Effects of acute and chronic exercise on vasoconstrictor responsiveness of rat abdominal aorta

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Spier, Scott A., M. Harold Laughlin, and Michael D. Delp. Effects of acute and chronic exercise on vasoconstrictor responsiveness of rat abdominal aorta. J. Appl. Physiol. 87(5): 1752–1757, 1999.—Reductions in blood pressure that are associated with exercise training have been hypothesized to be the result of a sustained postexercise vascular alteration following single bouts of exercise. The purpose of this study was to determine whether a decrease in vascular sensitivity to vasoconstrictor agonists occurs after a single bout of exercise and whether this vascular alteration is sustained through various periods of exercise training. Vascular responses of abdominal aortic rings to norepinephrine (NE; 10−9–10−4 M) were determined in vitro. Aortas were isolated from sedentary rats immediately after rats performed a single bout of treadmill exercise (30 m/min for 1 h); 24 h after the last exercise bout in rats exercised for 1 day; and 1, 2, 4, and 10 wk of training at 30 m/min, 60 min, 5 days/wk. Sensitivity to NE was only diminished after 10 wk of training. This diminished vascular sensitivity to NE was abolished with the removal of the endothelial cell layer. Furthermore, there were no reductions in developed tension or vascular sensitivity to the vasoconstrictor agonists KCl (10−9−10−4 M), phenylephrine (10−8–10−4 M), and arginine vasopressin (10−9−10−3 M) in vessels either with or without the endothelial layer after a single bout of exercise. These data indicate that a single bout of exercise does not diminish aortic responsiveness to the vasoconstrictor agonists and thus is not responsible for the diminished contractile responsiveness that occurs between 4 and 10 wk of moderate-intensity exercise training in rats. This vascular adaptation to exercise training appears to be mediated through an endothelium-dependent mechanism.

exercise; norepinephrine; vascular smooth muscle

POSTEXERCISE HYPOTENSION, reported to occur in both humans (1, 8, 11, 20) and animals (13), results from a decrease in systemic vascular resistance after exercise (3, 14). One possible mechanism for the decreased systemic vascular resistance is a diminished vascular sensitivity to constrictor agonists such as norepinephrine (NE). Support for this mechanism has been shown by Howard et al. (10), in which a single bout of exercise produced a diminished sensitivity of thoracic aortic rings to phenylephrine (PE) in vitro. In another study from the same laboratory, Howard and DiCarlo (9) measured changes in rabbit iliac blood flow velocity induced by bolus injections of PE during a control condition and after a single bout of exercise. They reported that the reduction in iliac blood flow velocity produced by PE was attenuated after exercise. This suggested a reduced ability of the hindlimb vasculature to constrict in response to activation of the α-adrenergic receptors after acute exercise. Similar results have been obtained in Dahl salt-sensitive rats (18).

In addition to a single bout of exercise, vascular control mechanisms have been shown to be modified by exercise training through adaptive changes in the responsiveness of endothelial cells to adrenergic-receptor activation (2, 6, 7, 19). For example, the binding of NE to α2-adrenergic receptors induces greater release of endothelium-derived nitric oxide (EDNO) after prolonged exercise training, thereby blunting NE-mediated vasoconstriction (6, 7).

The primary purpose of the present study was to determine whether a single bout of exercise induces a reduction in vascular sensitivity to NE and whether this reduced vascular sensitivity to NE is sustained with up to 10 wk of training. More specifically, we sought to determine whether the mechanism of diminished vasoconstrictor responsiveness after a single bout of exercise, like that after prolonged exercise training, involves an endothelium-dependent dilatory mechanism. It was hypothesized that a single bout of exhaustive exercise would produce a diminished vascular responsiveness to NE and that this reduced sensitivity would be evident at all training durations up to 10 wk. Furthermore, vascular responsiveness to PE, arginine vasopressin (AVP), and KCl would be studied immediately postexercise to determine whether a diminished vascular responsiveness to constrictor agonists might occur when constriction is mediated through a specific α1β-2-adrenergic-receptor mechanism, a non-α-adrenergic-receptor mechanism, or through a nonreceptor mechanisms, respectively.

MATERIALS AND METHODS

Animals and training. The methods employed in this study were approved by the Texas A&M University Institutional Animal Care and Use Committee. The investigation conformed to the Guide for the Care and Use of Laboratory Animals (DHEW Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892).

One hundred male Sprague-Dawley rats were obtained (Sasco) and housed in a temperature-controlled (23 ± 2°C) room with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum. The rats were randomly assigned to either a sedentary control (Sed) group, postexercise group (PostEx), or one of five additional groups, which consisted of animals exercised for 1 day (1 DAY), 1 wk (1 WK), 2 wk (2
PostEx and 1-DAY animals performed a single bout of treadmill running at 30 m/min (15° incline) for 1 h. Rats in the 1-, 2-, 4-, and 10-WK groups performed 30 m/min treadmill running up a 15° incline, 1 h/day, 5 days/week, for 1, 2, 4, and 10 wk, respectively. Vascular responses were determined immediately after exercise in aortic rings from PostEx animals. Vascular responses from all the other exercise-trained groups (including the 1-DAY group) were determined 24 h after the last exercise bout. Because there was no decrease in vascular sensitivity to NE after a single bout of exercise, vascular responsiveness to PE, AVP, and KCl was determined immediately after a single bout of exercise to compare receptor and nonreceptor responses. After the treatment period, the animals were anesthetized with pentobarbital sodium (35 mg/kg ip), euthanized by decapitation, and the aortas were dissected free. The time of all deaths occurred between 7:00 and 9:00 AM. The mean body mass at the time of death was similar among Sed (418 ± 14 g), PostEx (400 ± 10 g), 1 DAY, (412 ± 9 g), 1 WK (404 ± 9 g), and 2 WK (393 ± 13 g) groups; body mass of rats in the 4 WK (379 ± 6 g) and 10 WK (351 ± 6 g) groups was less than that of the Sed rats.

Oxidative enzyme activity. After the animals were euthanized and the abdominal aortas were removed, the soleus muscle was dissected free and stored at −70 °C. Citrate synthase activity, a marker of mitochondrial content, was measured from whole muscle homogenate by using the spectrophotometric method of Srere (16).

Vessel preparation. The segment of abdominal aorta distal to the renal artery and proximal to the bifurcation of the iliac arteries was carefully dissected, dissected free, and quickly (<5 min) placed in chilled (4°C) Krebs bicarbonate buffer solution. Segments were trimmed of fat and connective tissue with the aid of a stereomicroscope (Olympus, Hitachiell Instruments, St. Louis, MO).

Vessels used to determine vascular responsiveness were cut into four rings, each ∼2 mm in axial length. Adjacent to each ring, a thin-vessel cross section was cut to determine outer diameter, inner diameter, and wall thickness. Outer and inner diameters were calculated by measuring total vessel diameter and luminal diameter, respectively, through the transverse section of the thin-vessel ring by using a Filar-calibrated micrometer eyepiece. Total vessel and luminal diameters were measured at three points separated by equal angles and averaged. Wall thickness was calculated as the average of the difference between outer and inner diameters divided by two. Axial length of each vessel ring was measured with a Filar-calibrated micrometer eyepiece. In two of the four rings from each animal, the endothelial cell lining was removed by gently rubbing the luminal surface of the ring with fine-tipped forceps. The endothelial layer was considered removed if 10−4 M acetylcholine-induced relaxation of a NE-evoked (10−7 M) contraction was <5%.

Length-tension relationship. Vascular rings used to determine vascular responsiveness were mounted on two stainless steel wires (0.406-mm diameter) passed through the vessel lumen. One wire was attached to a force transducer (Grass FT03, Grass Instrument, Quincy, MA) and the other to a micrometer microdrive (Stoelting, Wood Dale, IL) to allow the vessel to be stretched by known increments. Each vessel apparatus was placed in an individual 20-ml tissue bath containing Krebs bicarbonate buffer equilibrated at 37°C with 95% O2-5% CO2. Isometric contractions were measured and recorded with a data-acquisition system (MacLab Chart v3.5 and Macintosh 6500/225 computer). The apex of the length-developed tension relationship (Lmax) was determined by repeated test exposures to 60 mM KCl at increasing vessel diameters. Rings were individually stretched by increments of 5–20% of the initial resting diameter. All subsequent pharmacological responsiveness studies were performed with the arterial rings at Lmax. The rings were allowed 30 min to stabilize at Lmax before further study.

Experimental design. Rats were divided into seven groups according to the length of their exercise-training period (Sed, n = 12; PostEx, n = 12; 1 DAY, n = 12; 1 WK, n = 12; 2 WK, n = 12; 4 WK, n = 12; 10 WK, n = 12). Maximal 10−4 M acetylcholine-induced dilation followed by a NE dose response was determined in the aortic rings from these animals. The acetylcholine responses and muscle citrate synthase activity data in a subset of animals from these groups (n = 8/group) have been previously reported (5).

When it was determined that there was no change in sensitivity to NE after a single bout of exercise, constrictions evoked by KCl, PE, and AVP were determined in a separate group of Sed (n = 8) and PostEx (n = 8) rats. Drug order was matched in vessels from the Sed and PostEx animals. NE, PE, AVP, and KCl were used to allow comparison of contractions resulting from receptor-mediated mechanisms [αa- and αa- adrenergic receptors (NE), αa- adrenergic receptors (PE), and V1 receptors (AVP)] and activation of voltage-gated Ca2+ channels (KCl). Concentration-response relationships were determined by cumulative addition of NE (10−9–10−4 M), PE (10−8–10−4 M), AVP (10−9–10−5 M), and KCl (10–100 mM). Recovery periods of 15–75 min were allowed between dose-response tests so that resting tension returned to baseline before administration of the next agent. Bathing medium was replaced every 5 min during recovery periods.

Solutions and drugs. The Krebs solution contained (in mM) 131.5 NaCl, 5 KCl, 1.2 NaH2PO4, 1.2 MgCl2, 2.5 CaCl2, 11.2 glucose, 13.5 NaHCO3, and 0.025 EDTA. Propanolol (0.003 mM) was added to oppose β2-adrenergic receptor-mediated vasoconstriction. Solutions were aerated with 95% O2-5% CO2 (pH 7.4) and maintained at 37 ± 0.5°C. Concentrated stock solutions of vasoactive agents were prepared in distilled water.

Statistical analysis. Contractile responses to drugs are presented as absolute values in grams of tension. Concentration-response curves were evaluated by using repeated analysis of variance with one-within (drug concentrations) and one-between (experimental groups) factor. Planned contrasts were conducted at each molar (or millimolar) concentration level to determine whether differences exist between experimental groups (Sed vs. PostEx). Vascular sensitivity, defined as the agonist concentration that produces 50% of the maximal vasoconstrictor response, was designated EC50. All EC50 values were converted to log values for statistical comparison. A one-way analysis of variance and a Student-Newman-Keuls post hoc test was used to determine the significance of differences among NE EC50 values for the time course study (Sed vs. PostEx vs. 1 DAY vs. 1 WK vs. 2 WK vs. 4 WK vs. 10 WK). An unpaired t-test was used to compare PE, AVP, and KCl EC50 values between groups (Sed vs. PostEx). Data were analyzed with each animal counted as one observation. For analyses in which several rings from one animal underwent identical protocols, the responses were averaged. All values are presented as means ± SE. P < 0.05 was required for significance.
**RESULTS**

Oxidative enzyme activity. Soleus muscle citrate synthase activity in Sed rats (28 ± 1 µmol·min⁻¹·g wet wt⁻¹) was similar to that in PostEx (27 ± 1 µmol·min⁻¹·g wet wt⁻¹), 1 DAY (28 ± 1 µmol·min⁻¹·g wet wt⁻¹), and 1 WK (29 ± 1 µmol·min⁻¹·g wet wt⁻¹) animals. However, there was a significant increase in citrate synthase activity in 2 WK (32 ± 1 µmol·min⁻¹·g wet wt⁻¹), 4 WK (34 ± 1 µmol·min⁻¹·g wet wt⁻¹), and 10 WK (35 ± 1 µmol·min⁻¹·g wet wt⁻¹) animals.

Vessel characteristics. Abdominal aortas from Sed and exercised animals had similar outer diameters, inner diameters, and wall thicknesses in the resting state (Table 1). The percent stretch to the apex of the length-active tension curve, calculated as the percent increase above initial resting inner diameter, was not different among groups. Arterial segment axial lengths and resting tensions, expressed as grams of tension, were also similar among groups.

Vasoconstrictor responses. Increasing concentrations of NE produced concentration-related increases in contractile tension. There were no differences in maximal tension developed by aortic segments from Sed and all exercise groups at any NE concentration (Fig. 1). Aortic sensitivity to NE was also similar among segments from Sed, PostEx, 1-DAY, 1-WK, 2-WK, and 4-WK groups. However, EC₅₀ of aortic segments from 10-WK rats was greater than that from Sed rats (Fig. 2). Removal of the endothelium abolished the diminished vascular responses to NE seen in 10-WK rats (Fig. 3).

Increases in extracellular KCl produced similar concentration-related increases in contractile tension in segments from Sed and PostEx rats (Fig. 4). There were no differences in sensitivity to KCl between Sed and PostEx vessel segments, either with or without the endothelial cell layer. Furthermore, the maximal KCl-induced force was not different among groups.

Increasing concentrations of PE also produced similar concentration-related increases in contractile tension in segments from Sed and PostEx rats (Fig. 5). Tension developed by aortic segments, with and without intact endothelium, from Sed and PostEx rats was similar at each concentration. Sensitivity to PE was also similar among groups.

Tension developed in response to AVP by vessel segments from Sed rats was lower than that from PostEx rats at each concentration. Removal of the endothelium abolished this difference (Fig. 6). There was no alteration in vascular sensitivity to AVP after exercise in vessels either with or without the endothelium.

**DISCUSSION**

Acute and chronic exercise have been shown to diminish postexercise and resting mean arterial pressures, respectively (13). It has been hypothesized that the decrease in arterial pressure results from an exer-
exercise-induced decrease in vasoconstrictor responsiveness of the arterial vasculature (9, 10). In support of this hypothesis, Howard et al. (10) have shown that PE-evoked tension of aortic ring segments is diminished after a single bout of exercise in rabbits. This decrease is PE response is similar to that reported in aortas of rats exercise trained for 10 wk (6, 7). The altered vasoconstrictor responses in trained rats appear to be the result of endothelium-dependent events. Therefore, the purpose of the present study was to determine whether a single bout of exercise induces a diminished response of rat aortas to adrenergic stimulation and whether this change is the result of endothelium-dependent adaptations. The results of the present study indicate that a single bout of exhaustive exercise does not decrease aortic sensitivity to NE, PE, AVP, or KCl. These results differ from those reported by Howard et al. (10). Possible explanations for the disparate results include differences in the anesthetic and method of euthanasia, the method of stretching the vessel rings (i.e., determining the length-tension relationship vs. stretching the vessel to a given tension), the intensity of the exercise, the species of animal investigated, or the section of aorta used in the study. For example, Howard et al. examined vascular responses of the thoracic aorta of normotensive rabbits that performed a single bout of treadmill running at 24 m/min until exhaustion (an average exercise duration of 16 min). In the present study, vascular responses were determined from the abdominal aorta of normotensive rats after a single bout of treadmill running at 30 m/min for 1 h. It is, therefore, possible that these differences in relative...
exercise intensity and duration could account for the disparate results.

A secondary purpose of this study was to determine the duration of training needed to diminish NE-evoked tension of arterial segments. Therefore, a time course study of up to 10 wk of treadmill exercise training was performed to determine when changes in vascular sensitivity to NE occur. A decrease in vascular sensitivity to NE was found by 10 wk of training, confirming previous results (6, 7). Although NE sensitivity appeared to decrease in all exercise groups (Fig. 2), the effect was only statistically significant after 10 wk of training.

The mechanism of chronic training-induced changes in vasoconstrictor properties has been previously described. Weigman et al. (19) first reported a decreased sensitivity to NE in the cremaster muscle microcirculation of rats swim-trained for 6 wk. Subsequently, Delp et al. (6) reported a diminished vascular sensitivity to NE in rats that were treadmill trained for 10 wk. These authors also found that vascular responses to the α₂-adrenergic agonist PE were not altered by chronic exercise and that the diminished vascular sensitivity to NE was abolished when the endothelium was removed. Collectively, the Delp et al. (6) and the present study indicate that the diminished response to NE in the abdominal aorta of rats trained for 10 wk is due to an endothelium-dependent mechanism involving the α₂-adrenergic receptor. Stimulation of α₂-adrenergic receptors on vascular endothelial cells causes release of EDNO through the nitric oxide synthase (NOS)-EDNO pathway (17). The upregulation of vascular smooth muscle relaxation mediated by EDNO occurs in vessels exposed long term to high blood flow (12), as is the case with exercise training. Therefore, exercise training appears to cause greater release of EDNO, which diminishes (or antagonizes to a greater extent) the vasoconstrictor effects of NE on smooth muscle cells. Chen and colleagues (2) have also found that the binding of NE to aortic vessels isolated from endurance-trained rabbits induced a greater release of EDNO. The greater release of EDNO that is evoked by NE after training has been hypothesized to involve an upregulation of endothelial cell NOS (eNOS) expression (4, 6).

To test this hypothesis, Delp and Laughlin (5) measured eNOS protein expression in rat aortas and reported an increase with 4–10 wk of training involving exercise of the same intensity as that used in the present study. These results are consistent with other studies that have reported an increase in NOS mRNA levels in the aorta of exercise-trained dogs (15) and greater expression of NOS mRNA in coronary resistance vessels of exercise-trained pigs (21).

Although we have previously reported greater endothelium-mediated dilation and greater expression of eNOS protein after 4 and 10 wk of training in the rat aorta (5), we did not observe a decreased sensitivity to NE until 10 wk of training. If an upregulation of eNOS contributes to the blunting of NE sensitivity in arteries, then a change in NE sensitivity might be expected to occur with both 4 and 10 wk of training. However, it is possible that because the NE response is the net result of opposing vasoconstrictor and vasodilatory influences, then it may be more difficult to detect an upregulation of an eNOS dilatory mechanism. In contrast, our previous studies demonstrating an enhanced training-induced endothelium-mediated dilation, the vasoconstrictor influence was held constant (NE preconstriction) while the dilatory stimulus (acetycholine) was incrementally increased (5, 6, 7). Thus the present study is consistent with the notion that the endothelium appears to diminish the vascular response of exercise-trained animals to NE through a greater release of EDNO, which, in turn, is mediated through a training-induced increase in the expression of eNOS.

Present results suggest that this adaptation is not fully manifest in rats until they have trained for 10 wk.

The above conclusions are based on experiments of vessels studied in vitro. The advantage of using an in vitro preparation to study possible alterations in vascular responsiveness with exercise is that potentially confounding neural, humoral, and metabolic influences present in vivo can be eliminated. For example, in studying vascular responsiveness to NE, the central effects of raising arterial pressure and the local effects of altering neuronal NE release via presynaptic α₂ receptors are avoided in vitro. Whereas the present study indicates that vascular responsiveness of conduit arteries to NE is unaltered by acute exercise and diminished with chronic exercise, it remains to be determined whether this also occurs in the resistance vasculature of skeletal muscle.

In conclusion, a single bout of moderate-intensity exhaustive exercise did not diminish the vasomotor responsiveness of rat arterial segments to vasoconstrictor agonists. Further, training for 1, 2, and 4 wk at 30 m/min, 60 min/day, 5 day/wk did not result in changes in the vasoconstrictor responsiveness of rat aorta. However, chronic exercise training for 10 wk did produce a diminished vascular sensitivity to α₁-adrenergic-mediated constriction. This adaptation requires more than 4 wk of exercise training to become manifest, and this appears to be mediated through an eNOS mechanism involving α₂-adrenergic receptors on the endothelium (present study and Refs. 5, 6). Thus the postexercise hypotension phenomenon does not appear to share the same vascular mechanism as that which occurs with chronic exercise training.

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