adenosine for any vessel order.

**DISCUSSION**

The results of this study indicate that both adrenergic vasodilator and vasoconstrictor responses are enhanced in some skeletal muscle tissues of aerobically trained rats (Figs. 2 and 3). These vascular adaptations were observed in the rat spinotrapezius muscle, despite the fact that the oxidative capacity of this muscle was not enhanced by the prescribed training regimen (Table 1). A recent study by Musch and Poole (19) demonstrated that blood flow to the rat spinotrapezius muscle is actually decreased, not increased, during treadmill running. Therefore, the enhancement of adrenergic...
vascular responses appears to have occurred in a skeletal muscle that is not substantially recruited during the training bouts. Previous studies of vascular responses to adrenergic activation in sedentary and trained humans and animals have produced varied results. Trained, compared with sedentary, humans have been found to have similar (28) or enhanced (27) constrictor responses to infused \( \alpha \)-adrenergic agonists and enhanced dilator responses to \( \beta \)-agonists (27). Whereas some studies of the isolated rat aorta (4, 6) and renal and femoral arteries (4) have found no differences between sedentary and trained groups in the responses to NE, Delp et al. have found suppression of vasoconstriction in response to NE in isolated aorta from trained healthy (2) and hypothyroid (3) rats. However, this suppression of the constrictor response was found to be endothelium dependent, and the authors concluded that there was an increase in the sensitivity of an \( \alpha_2 \)-endothelium-dependent vasodilator mechanism. With regard to skeletal muscle vascular beds, Lash et al. (12) found no differences in the hindlimb vascular response to infused phenylephrine between sedentary and treadmill-trained rats. However, Hudlicka and Fronek (7) found frequency-dependent adaptations of the rabbit anterior tibial artery to electrical stimulation and contraction of the fast hindlimb muscles: low-frequency stimulation resulted in increased vascular responsiveness to NE, whereas high-frequency stimulation resulted in decreased responsiveness to NE. Various studies of the coronary circulation have found either no change (22) or a decrease (20, 21) in the constrictor response to NE in trained, compared with sedentary, animals.

Only two previous studies have specifically examined the microvascular responses to adrenergic stimulation in sedentary and trained animals. A study by Sun et al. (26) found no differences in NE-induced contraction between isolated 2A arterioles from the gracilis muscles of sedentary and treadmill-trained rats. In contrast, Wiegman et al. (30) found a suppression of cremaster arteriolar constriction in response to locally applied NE in swim-trained rats. Wiegman et al. interpreted their results to reflect either a decrease in the sensitivity of \( \alpha \)-mediated constriction or an increase in the sensitivity of \( \beta \)-mediated vasodilation. The results of the present study suggest that aerobic training enhanced both adrenergic vasoconstriction and adrenergic vasodilation in the rat spinotrapezius muscle. It is likely that these results are a reflection of training-induced adaptations of both \( \alpha \) - and \( \beta \) -mediated vascular responses in this tissue. It remains possible, however, that none of these studies accurately reflects adaptations in “trained” skeletal muscle tissues. As previously noted, the train-
The enhanced adrenergic dilation at low doses of Epi was an unexpected finding. The preliminary work in this tissue found only vasoconstriction in response to Epi, which is consistent with the results obtained in Sed animals (Fig. 3). However, several studies have identified β-mediated vasoconstriction in skeletal muscle tissues (14, 15, 29), although these effects are often masked by a more potent α-mediated constriction. In this study, exercise training appeared to enhance β-mediated vasodilation proportionally more than α-mediated constriction, such that the dilation became evident at the lower current doses of Epi used in this study. These results are consistent with the previous work of Svedenhag et al. (27), who found the magnitude of isoproterenol-induced vasodilation to be greater in trained than in sedentary humans. As previously noted, the suppression of NE-induced constriction of cremaster arterioles in trained rats, as reported by Wiegman et al. (30), could also be interpreted as an enhancement of competing β-mediated vasodilation. A distinct difference between the present results and those of Wiegman et al. is that frank vasodilation was expressed in the 1A and 2A arterioles of the present study. Therefore, suppression of vasoconstriction cannot account for the present results.

The enhanced adrenergic vasodilation observed in the present study could be related to changes in receptor affinity or number, changes in second-messenger efficacy, or changes in the contractile response of the vascular smooth muscle. Our laboratory has previously demonstrated that exercise training enhances vasodilation in response to sodium nitroprusside in the larger arterial vessels in this tissue (11) and enhances the dilator response of all sizes of arteriolar vessels to muscle contractions (8, 10, 11) (Fig. 1). However, there is no generalized enhancement of vasodilation, as the response to adenosine was unchanged or slightly suppressed in the same vessels (Fig. 5). It is interesting to note that a similar suppression of adenosine-mediated vasodilation was apparent in comparably sized vessels from the gracilis muscle of trained swine (20), whereas it is unchanged in the smaller coronary resistance vessels (17).

The differences between the present results and those previously reported in the literature may be due to a variety of factors. First, previous work in both the rat spinotrapezius muscle (11) and the coronary circulation (13, 21) indicates that vessel size and location within the branching pattern may play significant roles in the adaptations expressed in response to exercise training. Therefore, it is not surprising that adaptations in the arteriolar vessels differ from those in the aorta and large arteries. In fact, differential adaptations between vessel orders are evidenced in the present study, as larger vessels reflect increased adrenergic vasodilation (Fig. 2) and smaller vessels reflect increased adrenergic vasoconstriction (Fig. 3) in Tr animals. Second, the specific tissue in which a vessel is located likely influences the resulting adaptations. The changes in blood flow, wall shear stress, local metabolic conditions, and neural vascular control experienced during a single exercise bout likely contribute to the vascular adaptations to training, and these changes differ substantially among the various vessels and muscles studied. Whereas aortic and femoral artery blood flows increase during treadmill running in the rat, evidence suggests that there is no such sustained elevation in flow in the rat spinotrapezius (19) or gracilis muscle (1). In fact, spinotrapezius muscle blood flow appears to decrease during treadmill running (19), which is likely a result of increased sympathetic constriction in a relatively inactive muscle. Third, neural innervation patterns and adrenergic receptor distributions likely mediate the vascular adaptations to exercise training. Conduit vessels, such as the aorta and large arteries, are not highly innervated, whereas the small arteries and larger arteriolar vessels are under sympathetic control; the smaller arterioles are less densely innervated and are more under metabolic control.

Our original intention was to determine what mechanisms may contribute to the enhanced functional vasodilation observed in the spinotrapezius muscle of Tr rats. We hypothesized that this could be due to enhancement of vasodilator, or suppression of vasoconstrictor, mechanisms. On the basis of the present results, it is possible to conclude that the enhancement of adrenergic vasodilation could be a contributing factor to the enhanced functional vasodilation observed in the arteriolar vessels. The results of this study suggest that...
exercise training enhances adrenergic vasodilation in the 1A and 2A arterioles to the extent that it is frankly expressed under conditions in which it is not apparent in Sed animals (Fig. 3). Therefore, the physiological importance of β-mediated vasodilation may be vastly different between Sed and Tr animals. It is important to note, however, that there is no evidence to support a role for enhanced β-mediated vasodilation in the training-related increase in terminal-feed artery functional dilation in the rat spinotrapezius muscle.

The observation that adrenergic constriction is enhanced in the larger arterial vessels of the rat spinotrapezius muscle may also have physiological relevance. The data of Musch and Poole (19) suggest that blood flow to the spinotrapezius muscle is reduced during treadmill running, which is typical of the blood flow responses observed in “inactive” skeletal muscle tissues. Teleologically, vasoconstriction in nonactive tissues supports perfusion of active tissues by increasing vascular resistance and maintaining perfusion pressure. During normal exercise, augmented vasoconstriction in inactive skeletal muscles of trained humans or animals may effectively “shunt” blood flow to active muscle tissues, allowing for increased perfusion of active tissues without additional demands on cardiac output. The present results indicate that this “shunting” may be accomplished by adaptations of adrenergic constrictor mechanisms in the larger “resistance” vessels (feed arteries and 1A arteriole) of the terminal circulation.

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