Effect of exercise training on resistance arteries in rats with chronic NOS inhibition

Oktay Kuru,1 Ümit Kemal Şentürk,2 Günner Koçer,2 Sadi Özdem,3 Oğuz K. Başkurt,2 Arzu Çetin,4 Akın Yeşilkaya,4 and Filiz Gündüz2

1School of Health Sciences, Muğla University, Muğla, Turkey; and 2Department of Physiology, 3Department of Pharmacology, and 4Department of Biochemistry, Medical Faculty, Akdeniz University, Antalya, Turkey

Submitted 24 September 2008; accepted in final form 19 May 2009

Regular exercise has blood pressure-lowering effects, as shown in different types of experimental hypertension models in rats, including the nitric oxide synthase (NOS) inhibition model. We aimed to investigate possible mechanisms implicated in the exercise effect by evaluating the vasoreactivity of resistance arteries. Exercise effects on agonist-induced vasodilatory responses and flow-mediated dilation were evaluated in vessel segments of the rat chronic NOS inhibition model. Normotensive and hypertensive rats were subjected to swimming exercise (1 h/day, 5 days/wk, 6 wk), while rats in other sedentary and hypertensive groups did not. Hypertension was induced by oral administration of the nonselective NOS inhibitor L-NAME (25 mg/kg day) for 6 wk. Systolic blood pressure, as measured by the tail-cuff method, was significantly decreased by the training protocol in exercising hypertensive rats. The vasoreactivity of resistance arteries was evaluated by both wire and pressure myography studies. An impaired nitric oxide-mediated relaxation pathway in untrained hypertensive rats led to decreased relaxation responses in vessels with intact endothelium. Exercise training significantly improved the responses to acetylcholine and flow-mediated dilation in exercise-trained hypertensive rats in parallel with a decrease in blood pressure. On the other hand contraction (norepinephrine and KCl) and relaxation (sodium nitroprusside) responses of vascular smooth muscle were not different between the groups. Vascular endothelial NOS protein expression was found to be increased in both exercising groups. In conclusion, these results revealed evidence of an increased role of the nitric oxide-dependent relaxation pathway in exercising hypertensive rats.

nitric oxide; hypertension; nitric oxide synthase

THE BLOOD PRESSURE-LOWERING effects of regular physical training in humans have been known for a long time (8). This beneficial effect of exercise has also been observed in various experimental hypertension models in animals (9, 15, 25). Despite some controversial results, it has been clearly demonstrated that low- to moderate-intensity exercise decreases elevated blood pressure or delays the onset of hypertension in spontaneously hypertensive rats (28), Dahl-salt hypertension (25), DOCA-induced hypertension (9), and Goldblatt hypertension (18). Besides the various hypotheses suggested for the blood pressure-lowering effect of exercise, it is widely accepted that this occurs via attenuation of peripheral vascular resistance in response to exercise (1, 10, 25). The most emphasized mediator involved in the decrease in total peripheral resistance is nitric oxide (NO) (22, 23). Elevation of NO production and release by the vascular endothelium are strongly induced by shear stress, an event that is prominent during exercise bouts, in conjunction with increased blood flow (19). Furthermore, it is also well known that regular physical activity induces genetic expression of the NO synthase enzyme (NOS) (22, 23, 24).

Systemic hypertension induced by chronic NOS inhibition in rats was first defined as a new experimental model of hypertension in 1992, allowing for further investigation of the involvement of NO in cardiovascular physiology (29). The effect of exercise on NOS-blockage hypertension has not been investigated in detail, and limited data are available regarding this issue. In a study performed by d’Avila et al. (4), a nonsignificant decrease in systolic blood pressure was observed in exercising rats under chronic NOS inhibition. The main focus of this study was investigation of insulin sensitivity in exercising rats with chronic NOS blockage. However, a significant reduction in elevated blood pressure during chronic NOS inhibition was first shown in our previous studies, in which our group examined the effect of exercise on hypertension (15, 16). Besides reduced blood pressure in exercising hypertensive animals, increased NOS enzyme activity was also detected in the gastrocnemius muscle of hypertensive rats after a 1-mo exercise regimen (15). However, it was still unclear whether the increased NOS enzyme activity could be effective enough to reduce peripheral vascular resistance, and responses of pressure regulating resistance arteries were also not evaluated.

In the present study, we further investigated the influence of exercise on resistance arteries that are involved in regulation of blood pressure in a NOS-blockage hypertension model in rats. In this direction, we examined the vasoreactivity of gastrocnemius muscle resistance arteries with both wire and pressure myography techniques, where responses to agonists and flow were evaluated in similar arterial segments. We hypothesized that relaxation response in hypertensive animals could be improved by exercise training. To explain the possible enhancement in vasodilatory responses, we also analyzed expression of NOS isoenzymes in active skeletal muscle and their arteries to detect the contribution of each specific type of NOS.

MATERIALS AND METHODS

Animals. Eighty adult female Wistar rats (8 wk old) weighing 200–220 g were used in the present study. The animals were housed at 23 ± 2°C on a 12:12-h light-dark cycle and had free access to standard rat chow and drinking water. Rats were assigned randomly to four different groups: sedentary control (C; n = 20), exercise training (E; n = 20), sedentary hypertensive (H; n = 20), and exercising...
hypertensive (HE; n = 20). In two of the groups (H and HE), hypertensive was induced by oral administration of the NOS inhibitor N\textsuperscript{\textnd}amino-nitro-L-arginine methyl ester (l-N\textsubscript{NAME}; 25 mg.kg\textsuperscript{-1}.day\textsuperscript{-1}), dissolved in drinking water, for 6 wk. l-N\textsubscript{NAME} was concomitantly given during the training protocol in the HE group. All other animals received normal tap water throughout the experiment (C and E groups). The animals in the training groups were subjected to swimming exercise (60 min/day, 5 days/wk for 6 wk) in a glass tank of 100 × 50 cm (depth = 50 cm) filled with tap water (32–34°C). The duration of the first swimming experience was limited to 10 min and increased by 10 min daily until 60 min was reached. The experimental protocol was approved by the Animal Care and Usage Committee of Akdeniz University and followed the guidelines for using animals in experimental research.

The systolic blood pressure of rats was measured using a noninvasive tail-cuff method; measurements were made at the beginning of the study and every 2 wk during the 6-wk period. Data were obtained with a MAY-BPHR 9610-FC unit and MP 150 data-acquisition system (BIOPAC Systems; Santa Barbara, CA). In exercising animals, the first measurements were performed 1 day after the last swimming session.

Responses to agonists (wire myography). All animals were killed under ether anesthesia by withdrawing the blood from the abdominal aorta 1 day after the last exercise bout in the training groups. The gastrocnemius muscle was excised from the legs of the animals and transferred to a dissecting dish filled with ice-cold physiological saline solution (PSS) containing (in mM) 110 NaCl, 5 KCl, 24 NaHCO\textsubscript{3}, 1 KH\textsubscript{2}PO\textsubscript{4}, 1 MgSO\textsubscript{4}, 2.5 CaCl\textsubscript{2}, 0.02 EDTA, and 10 glucose. The gastrocnemius feed arteries (200–250 μm in diameter) were carefully dissected free under a dissecting microscope (SZ61; OLYMPUS, Tokyo, Japan) and prepared for the study using wire myography equipment (EMKA Technologies, Paris, France). The isolated arterial segments were then placed on a mounting plate containing preheated PSS solution (37°C, pH 7.4). The fatty connective tissue attached to the blood vessels was removed, and the vessels were then cut into ~2-mm length rings for the experiments. Two fine tungsten wires (25 μm in diameter) were placed through the lumen of the rings. The rings were then horizontally mounted, being careful to avoid damage to the inner surface, in organ bath chambers containing 10 ml of PSS solution as described by the manufacturer. In some rings, the endothelium was removed by gently rubbing the intimal surface with the free wire before transferring and mounting the preparation in the vessel chamber. One wire was anchored to the removable mounting jaw of the equipment, and the other was connected to the isometric force transducer (ELG-S270B; Entran Sensors and Electronics, Toulouse, France), allowing the wires to be fixed in two steps. The bath medium was bubbled with a mixture of CO\textsubscript{2} (5%) and O\textsubscript{2} (95%) and was maintained at 37°C (pH 7.4). The optimal resting tension (90 mmHg) for each ring was determined by construction of a passive diameter-tension curve for which the vessel length was constant using computer software (Normaliz v1.0, EMKA Technologies). The preparations were allowed to equilibrate under an optimal resting tension for 60 min before the start of the experiments. During this period, the bath solution was changed every 15 min. After equilibration, tissue viability was confirmed by contraction in response to norepinephrine (NE; 10\textsuperscript{-6} M) in PSS containing 20 mM K\textsuperscript{+}. Several washes were performed to remove the constrictor agents, and the preparations were allowed to stabilize for 30 min. Endothelial integrity was then assessed by precontracting tissues with NE (10\textsuperscript{-6} M) before recording the relaxation elicited by acetylcholine (ACH; 10\textsuperscript{-6} M). Relaxation 70% greater than that of the precontraction value was accepted to indicate a functional endothelium; relaxation exhibited by tissues with endothelium removed was <5%. After an additional 30-min period that included washes every 15 min, the contractility of arteries at a high K\textsuperscript{+} concentration (80 mM) was evaluated. The tissues were then allowed to further re-equilibrate for 30 min, washing was repeated every 15 min during similar periods between the following experimental protocols, and the tension was allowed to return to baseline. In bath solutions containing high K\textsuperscript{+} levels, additional K\textsuperscript{+} was replaced with equivalent amounts of Na\textsuperscript{+} to obtain a certain K\textsuperscript{+} concentration.

In both endothelium-denuded and endothelium-intact vessels, concentration-response curves to NE (10\textsuperscript{-9} to 3 × 10\textsuperscript{-6} M) were constructed by cumulative addition of the drug. Concentration-relaxation response curves in response to sodium nitroprusside (SNP; 10\textsuperscript{-9} to 10\textsuperscript{-3} M) were also obtained for the same set of rings precontracted with NE (10\textsuperscript{-6} M). To analyze the influence of endothelial factors, relaxation studies were performed using a different series of vessels with intact endothelium, which underwent the same normalization and equilibration periods. The preparations were then precontracted with a single submaximal concentration of NE (10\textsuperscript{-6} M). When the contractile response to the agonist was stable, increasing concentrations of ACh were applied in a cumulative fashion (10\textsuperscript{-9} to 10\textsuperscript{-6} M). The NE-induced steady state of contraction was considered to be 100%, and the relaxation responses were calculated as a percentage of this contraction. The same experiment used to examine relaxation in response to ACh was carried out in the presence of different drugs, under the following conditions: incubation with the nonselective NOS inhibi-l-N\textsubscript{NAME} (10\textsuperscript{-4} M, 20 min), the selective inhibitor of the soluble guanylate cyclase enzyme IH-(1,2,4)oxadiazolo(4,3-a) quinnoxalin-1-one (ODQ; 10\textsuperscript{-5} M, 30 min), and l-N\textsubscript{NAME} (10\textsuperscript{-4} M) plus the cyclooxygenase inhibitor indomethacin (10\textsuperscript{-7} M), the K\textsuperscript{+} channel blocker tetraethylammonium (10\textsuperscript{-3} M), and 4-aminopyridine (10\textsuperscript{-3} M) together for 20 min. The last protocol was applied to inhibit all endothelium-derived vasodilators including NO, PGI\textsubscript{2}, and the endothelium-derived hyperpolarizing factor.

Flow-mediated dilation (pressure myography). The arterial segments for pressure myograph preparations (~200 μm in diameter) were isolated similarly to those described above. The vessels were then transferred to a vessel chamber (CH/2SH; Living Systems, Burlington, VT), mounted between two glass micropipettes, and superfused with oxygenated PSS at 37°C. The micropipettes were connected to a pressure servo-control system composed of two roller pumps, two pressure transducers that monitored the inflow and outflow pressures, and a control unit (PS/200/Q, Living Systems). The vessel segment was perfused from a separate source of the oxygenated MOPS-buffered PSS containing (in mM) 145 NaCl, 4.7 KCl, 2 CaCl\textsubscript{2}, 1.17 MgSO\textsubscript{4}, 1.2 NaH\textsubscript{2}PO\textsubscript{4}, 5.0 glucose, 2 pyruvate, 0.02 EDTA, and 25 MOPS supplemented with BSA (1 g/100 ml). The servo-control system allowed us to set the flow rate through the arterial segment from 0 to 370 μl/min. The arterial segment preparation was placed on an inverted microscope (Eclipse TS100; Nikon, Tokyo, Japan) equipped with a charge-coupled device camera (XC73CE; Sony, Tokyo, Japan). The camera was connected to a video dimension-analysis system (model V94, Living Systems), which allowed for continuous recording of the vessel diameter. The intraluminal and perfusion pressures, fluid flow rate through the arterial segment, and vessel diameter were continuously recorded via a data-acquisition system (MP 100A-CE, BIOPAC Systems) connected to a personal computer.

The preparations were left to stabilize for 1 h while the bath solution was refreshed every 15 min. Before it was studied, the length of the arterial segment was adjusted to its in vivo length, and the arterial segment was equilibrated to 45 mmHg intraluminal pressure for 20 min, followed by a 40-min period at 60 mmHg intraluminal pressure and a zero flow rate for a total of 60 min of stabilization. Most of the vessels developed a spontaneous tone of at least a 25% increase in initial diameter. Vessels with intact endothelium were used in this study, and the endothelial integrity of pressurized arterial segments was tested by adding ACh (10\textsuperscript{-6} M) to the bath solution to assess relaxation after precontraction with NE (10\textsuperscript{-6} M). The following two types of studies were carried out: flow-mediated dilation (FMD) and FMD plus l-N\textsubscript{NAME}.

The arterial segments were washed with oxygenated PSS. Arteries that did not develop at least 25% spontaneous tone at the end of the
equilibration period (3 from C and 2 from each H, E, and HE groups) were constricted with NE (10^{-8} to 10^{-6} M). FMD was assessed at a series of fluid flow rates between 7 and 62 µl/min, while the intraluminal pressure was kept constant at 65 mmHg by the pressure servo-control system. Each flow rate was maintained for 5 min to obtain a steady vessel diameter. The arterial segment was washed again, and the procedure to assess FMD was repeated after a 20-min incubation with L-NAME (10^{-4} M). The results for FMD are expressed as the percent change in diameter relative to NE-induced preconstriction.

**Determination of NOS isoform expression.** The gastrocnemius muscle from the other leg of the animal was also excised and transferred to a dissecting dish containing cold PSS. The arterial vasculature across the sample was carefully dissected free and immediately isolated under a microscope. All other visible vascular remnants were removed, and a small piece of striated muscle tissue was also isolated. Tissue samples were flash-frozen in liquid nitrogen and stored at −80°C. The expression of NOS isoforms was assessed separately in arterial tissues and vessel-free muscle samples by Western blot.

Arterial tissues were pooled as three samples from the same group to obtain a sample containing enough protein for blotting. Tissue samples were homogenized by sonication (Sonopuls; Bandelin Electronic, Berlin, Germany) for 10 s in cold 1% Triton X-100-HEPES lysis buffer (600 µl) on ice. The homogenate was then centrifuged at 4°C (8,000 rpm for 10 min and 15,000 rpm for 15 min), and the protein content of the supernatant was determined by Bradford reagent (Bio-Rad Laboratories, Hercules, CA). Samples containing equal amounts of protein were mixed in Laemmli sample buffer (Sigma-Aldrich, St. Louis, MO), loaded for resolution via SDS-PAGE (7.5%), and transferred to nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). The membranes were blocked in 5% fat-free milk powder solution prepared in 0.1% Tween 20-containing phosphate buffer (pH 7.4) for 2 h at room temperature and then incubated overnight at 4°C with endothelial (eNOS), neuronal (nNOS), or inducible (iNOS) NOS rabbit antibodies (1:1,500, 1:2,000, and 1:1,000, respectively; Sigma-Aldrich), followed by horseradish peroxidase-conjugated secondary antibody (Chemicon). The same procedure using the appropriate primary and secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) was used for detection of GAPDH protein as a positive control and for standardization of the blotting results. Enhanced chemiluminescent (ECL) labeling (Amer sham-RPN2132, GE Healthcare UK Limited) was used to visualize the labeled proteins by exposure to ECL-sensitive films (Amersham Hyperfilm ECL, GE Healthcare). The density of digitized images was calculated with Scion Image software (Scion, Frederick, MD).

**Statistical analyses.** The concentration-response curves in different protocols were constructed using the results from six to nine preparations obtained from different animals. The results are presented as means ± SE. Statistical significance between the curves was tested using two-way ANOVA for repeated measures followed by the Newman-Keuls test. The maximal effect of the drug in a given protocol was determined. The results for blood pressure, KCl contraction, and body weight are reported as means ± SE and were analyzed by one-way ANOVA with Newman-Keuls post hoc test for significance. A P value <0.05 was considered significant.

**RESULTS**

Body weight was not different between the groups at the end of the study. Initial levels of systolic blood pressure were not different between the groups. Blood pressure was found to be elevated after the first 2 wk in both groups receiving L-NAME, and these high levels were maintained during the experimental period (Fig. 1). H and HE groups exhibited significantly higher systolic blood pressure levels than controls (P < 0.001) at the end of the study, whereas exercise training induced a significant decrease in systolic blood pressure in HE animals compared with the H group (P < 0.01).

**Responses to agonists.** Vasoconstrictor responses induced by NE (10^{-9} to 3 × 10^{-6} M) or KCl (80 mM) were not different between the groups (Fig. 2; results for KCl-induced contraction are not shown). Endothelium-independent vasodilator responses to the NO donor SNP (10^{-9} to 10^{-5} M) were also similar among the groups (Fig. 3). However, endothelium-dependent relaxation responses induced by cumulative doses of ACh (10^{-9} to 10^{-6} M) in vessel rings precontracted with NE from H rats were significantly reduced (P < 0.05) compared with that shown in controls (Fig. 4). Vasodilation in response to ACh in vessel rings from HE rats was restored to the levels obtained in C and E animals as observed by a statistical significance was obtained for the means of the maximal response in these two groups (Table 1).

The effect of nonselective NOS inhibition was observed in all experimental groups, and maximal relaxation responses were all reduced by L-NAME inhibition in the present protocol (Fig. 4). The H group exhibited a significantly reduced relaxation response to ACh after incubation with L-NAME (10^{-4} M) compared with the C group (P < 0.05), whereas the response patterns were not different among the remaining groups. The training protocol led to a greater maximal relaxation in rings from HE animals (P < 0.05) than in those of the H group (Table 1). Dilation responses to ACh were markedly inhibited by the guanylate cyclase inhibitor ODQ (10^{-5} M) in all groups (Fig. 4), and relaxation patterns induced by ACh were not different between the groups.

The vessel preparations in all of our groups exhibited an apparent inhibition of ACh-induced dose-dependent relaxation in the protocol used for total inhibition of dilators, in which L-NAME (10^{-4} M), indomethacin (10^{-5} M), tetroxethyllammonium (10^{-3} M), and 4-aminopyridine (10^{-3} M) were used together (Fig. 4). Maximal responses were similar between the groups, and no differences were observed between the relax-
Independent vasodilator responses to the NO donor SNP (10⁻⁹ M) did not differ between the groups, and endothelium- and H groups (P < 0.01). Exercise training caused an elevation in the relaxation responses of C vessels were similar to those of the E group, whereas exercise training led to an improvement in FMD of HE group vessels, an effect observed as a significant difference between the HE and H groups (P < 0.01). Incubation with L-NAME dramatically attenuated the FMD in all experimental groups. However, the responses of the H group were not different from that of the C group (Fig. 6). The expression of both nNOS and iNOS in skeletal muscle did not differ between the experimental groups (Table 2). In addition to these findings, we could not detect eNOS protein in skeletal muscle samples.

**DISCUSSION**

Numerous studies have investigated in detail the relationships between exercise and hypertension in humans (20). The blood pressure-lowering effects of regular exercise are also apparent in animal models of experimental hypertension. The present study indicates a similar effect in accordance with our previous studies on hypertensive rats under chronic NOS inhibition, in which endothelial dysfunction was shown to prevail (15, 16). We attempted to evaluate the contribution of the NO pathway to exercise-induced blood pressure reduction in the present study, and our findings support the idea that impaired vascular responses to ACh and FMD could be partly restored based on increased expression of eNOS enzyme in the muscle microvascular bed.

Endothelial dysfunction is an important hypothesis that is suggested to be involved in the pathogenesis of essential hypertension. This process occurs as an insufficiency in the production of endothelium-derived relaxing agents in response to ACh, as demonstrated in both humans and experimental animals (6). Hypertension induced by chronic NOS inhibition has also been attributed as a state related to endothelial dysfunction (29). Chronic NOS inhibition, as applied by using the nonspecific NOS enzyme inhibitor L-NAME, induced an apparent elevation in blood pressure after the first week in our study. Although blood pressure in our HE group was higher than in the C group, it was found to be lower than the hypertensive group that did not exercise (H group). The latter difference was detected during the fourth week of our study and was maintained until the end. The idea for exercise-related improvements in responses of the vascular endothelium is widely supported by research data obtained during recent years.

**Expression of NOS isoforms.** GAPDH protein expression in both vascular and skeletal muscle tissues was similar among the groups, and the results represent the mean levels calculated for NOS expression in the experimental groups. The nNOS protein expression in vessel tissues was found to be unaltered among the groups (Table 2). In contrast, it was not possible to detect iNOS protein in isolated vascular tissues. Exercise training significantly elevated the expression of vascular eNOS enzyme expression in both the E and HE groups compared with the C group (P < 0.05), whereas expression in the H group did not differ from that of the C group (Fig. 6). The expression of both nNOS and iNOS in skeletal muscle did not differ between the experimental groups (Table 2). In addition to these findings, we could not detect eNOS protein in skeletal muscle samples.

**DISCUSSION**

Numerous studies have investigated in detail the relationships between exercise and hypertension in humans (20). The blood pressure-lowering effects of regular exercise are also apparent in animal models of experimental hypertension. The present study indicates a similar effect in accordance with our previous studies on hypertensive rats under chronic NOS inhibition, in which endothelial dysfunction was shown to prevail (15, 16). We attempted to evaluate the contribution of the NO pathway to exercise-induced blood pressure reduction in the present study, and our findings support the idea that impaired vascular responses to ACh and FMD could be partly restored based on increased expression of eNOS enzyme in the muscle microvascular bed.

**Expression of NOS isoforms.** GAPDH protein expression in both vascular and skeletal muscle tissues was similar among the groups, and the results represent the mean levels calculated for NOS expression in the experimental groups. The nNOS protein expression in vessel tissues was found to be unaltered among the groups (Table 2). In contrast, it was not possible to detect iNOS protein in isolated vascular tissues. Exercise training significantly elevated the expression of vascular eNOS enzyme expression in both the E and HE groups compared with

**DISCUSSION**

Numerous studies have investigated in detail the relationships between exercise and hypertension in humans (20). The blood pressure-lowering effects of regular exercise are also apparent in animal models of experimental hypertension. The present study indicates a similar effect in accordance with our previous studies on hypertensive rats under chronic NOS inhibition, in which endothelial dysfunction was shown to prevail (15, 16). We attempted to evaluate the contribution of the NO pathway to exercise-induced blood pressure reduction in the present study, and our findings support the idea that impaired vascular responses to ACh and FMD could be partly restored based on increased expression of eNOS enzyme in the muscle microvascular bed.

**Expression of NOS isoforms.** GAPDH protein expression in both vascular and skeletal muscle tissues was similar among the groups, and the results represent the mean levels calculated for NOS expression in the experimental groups. The nNOS protein expression in vessel tissues was found to be unaltered among the groups (Table 2). In contrast, it was not possible to detect iNOS protein in isolated vascular tissues. Exercise training significantly elevated the expression of vascular eNOS enzyme expression in both the E and HE groups compared with
We investigated the vasoreactivity of resistance arteries, which play an important role in the regulation of blood pressure, by evaluating their responses to agonists and FMD. The results obtained from hypertensive rats with significantly lowered blood pressure after a 6-wk-long exercise training program are particularly important.

In the first steps of the wire myograph portion of our study, we examined the vasoreactivity of vascular smooth muscle of resistance arteries in intact endothelium and endothelium-denuded vessel preparations. The contracting and relaxing responses of vascular smooth muscle cells did not exhibit a significant difference between the groups after application of the agonists KCl, NE, or SNP. However, there was a significant difference in the endothelial response of resistance arteries between the groups. It is well known that impairment in endothelial function develops and accompanies the hypertension based on chronic NOS administration (5, 7, 29). We also obtained significantly reduced vasorelaxation responses to ACh in the H group, which received L-NAME for 6 wk. Concomitantly performed exercise training normalized the responses to ACh in the HE group. Although some studies have already reported this corrective effect of physical exercise on endothelial function in different experimental models of hypertension (21, 26, 28), this is the first time that it has been demonstrated in the NOS inhibition model. ACh-induced dilation response was decreased in all groups after L-NAME incubation. However, the difference between sedentary and trained hypertensive animals remains after L-NAME treatment (Fig. 4B). This result suggests that not only the increase in NO levels but also the factors, such as prostaglandins and/or endothelium-derived hyperpolarizing factor, might be involved in dilation response of HE group. This finding was also correlated with the disappearance of the differences between the ACh-induced relaxation responses shown in the H and HE groups with the following total vasodilator blockage (Fig. 4D).

Table 1. Maximal response values to NE, SNP, and ACh alone and in the presence of other inhibitors in isolated arterial ring preparations

<table>
<thead>
<tr>
<th>Protocol</th>
<th>C</th>
<th>E</th>
<th>H</th>
<th>HE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE, g</td>
<td>1.48±0.23</td>
<td>1.18±0.14</td>
<td>1.11±0.29</td>
<td>1.38±0.09</td>
</tr>
<tr>
<td>SNP, %</td>
<td>95.3±1.8</td>
<td>95.2±1.1</td>
<td>95.9±2.5</td>
<td>94.5±1.2</td>
</tr>
<tr>
<td>ACh, %</td>
<td>70.1±6.9</td>
<td>74.4±4.9</td>
<td>47.8±5.1†</td>
<td>70.8±4.5†</td>
</tr>
<tr>
<td>L-NAME, %</td>
<td>56.4±8.5</td>
<td>57.7±8.2</td>
<td>23.7±7.5†</td>
<td>58.3±4.4†</td>
</tr>
<tr>
<td>ODQ, %</td>
<td>55.3±6.1</td>
<td>60.3±8.2</td>
<td>43.4±8.8</td>
<td>49.8±6.4</td>
</tr>
<tr>
<td>L-NAME + indomethacin + TEA + 4-AP, %</td>
<td>18.8±6.0</td>
<td>17.7±9.3</td>
<td>14.6±14.6</td>
<td>17.3±7.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6–9 animals per group. 4-AP, 4-aminopyridine; L-NAME, N^o-nitro-l-arginine methyl ester; NE, norepinephrine; ODQ, 1H-(1,2,4)oxadiazolo(4,3-a) quinoxalin-1-one; SNP, sodium nitroprusside; TEA, tetraethylammonium. Groups are sedentary control (C), exercise training (E), sedentary hypertensive (H), and exercising hypertensive (HE). *P < 0.05 vs. C group; †P < 0.05 vs. H group.
On the other hand, the responses to ACh were almost equally attenuated in all groups with the ODQ protocol (Fig. 4C). Interestingly, dilation responses with the L-NAME and ODQ protocols were similar in all groups except in the H group. This different response to L-NAME and ODQ incubations support the possibility that L-NAME is blocking more than just eNOS in the H group. It has been demonstrated that L-NAME also blocks nNOS and reduces its production of hydrogen peroxide in mouse aorta (3).

The resistance arteries obtained from H animals exhibited a reduced FMD. However, a 6-wk training period in parallel with hypertension induced a significant improvement of the impaired FMD results, which is another novel finding of the present study. It is well known that exercise training provides significant beneficial support in conditions where endothelial function is impaired (hypertension, aging, smoking, hypercholesterolemia, obesity, and diabetes mellitus) (2). The fact that FMD responses were significantly depressed by L-NAME incubation reflects the important involvement of NO in vasodilation induced by increased shear stress. The cytoskeletal components of the vascular endothelial layer act as a mechanotransducer that perceives the increase in shear stress, resulting in activation of the mechanisms responsible for NO release (12, 17). Consecutively repeated inductions led to a rearrangement in the regulation of NO production as an increase in the expression and activity of NOS enzyme. The possible alterations in NOS enzyme expression as related to the effect of exercise in the experimental NOS inhibition model were evaluated in the last part of our study.

The results of our previous study evaluating the effect of exercise on hypertension during chronic NOS inhibition revealed that NOS enzyme activity was augmented in trained hypertensive rats compared with sedentary hypertensive rats.

Table 2. Expression levels of eNOS, nNOS, and iNOS enzymes (normalized to GAPDH expression values) calculated by densitometric analysis of the Western blot images

<table>
<thead>
<tr>
<th>NOS Isoform</th>
<th>Arteries</th>
<th>C</th>
<th>E</th>
<th>H</th>
<th>HE</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS</td>
<td>10.3±2.2</td>
<td>19.4±1.4*</td>
<td>15.9±2.7</td>
<td>20.6±3.2*</td>
<td></td>
</tr>
<tr>
<td>nNOS</td>
<td>118.8±18.5</td>
<td>134.3±11.9</td>
<td>117.5±13.9</td>
<td>111.5±15.7</td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>Not detected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eNOS</td>
<td>Not detected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nNOS</td>
<td>29.5±4.6</td>
<td>28.4±2.7</td>
<td>31.4±2.5</td>
<td>29.3±3.7</td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>22.4±2.5</td>
<td>20.35±2.7</td>
<td>18.1±4.3</td>
<td>9.6±4.6</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eNOS</td>
<td>Not detected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nNOS</td>
<td>25.9±4.6</td>
<td>24.8±2.7</td>
<td>31.4±2.5</td>
<td>29.3±3.7</td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>22.4±2.5</td>
<td>20.35±2.7</td>
<td>18.1±4.3</td>
<td>9.6±4.6</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6–9 animals per group). eNOS, iNOS, and nNOS, endothelial, inducible, and neuronal nitric oxide synthase, respectively. *P < 0.05 vs. C group.
(15). These results also had some limitations, as we did not evaluate whether the increase in NOS activity resulted from induced genetic expression or posttranslational modification. In addition, the NOS enzyme isoform responsible for this increase remains uncertain. Both endothelial and skeletal muscle NOS enzymes are suggested to be the source of elevated NO release during muscle contractions (2). On the other hand, it is also known that NOS enzyme expression increases in skeletal muscle with exercise training (27). The latter finding was not confirmed by our present results as there were no significant differences between the skeletal muscle NOS expression levels in our groups.

Expression of iNOS was not detected in resistance arteries, and there were no significant differences between the vascular nNOS enzyme expression levels among the experimental groups. Expression of eNOS in arterial segments was found to be significantly higher in both exercising groups than in the control. Upregulation of eNOS expression was expected because the increased shear stress during exercise is known to induce eNOS expression based on previous studies (11, 12). The results of the present study provide strong evidence that this finding is also valid for the NOS inhibition-induced hypertension model. On the other hand, we did not observe a further increase in both ACh and FMD responses in our normotensive exercising group, although the expression of eNOS was increased in both exercising groups. This finding indicates that not solely eNOS expression is involved in current responses, but some other alterations in protein regulation (phosphorylation and/or heat shock protein 90 interaction) could also be implicated (13).

In conclusion, the present study confirmed the findings that regular physical training decreases elevated blood pressure in hypertensive rats with chronic NOS inhibition. To reveal the mechanism of this finding, we demonstrated that vasorelaxation responses in resistance arteries were improved in response to chemical stimulation by ACh or physical stimulation by flow. Increased eNOS expression in the resistance arteries of trained animals appears to be an important component of this improvement.

GRANTS

This study was supported by the Akdeniz University Research Projects Unit (project no. 2004.01.0200.042 and 2005.03.122.006).

REFERENCES