Exercise training improves aortic endothelium-dependent vasorelaxation and determinants of nitric oxide bioavailability in spontaneously hypertensive rats

Drew A. Graham and James W. E. Rush
Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Submitted 21 November 2003; accepted in final form 26 January 2004

Graham, Drew A., and James W. E. Rush. Exercise training improves aortic endothelium-dependent vasorelaxation and determinants of nitric oxide bioavailability in spontaneously hypertensive rats. J Appl Physiol 96: 2088–2096, 2004.—The present study examined in vitro vasomotor function and expression of enzymes controlling nitric oxide (NO) bioavailability in thoracic aorta of adult male normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) that either remained sedentary (Sed) or performed 6 wk of moderate aerobic exercise training (Ex). Training efficacy was confirmed by elevated maximal activities of both citrate synthase (P = 0.0024) and β-hydroxyacyl-CoA dehydrogenase (P = 0.0073) in the white gastrocnemius skeletal muscle of Ex vs. Sed rats. Systolic blood pressure was elevated in SHR vs. WKY (P < 0.0001) but was not affected by Ex. Despite enhanced endothelium-dependent relaxation to 10^{-6} M ACh in SHR vs. WKY (P = 0.0061), maximal endothelium-dependent relaxation to 10^{-4} M ACh was blunted in Sed SHR (48 ± 12%) vs. Sed WKY (84 ± 6%, P = 0.0067). Maximal endothelium-dependent relaxation to 10^{-4} M ACh was completely restored in Ex SHR (93 ± 9%) vs. Sed SHR (P = 0.0011). N^\text{G}-nitro-L-arginine abolished endothelium-dependent relaxation in all groups (P ≤ 0.0001) and caused equal vasoconstriction to maximal ACh in Sed SHR and Ex SHR. Endothelium-independent relaxation to sodium nitroprusside was similar in all groups. Protein levels of endothelial NO synthase were higher in SHR vs. WKY (P = 0.0157) and in Ex vs. Sed (P = 0.0536). Protein levels of the prooxidant NAD(P)H oxidase subunit, gp91phox, were higher in SHR vs. WKY (P < 0.0001) and were diminished in Ex vs. Sed (P = 0.0557). Levels of the antioxidant SOD-1, -2, and catalase enzymes were lower in SHR vs. WKY (all P ≤ 0.0005) but were not altered by Ex. Thus elevated gp91phox-dependent oxidative stress and reduced antioxidant capacity likely contributed to impaired endothelium-dependent vasorelaxation in Sed SHR. Furthermore, reduced gp91phox-dependent oxidative stress and enhanced endothelial NO synthase-derived NO likely contributed to restored endothelium-dependent vasorelaxation in Ex SHR.

nitric oxide synthase; oxidative stress; vascular smooth muscle; blood pressure; cardiovascular diseases

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
decrease vascular prooxidant enzyme [NAD(P)H oxidase] capacity in both adult WKY rats and adult SHR.

MATERIALS AND METHODS

Experimental animals and training protocol. Male WKY rats (n = 24) and SHR (n = 24) were obtained from Harlan (Indianapolis, IN) and group housed at constant air temperature (20–21 °C) and humidity (~50%) in a 12:12-h reverse light-dark cycle. Rats had free access to standard 22/5 Rodent Diet (W) lab chow (Harlan) and tap water. The University of Waterloo Animal Care Committee approved all animal-related procedures in this study. Chemicals, drugs, and reagents were obtained from Sigma Chemical (St. Louis, MO) unless otherwise stated.

At 11 wk of age, all rats were introduced to treadmill exercise (1 bout: 10 min, 5–10 m/min, 0% grade) on a motorized treadmill (Wood’s, Chambersburg, PA). Rats were assigned to sedentary (Sed; n = 24) and aerobically exercise-trained (Ex; n = 24) groups, which resulted in four treatment groups: Sed WKY (n = 12), Sed SHR (n = 12), Ex WKY (n = 12), and Ex SHR (n = 12). Ex groups underwent 6 wk of progressive aerobic exercise training on a motorized treadmill (5 days/wk, 6 wk) and reached the desired final training intensity and duration (21 m/min, 4.5% grade, 45 min/day) by the end of week 3. Although well-controlled studies examining oxygen uptake in WKY rats and SHR are limited, the work rate associated with the developed tension (i.e., no L-NAME or 10^{-7} M L-NAME) and developed tension to KCl at 60 mm KCl at increasing resting lengths, as described previously (39), and all subsequent experiments were performed at L0. All rings were initially exposed to cumulative 10^{-7} and 10^{-6} M doses of phenylephrine (PE; \alpha_1-adrenoceptor agonist), washed out several times with warmed Krebs-bicarbonate buffer (pH 7.4, 37°C), and allowed to relax back to baseline to ensure that the lower dose elicited submaximal vasoconstriction. Two of the four rings from each animal were then incubated for 30 min in 10^{-4} M Nω-nitro-L-arginine methyl ester (L-NAME; L-arginine analog that inhibits NO synthase). All rings were precontracted with submaximal (10^{-5} M) PE, and endothelium-dependent responses to cumulative doses of ACh (muscarinic receptor agonist; 10^{-10}, 10^{-8}, 10^{-6}, and 10^{-4} M) were assessed. After an ~25 min washout with warmed Krebs-bicarbonate buffer (pH 7.4, 37°C), rings were precontracted again with submaximal (10^{-7} M) PE, and endothelium-independent responses to cumulative doses of sodium nitroprusside (SNP; exogenous NO donor; 10^{-10}, 10^{-8}, 10^{-6}, and 10^{-4} M) were assessed.

Immunoblot analyses. Frozen segments of thoracic aorta were homogenized and immunoblots performed as described previously (37). Primary antibodies were used as follows: mouse anti-eNOS and mouse anti-gp91phox (both 1:1,000; Transduction Laboratories, Lexington, KY); goat anti-p22phox (1:500; Santa Cruz, Santa Cruz, CA); rabbit anti-Cu/Zn SOD (SOD-1) and rabbit anti-Mn SOD (SOD-2; 1:1,000 and 1:5,000, respectively; Stressgen, Victoria, BC); rabbit anti-catalase (1:3,000; Chemicon, Temecula, CA); and rabbit anti-soluble guanylate cyclase (sGC; 1:1,000; Calbiochem, San Diego, CA). Horseradish peroxidase-conjugated secondary antibodies were used in combination with enhanced chemiluminescence (Amersham, Little Chalfont, UK) and gel documentation (Syngene, Cambridge, UK) for detection of protein signals.

Preparation of skeletal muscle homogenates. Samples of snap-frozen white gastrocnemius skeletal muscle (~5–10 mg wet wt) were homogenized as described previously (10), sonicated on ice (60% power, 2 s on, 5 s off duty cycle, 20-s total “on” time; Sonics and Materials, Danbury, CT), and total protein concentrations measured as described by Lowry et al. (31). V_{max} of the mitochondrial marker enzymes citrate synthase and \beta-hydroxyacyl-CoA dehydrogenase were determined in homogenates as described previously (10).

Measurement of plasma nitrate levels. Plasma was harvested from whole blood obtained via cardiac puncture and stored at ~80°C. Plasma NO levels were determined from converted nitrate and nitrite (Sieves Instruments, Boulder, CO) as described previously (49).

Statistical procedures. Most statistical analyses were performed by two-way ANOVA for strain (WKY vs. SHR) and training status (Sed vs. Ex), and the least squares means post hoc procedure was used to compare between-group permutations where strain \times train interaction terms reached significance. Effects of L-NAME were tested using orthogonal contrasts. Individual ring data within each L-NAME treatment (i.e., no L-NAME or 10^{-4} M L-NAME) and within each rat (n = 2 rings per treatment per rat) were averaged and used to calculate group means and SE values. EC_{50} values were not calculated due to the small number (i.e., 4) of distinct ACh and SNP concentrations used. Data are expressed as group means ± SE, and a 95% confidence interval was accepted as significant. SAS analysis software was used for all statistical procedures (SAS Institute, Cary, NC).

RESULTS

Characterization of SHR and Ex models. SBP was elevated in SHR vs. WKY rats but was not altered in Ex vs. Sed animals (Table 1). BM-standardized left ventricular (LV; interventricular septum and free LV wall) mass and BM-standardized total heart mass were elevated in SHR vs. WKY rats. Ex was also associated with BM-standardized left ventricular mass hyper trophy vs. Sed (Table 1). Citrate synthase V_{max} and \beta-hydroxyacyl-CoA dehydrogenase V_{max} were elevated in white gastrocnemius muscles of SHR vs. WKY rats and in Ex vs. Sed rats (Table 2). Thus the classic hypertension-associated adaptation of LV hypertrophy and the hallmark exercise training effects of increased capacity of skeletal muscle mitochondrial enzymes and LV hypertrophy occurred.

Vasomotor function in thoracic aortic rings. Resting tension at L0 and developed tension to KCl at L0 were similar in WKY vs. SHR and in Sed vs. Ex rats (Table 3). Tension development to maximal (10^{-4} M) PE was higher in WKY vs. SHR but similar in Sed vs. Ex animals (Table 3). Similarly, tension development to submaximal (10^{-7} M) PE was higher in WKY vs. SHR during preconcentration before assessment of relaxation to ACh in the absence and presence of L-NAME (P < 0.0001 and P = 0.0026; Fig. 1A and C, respectively) and SNP in the absence and presence of L-NAME (P < 0.0001 and P =
Relative submaximal vasorelaxation to $10^{-8}$ M ACh was greater in SHR vs. WKY ($P = 0.0061$; Fig. 1B). However, consistent with previous reports, maximal ($10^{-4}$ M) relaxation to ACh was blunted in Sed SHR (48 ± 12%) vs. Sed WKY (84 ± 6%, $P = 0.0067$; Fig. 1B). Notably, exercise training completely restored maximal relative relaxation to $10^{-4}$ M ACh in SHR (93 ± 9%), such that it was greater than Sed SHR ($P = 0.0011$) and no different from Sed WKY or Ex WKY (89 ± 2%; Fig. 1B). Overall relative ACh-induced relaxation was completely blocked by l-NAME in all groups ($P < 0.0001$ and $P = 0.0001$ by linear and quadratic orthogonal contrasts, respectively; Fig. 1. B and D). In fact, maximal stimulation with $10^{-6}$ and $10^{-4}$ M ACh caused significant vasoconstriction in SHR vs. WKY in the presence of l-NAME ($P = 0.0266$; Fig. 1D).

Relative submaximal vasorelaxation to SNP ($10^{-10}$ M) was greater in SHR vs. WKY both in the absence ($P = 0.0320$; Fig. 2B) and presence ($P = 0.0268$; Fig. 2D) of l-NAME. Ex rats had slightly blunted relative maximal SNP-induced relaxation ($10^{-6}$ and $10^{-4}$ M) vs. Sed rats ($P = 0.0165$ and $P = 0.0191$, respectively; Fig. 2B); however, this effect was abolished in the presence of l-NAME (Fig. 2D). Overall relative vasorelaxation to SNP was enhanced by l-NAME ($P = 0.0004$ by linear orthogonal contrast; Fig. 2. B and D).

**Enzyme levels in thoracic aorta.** Levels of the endothelium-specific enzyme, eNOS, were elevated in SHR vs. WKY and in Ex vs. Sed animals (Fig. 3A), and of the catalytic prooxidant NAD(P)H oxidase subunit gp91phox (expressed in endothelial cells) were higher in SHR vs. WKY but lower in Ex vs. Sed rats (Fig. 3B). In contrast, protein levels of another catalytic NAD(P)H oxidase subunit, p22phox (expressed in endothelial and VSM cells), were similar in WKY vs. SHR and in Sed vs. Ex rats (data not shown). Levels of the antioxidant enzymes SOD-1, -2, and catalase were all lower in SHR vs. WKY but similar in Sed vs. Ex rats (Table 4). Protein levels of sGC were elevated in SHR vs. WKY and in Ex vs. Sed rats (Fig. 3C).

**DISCUSSION**

The purpose of this study was to examine the effects of 6 wk of moderate aerobic Ex on aortic endothelium-dependent vasorelaxation and expression of enzymes controlling NO bioavailability in the vascular wall in a rodent model of essential hypertension. We confirmed previous reports that Sed SHR exhibited nonsignificantly lower plasma nitrate levels (33.6 ± 1.3 µM) compared with Sed WKY (39.7 ± 1.7 µM), Ex WKY (38.6 ± 2.6 µM), and Ex SHR (39.4 ± 1.5 µM).

### Table 1. General characteristics of experimental animals

<table>
<thead>
<tr>
<th>Strain</th>
<th>BM, g</th>
<th>LV/BM, mg/g</th>
<th>RV/BM, mg/g</th>
<th>H/BM, mg/g</th>
<th>SBP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>304 ± 9</td>
<td>2.09 ± 0.06</td>
<td>0.61 ± 0.06</td>
<td>2.69 ± 0.11</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>Ex</td>
<td>309 ± 7</td>
<td>2.15 ± 0.03</td>
<td>0.61 ± 0.02</td>
<td>2.76 ± 0.03</td>
<td>118 ± 6</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>0.0011</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE of $n = 8$–12 animals. BM, body mass; LV/BM, left ventricle-to-body mass ratio; RV/BM, right ventricle-to-body mass ratio; H/BM, total heart-to-body mass ratio; SBP, systolic blood pressure; Sed, Sedentary; Ex, exercise trained; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; Str, strain; Tr, training.

### Table 2. $V_{\text{max}}$ of 2 mitochondrial enzymes in white gastrocnemius skeletal muscle

<table>
<thead>
<tr>
<th></th>
<th>Sed</th>
<th>Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>CS $V_{\text{max}}, \text{mol} \cdot \text{h}^{-1} \cdot \text{kg}^{-1} \cdot \text{protein}^{-1}$</td>
<td>1.67 ± 0.14</td>
<td>1.23 ± 0.18</td>
</tr>
<tr>
<td>β-HAD $V_{\text{max}}, \text{mol} \cdot \text{h}^{-1} \cdot \text{kg}^{-1} \cdot \text{protein}^{-1}$</td>
<td>2.54 ± 0.29</td>
<td>1.67 ± 0.19</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.0009</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

Values are means ± SE of $n = 4$–6 in triplicate. CS, citrate synthase; β-HAD, β-hydroxyacyl-CoA dehydrogenase.
and exposure of aortic rings from SHR to exogenous SOD corrected this relaxation response such that it was no different from that of WKY (6). Increased aortic protein levels of the prooxidant gp91phox subunit (Fig. 3B) and decreased levels of the antioxidant enzymes SOD-1, -2, and catalase (Table 4) observed in SHR vs. WKY in the present study suggest that an imbalanced pro- and antioxidant status, leading to increased destruction of NO, could be partially responsible for the impaired maximal endothelium-dependent vasorelaxation exhibited by Sed SHR vs. all other groups (Fig. 1B).

Interestingly, SHR showed greater endothelium-dependent relaxation to $10^{-8}\text{M}$ ACh compared with WKY (Fig. 1B). Higher protein levels of eNOS (Fig. 3A and Ref. 19) and sGC (Fig. 3C) exhibited in SHR vs. WKY could have contributed to this observation by increased relative NO production and VSM sensitivity to NO, respectively. It is important to note that Sed SHR actually achieved near-maximal relaxation to $10^{-6}\text{M}$ ACh, although this dose clearly results in a submaximal response in all other groups (Fig. 1B). Additionally, aortic rings from SHR produced significant vasoconstriction compared with WKY in response to maximal ($10^{-6}$ and $10^{-4}\text{M}$) ACh in the presence of L-NAME (Fig. 1D), consistent with other studies (24, 28). Greater production of endothelium-derived vasoconstricting prostanoids in adult SHR (1, 19, 24, 28) could contribute to the vasocontraction elicited by ACh in the presence of L-NAME in the present study.

**Table 3. Absolute resting tension at $L_o$, developed tension to KCl at $L_o$, and developed tension to maximal PE in thoracic aortic rings**

<table>
<thead>
<tr>
<th></th>
<th>Sed</th>
<th>Ex</th>
<th>Main Effects</th>
<th>Interaction, Strain × Train</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting tension at $L_o$, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>6.95±0.49</td>
<td>6.92±0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>7.17±0.39</td>
<td>6.74±0.42</td>
<td>0.6358</td>
<td>0.9704 0.6824</td>
</tr>
<tr>
<td>Developed tension to 60 mmol/l KCl at $L_o$, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>1.70±0.10</td>
<td>1.59±0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>1.78±0.09</td>
<td>1.75±0.12</td>
<td>0.4695</td>
<td>0.2465 0.6897</td>
</tr>
<tr>
<td>Developed tension to maximal ($10^{-4}\text{mol/l}$) PE, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>2.29±0.07</td>
<td>1.90±0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>2.42±0.11</td>
<td>1.99±0.17</td>
<td>0.0028</td>
<td>0.3954 0.8503</td>
</tr>
</tbody>
</table>

Values are means ± SE of $n = 6$ in duplicate. $L_o$, optimal length for tension development; PE, phenylephrine.
Consistent with work using the endothelium-independent sGC agonist YC-1 (36), SHR and WKY in the present study exhibited similar vasorelaxation responses to both submaximal and maximal SNP stimulation (Fig. 2B). Although higher aortic levels of sGC observed in SHR vs. WKY (Fig. 3C) do not appear to enhance maximal endothelium-independent response in the former, these immunoblot data suggest that SHR may partially compensate for diminished NO release from the endothelium by upregulating sGC in the VSM to protect VSM sensitivity to NO.

Improved endothelium-dependent vasorelaxation in the peripheral vasculature of both humans (18, 20, 22, 25, 45) and animals (8, 12, 13, 32, 35, 46, 48, 50) has been shown after exercise training. Although all of the mechanisms responsible for this phenomenon are not known, NO synthase inhibitors eliminate the effect in rodent aorta (8, 12, 50), suggesting a major contribution of improved NO production. Indeed, Ex is also associated with enhanced eNOS mRNA expression (38, 47) and protein content (11, 12, 16, 30, 48) in various vessels and species. Ex fully restored maximal ACh-induced vasorelaxation in aortic rings from adult SHR in the present study to the level of both Sed WKY and Ex WKY (Fig. 1B), an effect that was completely abolished in the presence of L-NAME such that the response of Ex SHR was no different from that of Sed SHR (Fig. 1D). In addition, elevated aortic eNOS protein content was observed in Ex vs. Sed rats (Fig. 3A). These data further support that Ex-induced increases in endothelial NO production contribute to the improvement in endothelium-dependent, NO-mediated vasorelaxation in adult SHR.

Additionally, aortic protein levels of gp91phox, the endothelium-specific catalytic subunit of the prooxidant NAD(P)H oxidase enzyme (17), were decreased after Ex in the present study (Fig. 3B). The authors are unaware of other studies examining the effects of Ex on vascular levels of gp91phox; however, it is notable that endothelial cell cultures exposed to pulsatile shear stress (23) and aortas from SHR exposed to angiotensin II receptor blockade (7) exhibit diminished levels of gp91phox and p22phox mRNA, respectively. Thus the elevated pulsatile flow (cardiac output) and diminished angiotensin II signaling (5) associated with Ex could have been responsible for the diminished aortic gp91phox protein levels observed in the present study. However, this is merely speculative, and further investigation is needed to determine the mechanisms responsible for this observation. Due to the multimeric nature and complex activation cascade of NAD(P)H oxidase, the present data do not allow for a definitive conclusion of whether Ex altered vascular wall oxidative stress in SHR or WKY rats through a gp91phox-dependent mechanism.
In contrast, moderate Ex did not alter aortic levels of the antioxidant SOD-1, -2, or catalase enzymes in the present study (Table 4). Although these observations are consistent with the lack of Ex-induced alterations in levels of SOD-1 in peripheral arteries from pigs (48) and mice (16) and SOD-2 and catalase protein levels in porcine AEC (37), they are inconsistent with previous reports of elevated SOD-1 protein in porcine AEC after exercise training (37). These antioxidant enzymes are expressed in both endothelial and VSM tissues; thus the limitations of using immunoblot analyses of whole aortic homogenates in the present study in an attempt to identify possible endothelium-specific adaptations should be recognized. Collectively, these data suggest equal or diminished pro-oxidant destruction of NO in the vascular walls of Ex SHR compared with Sed SHR, which, when coupled with potentially elevated NO production, could result in improved NO bioavailability and contribute to the enhanced maximal endothelium-dependent vasorelaxation observed in Ex SHR.

Table 4. Relative levels of 3 antioxidant enzymes as determined by immunoblot analysis in thoracic aortic homogenates

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sed WKY</th>
<th>Sed SHR</th>
<th>Ex WKY</th>
<th>Ex SHR</th>
<th>Main Effects</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD-1 protein level, arbitrary units</td>
<td>1.00 ± 0.03</td>
<td>0.87 ± 0.02</td>
<td>0.94 ± 0.03</td>
<td>0.87 ± 0.02</td>
<td>0.0005</td>
<td>0.1787</td>
</tr>
<tr>
<td>SOD-2 protein level, arbitrary units</td>
<td>1.00 ± 0.03</td>
<td>0.84 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.87 ± 0.03</td>
<td>&lt;0.0001</td>
<td>0.7433</td>
</tr>
<tr>
<td>Catalase protein level, arbitrary units</td>
<td>1.00 ± 0.07</td>
<td>0.82 ± 0.04</td>
<td>1.09 ± 0.06</td>
<td>0.74 ± 0.05</td>
<td>&lt;0.0001</td>
<td>0.9640</td>
</tr>
</tbody>
</table>

Values are means ± SE of n = 6 in duplicate.
Although thoracic aortic rings from Sed WKY in the present study exhibited ~84% maximal vasorelaxation to ACh, and this was not significantly improved in Ex WKY (89%; Fig. 1B), studies using adult male Sprague-Dawley rats reported improved maximal ACh-induced vasorelaxation in abdominal aortic rings (~60 vs. ~80% relaxation in Sed vs. Ex rats, respectively) after 10 wk of moderate- to high-intensity aerobic treadmill training (30 m/min, 15% incline, 60 min/day, 5 days/wk) (12, 13). It is possible that these discrepancies in Ex-induced improvements in maximal endothelium-dependent vasorelaxation are either strain- and/or vessel dependent and that the differences could arise from either an altered maximal response of Sed animals to ACh and/or in some unidentified disparity in the manner of response to Ex. Of note, the present study used a shorter and more moderate training protocol than the work by Delp and colleagues (12, 13), possibly contributing to the lack of significant Ex-induced improvements in endothelium-dependent vasorelaxation in WKY rats.

It is interesting that Ex slightly but significantly decreased the maximal SNP-induced relaxation in the present study (Fig. 2B), an effect that was abolished in the presence of L-NAME (Fig. 2D). The mechanisms responsible for these alterations were not examined in this study but warrant further investigation.

Although the thoracic aorta provides a convenient and suitable tissue for studying in vitro vasomotor function in rats, it can be difficult to extrapolate these findings to the level of the resistance arteries. For instance, although the present data indicate that moderate Ex results in enhanced maximal endothelium-dependent, NO-mediated vasorelaxation in thoracic aortic rings from adult male SHR, no concomitant blunting of SBP was observed in these animals. This latter finding was unexpected because it is well established that prolonged moderate Ex reduces end-point SBP in SHR (2, 9, 14, 40–42).

However, it seems that the duration of the training regimen plays a significant role in the hypotensive effect of chronic exercise, because several studies report only small (14, 40, 41) or no (42) reductions in SBP after 6 wk of a training protocol that is similar in intensity to that in the present study. It could be that if the present SHR had continued the training protocol for several more weeks, a reduction in pressure would have been observed. This idea is supported by reports that training protocols of similar intensity but longer (10–20 wk) duration result in a lowering of SBP in SHR (9, 14, 40–42). This may suggest that improvement in conduit artery endothelium function is an early adaptation to training in hypertensive animals and that this precedes adaptations in resistance vasculature and improvements in blood pressure per se. In addition, SHR in many of the studies cited above were exercised from a younger age (3–6 wk old) (9, 14, 41, 42) and did not have hypertension as severe as those in the present study before beginning the training. Thus the comparison of these previous studies with the present data is complicated by interpretation of prevention vs. reversal of hypertension of varying severities in young and adult SHR, respectively. Observations of enhanced vasomotor function in the forearms of Ex subjects as measured by venous occlusion plethysmography (25) and improved vasorelaxation of coronary resistance arteries of Ex pigs (32) both suggest enhanced NO bioavailability in the resistance vasculature. Perhaps an exercise training regimen of similar intensity but longer duration would have elicited improved resistance artery endothelial vasomotor function as well as reduced SBP. There is precedent for earlier vasomotor adaptations to Ex in conduit arteries (29), whereas similar adaptations occur only later in resistance arteries (32). In the absence of experiments using isolated resistance arteries, we cannot speculate further regarding their possible responses to Ex in this model.

In addition to being an index of endothelial vasomotor function, the present observation that maximally stimulated vasorelaxation through an endothelium-dependent, NO-mediated pathway was improved in Ex SHR has implications for improvements of other functions of NO in the vasculature (e.g., regulation of leukocyte adhesion, platelet aggregation, thrombosis, vascular cell growth). Thus Ex-induced improvements in NO bioavailability, as measured by assessment of endothelium-dependent vasomotor function, can imply or be a marker of beneficial vascular adaptations other than those directly related to improved control of vascular tone.

In conclusion, blunted endothelium-dependent vasomotor function in Sed SHR was likely due to a combination of elevated oxidative stress (increased gp91phox protein levels and decreased SOD-1, -2, and catalase protein levels) and elevated vasoconstricting prostaglandin synthesis. Because maximal vasorelaxation to ACh was fully restored in Ex SHR vs. Sed SHR, and this improvement was abolished in the presence of L-NAME, it seems likely that Ex-induced adaptations in vasomotor function in SHR are largely NO mediated. The increased aortic protein levels of eNOS and decreased aortic protein levels of gp91phox after Ex support this interpretation; however, activity measurements of these enzymes would help elucidate the functional roles of various pathways controlling NO bioavailability in response to an Ex stimulus.

ACKNOWLEDGMENTS

The authors thank Lisa Code, Susan Teschke, Bryon Hughson, and Seema Bhandarkar for excellent technical assistance.

GRANTS

This research was supported by the Heart and Stroke Foundation of Ontario (NA 4604 and T5210), the Canadian Foundation for Innovation, and the Ontario Innovation Trust. J. W. E. Rush is the Canadian Institutes of Health Research-Canada Research Chair in Integrative Vascular Biology. D. A. Graham is supported by a Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship B Award.

REFERENCES


