Influence of exercise on dilatation of the basilar artery during diabetes mellitus

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Mayhan, William G., Hong Sun, Jill F. Mayhan, and Kaushik P. Patel. Influence of exercise on dilatation of the basilar artery during diabetes mellitus. J Appl Physiol 96: 1730–1737, 2004.—Our goal was to examine whether exercise training alleviates impaired nitric oxide synthase (NOS)-dependent dilatation of the basilar artery in Type 1 diabetic rats. To test this hypothesis, we measured in vivo responses of the basilar artery in sedentary and exercised nondiabetic and diabetic rats. We found that acetylcholine produced dilatation of the basilar artery that was similar in sedentary and exercised nondiabetic rats. Acetylcholine produced only minimal vasodilatation in sedentary diabetic rats. However, exercise alleviated impaired acetylcholine-induced vasodilatation in diabetic rats. Nitroglycerin produced dilatation of the basilar artery that was similar in sedentary and exercised nondiabetic and diabetic rats. l-NMMA produced similar inhibition of acetylcholine-induced dilatation of the basilar artery in sedentary and exercised nondiabetic and diabetic rats. Finally, we found that endothelial NOS (eNOS) protein in the basilar artery was higher in diabetic compared with nondiabetic rats and that exercise increased eNOS protein in the basilar artery of nondiabetic and diabetic rats. We conclude that 1) exercise can alleviate impaired NOS-dependent dilatation of the basilar artery during diabetes mellitus, 2) the synthesis and release of nitric oxide accounts for dilatation of the basilar artery to acetylcholine in sedentary and exercised nondiabetic and diabetic rats, and 3) exercise may exert its effect on cerebrovascular reactivity during diabetes by altering levels of eNOS protein in the basilar artery.

METHODS

Induction of diabetes. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (180–200 g body wt) were randomly divided into one of four groups: sedentary nondiabetic, exercised nondiabetic, sedentary diabetic, and exercised diabetic. All rats had access to food and water ad libitum. The diabetic groups of rats were injected with streptozotocin (50–60 mg/kg ip) to induce diabetes, and the nondiabetic groups were injected with saline. All rats were housed at 22°C with a 12-h light-dark cycle and allowed free access to food and water. The rats were killed at 6 weeks after diabetes induction.

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of rats were injected with vehicle (sodium citrate buffer). Blood samples, for measurement of blood glucose concentration, were obtained 5–7 days after injection of streptozotocin or vehicle and on the day of the experiment. Blood glucose concentration was determined by using an Accu-Chek II blood glucose monitor (Boehringer Mannheim Diagnostics, Indianapolis, IN), and an animal with a blood glucose concentration of >300 mg/dL was considered to be diabetic. We have used similar methods in previous studies (21, 22, 29).

Exercise training. Rats were exercised by using standard techniques similar to those described by others (2, 13, 31, 35, 42). Treadmill exercise was started 3 days after injection of streptozotocin or vehicle and was carried out 5 days/wk until the day before the experiment. Experiments were conducted 2–2.5 mo after injection of streptozotocin or vehicle. Thus the rats in the exercised groups were exercised for ~2–2.5 mo. The length of time on the treadmill was initially 10 min/day for the first 5 days at 0% grade at a speed of 15–20 m/min. Then, over a 10-day period, the speed was increased to 25 m/min, the grade was increased to 10% and the duration on the treadmill was increased to 60 min. To assess the level of exercise in rats, we measured citrate synthase activity in skeletal muscle (soleus muscle) by using methods described by others (3, 40, 45). We found that our regimen for exercise resulted in an ~45% increase in citrate synthase activity in the soleus muscle of rats (11.9 ± 1.4 in sedentary rats; 17.2 ± 2.5 in exercised rats; P < 0.05). This is similar to that reported by others (10, 40).

Preparation of animals. Rats were prepared for in vivo studies 2–2.5 mo after injection of streptozotocin or vehicle. Rats were anesthetized [thiobutabarbitral (Inactin); 100 mg/kg ip], and a tracheotomy was performed. The animals were ventilated mechanically with room air and supplemental oxygen. A catheter was inserted into a femoral vein for injection of supplemental anesthesia. A femoral artery was cannulated for measurement of arterial pressure and to obtain a blood sample for the measurement of blood glucose concentration.

After placement of all catheters, the animal was placed in a head holder in a supine position. The larynx and esophagus were retracted rostrally and laterally, and the musculature covering the basioccipital bone was removed. Then, a craniotomy was made in the bone at the base of the skull. The dura was incised to expose the basilar artery. We (23, 24) and others (4) have used this method to expose the basilar artery. The cranial window was suffused with artificial cerebral spinal fluid (37 ± 1°C) and bubbled continuously to maintain gases within normal limits. Blood gases were monitored and maintained within normal limits throughout the experiment. The diameter of the basilar artery was measured online by using a video image-shearing device (model 908, Instrumentation for Physiology and Medicine, San Diego, CA).

Experimental protocol. The basilar artery preparation was allowed to equilibrate for 30–45 min before examination of responses to the agonists. Then, we examined dilatation of the basilar artery in sedentary nondiabetic (n = 6), sedentary diabetic (n = 8), exercised nondiabetic (n = 9), and exercised diabetic (n = 9) rats in response to acetylcholine (1.0 and 10 μM) and to nitroglycerin (0.1 and 1.0 μM). Agonists were mixed in artificial cerebral spinal fluid and superfused over the basilar artery preparation. The application of vehicle did not affect vessel diameter, and the application of agonists was randomized. The diameter of the basilar artery was measured immediately before the application of agonists and every minute for 5 min during application of the agonists. Steady-state responses were reached within 2–3 min after application, and the diameter of the basilar artery returned to baseline within 5 min after application of the agonists was stopped.

After this initial examination of reactivity of the basilar artery in the various groups of rats, we then examined responses of the basilar artery to topical application of L-arginine (1-NNMA: 1 and 10 μM). Thus we measured the diameter of the basilar artery at 5-min intervals during a 30-min topical application of L-NNMA (1.0 and 10 μM) in sedentary and exercised nondiabetic and diabetic rats.

Finally, we examined the role of nitric oxide in responses of the basilar artery to the agonists. Thus, during a continuous suffusion of L-NNMA (10 μM), we again measured responses of the basilar artery to acetylcholine and nitroglycerin in sedentary and exercised nondiabetic and diabetic rats.

Western blot analysis of eNOS protein. After our functional studies, basilar arteries were isolated from brain tissue and stored at −80°C until the examination of eNOS protein could be completed. Samples (basilar arteries) were homogenized in 20% (wt/vol) ice-cold buffer containing 10 mM Tris-HCl (pH 7.4), 1% SDS, 1 mM sodium vanadate, 10 μg/ml aprotinin, 10 μg/ml leupatin, and 1 mM phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 12,000 g for 20 min at 4°C, and protein concentration in supernatant was determined by the Bradford method (Bio-Rad, Richmond, CA) with BSA as the standard. SDS-PAGE was performed on a 7.5% gel onto which 1.5 μg of total protein/well were loaded. After SDS-PAGE, the proteins were transferred onto a polyvinylidene difluoride membrane. Immunoblot analysis was performed by using a mouse monoclonal anti-eNOS as the primary antibody (1:1,000) and a horseradish-peroxidase-conjugated goat anti-mouse IgG (1:2,000) as the second antibody. The bound antibody was detected using an enhanced chemiluminescence kit and quantified by scanning densitometry. We have used these methods previously (39, 44, 46).

Statistical analysis. Analysis of variance with Fisher’s test for significance was used to compare responses of the basilar artery to the agonists, baseline diameter of the basilar artery, body weight, blood glucose concentration, and eNOS protein between sedentary and exercised nondiabetic and diabetic rats. A paired t-test was used to compare responses of the basilar artery before and after application of L-NNMA within sedentary and exercised nondiabetic and diabetic rats. A P value of 0.05 or less was considered to be significant.

RESULTS

Responses in sedentary and exercised nondiabetic and diabetic rats. Baseline diameter of the basilar artery, blood glucose concentration, and body weight of sedentary and exercised nondiabetic and diabetic rats are shown in Table 1. We found that baseline diameter of the basilar artery was similar in all groups of rats. In addition, exercise did not influence blood glucose concentration in nondiabetic or diabetic rats. Blood glucose concentration remained significantly higher in diabetic compared with nondiabetic rats regardless of the exercise regimen. Body weight was similar in sedentary and exercised nondiabetic rats. However, body weight was significantly lower in exercised diabetic rats than in sedentary diabetic rats. Furthermore, body weight in sedentary and exercised diabetic

Table 1. Baseline diameter of the basilar artery, blood glucose concentration, and body weight in sedentary and exercised nondiabetic and diabetic rats

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<th>Sedentary</th>
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<td></td>
<td>Nondiabetic</td>
<td>Diabetic</td>
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<tr>
<td>Blood pressure, mmHg</td>
<td>107 ± 4</td>
<td>109 ± 7</td>
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<tr>
<td>Baseline diameter, μm</td>
<td>258 ± 23</td>
<td>225 ± 11</td>
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<tr>
<td>Blood glucose, mg/dl</td>
<td>132 ± 18</td>
<td>400 ± 9†</td>
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<tr>
<td>Body weight, g</td>
<td>381 ± 32</td>
<td>276 ± 15*</td>
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Values are means ± SE. *P < 0.05 vs. sedentary nondiabetic rats. †P < 0.05 vs. exercised nondiabetic rats. ‡P < 0.05 vs. sedentary diabetic rats.
rats was significantly lower that that found in sedentary and exercised nondiabetic rats.

Dilatation of the basilar artery in response to acetylcholine in sedentary and exercised nondiabetic and diabetic rats is shown in Fig. 1. We found that acetylcholine-induced dilatation of the basilar artery was similar in sedentary and exercised nondiabetic rats. In addition, dilatation of the basilar artery in response to acetylcholine was significantly less in sedentary diabetic rats compared with sedentary and exercised nondiabetic rats (P < 0.05). Finally, we found that exercise alleviated impaired vasodilatation in response to acetylcholine in diabetic rats (P < 0.05 vs. response in sedentary diabetic rats). In contrast to that observed during suffusion with acetylcholine, nitroglycerin produced similar dose-related dilatation of the basilar artery in sedentary and exercised nondiabetic and diabetic rats (Fig. 2). Thus it does not appear that the effects of exercise on acetylcholine-induced vasodilatation are related to a nonspecific effect of exercise.

Responses after L-NMMA. Topical application of L-NMMA (1.0 μM) produced modest changes in baseline diameter of the basilar artery that were similar in sedentary and exercised nondiabetic and diabetic rats (Fig. 2). In addition, topical application of L-NMMA (10 μM) produced similar constriction of the basilar artery in sedentary and exercised nondiabetic and diabetic rats, and in sedentary diabetic rats (Fig. 2). However, there was a significant increase in vasoconstriction produced by L-NMMA (10 μM) in exercised diabetic compared with sedentary diabetic rats (Fig. 2).

Next, we examined responses of the basilar artery to the agonists in sedentary and exercised nondiabetic and diabetic rats after topical application of L-NMMA (10 μM). In sedentary nondiabetic and diabetic rats, we found that L-NMMA significantly inhibited responses of the basilar artery to acetylcholine (Fig. 3), but it did not influence vasodilatation in response to nitroglycerin (Fig. 3). Similarly, we found that L-NMMA (10 μM) significantly inhibited dilatation of the basilar artery in response to acetylcholine (Fig. 4) in exercised nondiabetic and diabetic rats, but it did not influence vasodilatation to nitroglycerin (Fig. 4). Finally, we calculated the percent inhibition by L-NMMA (10 μM) in acetylcholine-induced dilatation of the basilar artery in sedentary and exercised nondiabetic and diabetic rats (Fig. 5). We found that L-NMMA produced similar inhibition in acetylcholine-induced dilatation of the basilar artery regardless of the experimental group (Fig. 5).

Measurement of eNOS protein in the basilar artery. To further examine whether exercise influenced dilatation of the basilar artery in diabetic rats via an effect on NOS, we measured eNOS protein in the basilar artery of sedentary and exercised nondiabetic and diabetic rats (Fig. 6). First, we found that eNOS protein was significantly elevated in basilar arteries from sedentary diabetic compared with sedentary nondiabetic rats. Second, we found that exercise significantly increased eNOS protein in the basilar artery from nondiabetic and diabetic rats.

DISCUSSION

This is the first study that we are aware of that has examined the beneficial effects of exercise on reactivity of cerebral blood vessels in Type 1 diabetic animals. There are three major new findings of the present study. First, we found that exercise training alleviates impaired NOS-dependent dilatation of the basilar artery in diabetic rats. This effect of exercise appears to be specific because exercise did not alter dilatation of the basilar artery to nitroglycerin, a NOS-independent agonist. Second, the cellular pathway by which exercise influences impaired dilatation of the basilar artery in response to acetylcholine during diabetes appears to involve NOS, presumably eNOS. We found that the degree of inhibition by L-NMMA on NOS-dependent vasodilatation was similar in nondiabetic sed-

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Fig. 1. Responses of the basilar artery to acetylcholine (A) and nitroglycerin (B) in sedentary nondiabetic and diabetic rats and in exercised nondiabetic and diabetic rats. Values are means ± SE. *P < 0.05 vs. nondiabetic rats. †P < 0.05 vs. diabetic rats.

Fig. 2. Responses of the basilar artery to Nω-monomethyl-L-arginine (L-NMMA; 1.0 and 10 μM) in sedentary nondiabetic and diabetic rats and in exercised nondiabetic and diabetic rats. Values are means ± SE. *P < 0.05 vs. diabetic rats.
entary rats, nondiabetic exercised rats, and diabetic exercised rats. Thus dilatation of the basilar artery in exercised diabetic rats appears to be related to the synthesis/release of nitric oxide. Third, complementary to our functional studies, we found that eNOS protein of the basilar artery is increased by exercise in nondiabetic and diabetic rats. On the basis of these findings, we suggest that exercise has beneficial effects on cerebral blood vessels, vessels not normally viewed as belonging to exercising tissue, and that exercise may have potential therapeutic value for the treatment of diabetes-induced dysfunction of cerebral vessels.

**Responses to acetylcholine and nitroglycerin.** Several investigators, using in vitro methodologies, have shown that acetylcholine produces marked relaxation of the basilar artery in rats (18, 41), rabbits (7, 32), cats (48), and humans (14, 49). In addition, we (23, 25) and others (4, 5) have examined in vivo dilatation of the rat basilar artery in response to topical application of acetylcholine. The results of these previous studies also showed that acetylcholine-induced dilatation of the basilar artery could be inhibited by L-NMMA, an enzymatic inhibitor of NOS (4, 5, 23). In contrast, vasodilatation in response to nitrovasodilators was not altered by L-NMMA (4, 5, 22). The results of the present study extend those of previous studies by supporting a role for nitric oxide in dilatation of the basilar artery in sedentary, as well as exercised nondiabetic and diabetic rats.

*eNOS protein.* Although we did not find a difference in NOS-dependent reactivity of the basilar artery between sedentary and exercised nondiabetic rats, we did find a significant increase in eNOS protein in the basilar artery of exercised nondiabetic rats compared with sedentary nondiabetic rats. Because we did not measure eNOS activity in our studies, it is difficult for us to determine whether the increase in eNOS protein represents an increase in total eNOS activity of the basilar artery in exercised nondiabetic rats. In addition, this apparent discrepancy between NOS-dependent reactivity of the basilar artery and eNOS protein in nondiabetic rats may suggest that levels of eNOS observed in sedentary nondiabetic rats are sufficient to produce a maximum increase in NOS-dependent vasoreactivity, and thus an increase in eNOS protein and/or activity may not be expected to increase agonist-induced changes in vascular diameter during physiological states. This finding is similar to the observation that exogenous application of L-arginine, an important cofactor for NOS, did not influence NOS-dependent reactivity of the basilar artery (4, 27). Thus levels of L-arginine, and presumably eNOS, during physiological conditions may not limiting.

We also found that eNOS protein was significantly elevated in sedentary diabetic compared with sedentary nondiabetic rats. This finding, which is similar to that reported in previous studies (12, 46), appears to suggest a compensatory response to altered vascular reactivity in diabetic animals and/or an impor-
tant role for eNOS and superoxide anion produced via activation of eNOS in impaired responses of the basilar artery during diabetes.

Finally, we found that eNOS protein was significantly elevated in basilar arteries from exercised diabetic compared with sedentary diabetic rats. On the basis of these findings, we suggest that the effects of exercise on NOS-dependent reactivity of the basilar artery during diabetes may be related to an increase in the synthesis and release of nitric oxide in response to acetylcholine. Again, however, we did not measure eNOS activity in our studies, and it is possible that increases in eNOS protein may not coincide with an increase in eNOS activity and an increase in the synthesis and release of nitric oxide. In addition, it is conceivable that exercise training has significant effects on other cellular processes, including those that may contribute to oxidative stress during diabetes. Because impaired responses of cerebral blood vessels during diabetes appear to be related to oxidative stress (26, 28), alterations in these pathways by exercise could presumably influence nitric oxide bioavailability and thus cerebrovascular reactivity. Studies have suggested that exercise increases antioxidant capacity in the peripheral circulation (20, 47). However, the effects of exercise on oxidant and/or antioxidant pathways have not been investigated in the cerebral circulation and additional studies will be required to determine the precise role of these pathways in diabetes-induced cerebrovascular dysfunction and the influence of exercise on these cellular pathways.

Effects of exercise on vasoreactivity during physiologic states. Several investigators have examined the effects of exercise on NOS-dependent reactivity in animals and in human subjects (8, 16, 17, 19, 42, 43). Sun et al. (42) found that short-term daily exercise for 2–4 wk enhanced NOS-dependent, but not -independent, dilatation of skeletal muscle arterioles. In addition, Kvernmo et al. (17) report that NOS-dependent dilatation of human skin vasculature is enhanced after physical conditioning. Furthermore, Koller et al. (16) report that exercise training augments flow-dependent dilatation of rat skeletal muscle arterioles. Finally, studies by Laughlin and colleagues (8, 19) report that endothelial-mediated relaxation of coronary arteries is improved by exercise training. Mechanisms by which exercise potentiates NOS-dependent relaxation of blood vessels are varied and have been reported to be related to an increase in shear across endothelium to increase eNOS activity and thus nitric oxide release (35, 42), an increase in the release of prostanoids (16), an increase in the activity of superoxide dismutase (20, 37) or other antioxidant enzymes (glutathione peroxidase and catalase) (34), and/or mechanisms unrelated to nitric oxide synthase, prostaglandins, and antioxidant enzymes (19, 50).

However, not all studies are in agreement regarding the effects of exercise on NOS-dependent reactivity of blood vessels during physiological states. Franke et al. (6) found that, whereas exercise enhanced NOS-dependent increases in forearm vascular conductance in human subjects, exercise did not...

Fig. 4. Responses of the basilar artery to acetylcholine and nitroglycerin in exercised nondiabetic (open bars) and diabetic (light gray bars) rats before application of l-NMMA (10 μM) and in exercised nondiabetic (dark gray bars) and diabetic (dark gray bars) rats after application of l-NMMA (10 μM). A: nondiabetic exercised rats. B: diabetic exercised rats. Values are means ± SE. *P < 0.05 vs. response before l-NMMA.
appear to alter vascular responses to NOS-dependent agonists. In addition, Oltman et al. (33) found that exercise training did not influence NOS-dependent responses of porcine coronary arteries. Furthermore, Rogers et al. (36) found that exercise training produced a decrease in responsiveness of isolated canine coronary arteries to $\beta$-adrenergic agonists and to vaso-active intestinal polypeptide. In the present study, we did not find a difference in the reactivity of the basilar artery to acetylcholine between nondiabetic sedentary and nondiabetic exercised rats. The discrepancy between studies that have reported an increased NOS-dependent vasoreactivity and those that do not observe a change in NOS-dependent reactivity during physiological states is difficult to determine, but it may be related to the exercise regimen, the vascular bed examined, and/or species differences regarding the effects of exercise on vascular reactivity. In addition, it is possible that exercise training produces an increase in the overall level of stress in the animals. In the present study, we did not measure any index of potential stress produced by exercise training, and thus we cannot rule out an effect of stress on vascular function during exercise training.

Effects of exercise on vasoreactivity during pathophysiological states. In addition to examining the effects of exercise during physiological states in animals and humans, investigators have examined the potential therapeutic effects of exercise during pathophysiological states. Although no studies to our knowledge have examined the effects of exercise on responses of cerebral arteries during Type 1 diabetes mellitus, other have examined the effects of exercise on responses of peripheral blood vessels during chronic hypertension (1, 11), heart failure (9, 15, 47), hyperlipidemia (50), and Type 2 diabetes mellitus (30, 38). Arvola et al. (1) found that daily exercise enhanced relaxation to nitroprusside in the mesenteric and carotid arteries and to acetylcholine in the carotid, but not mesenteric, arteries of normal rats and that it enhanced NOS-dependent relaxation of mesenteric and carotid arteries in hypertensive obese rats compared with normal rats. The mechanism for the effects of exercise on improving relaxation of arteries from hypertensive obese rats appeared to be related to an increase in the synthesis and release of nitric oxide because inhibition of NOS with l-NAME abolished this improvement. It is also important to note that exercise enhanced responses of carotid and mesenteric arteries. Thus the effects of exercise during pathophysiological conditions do not appear to be limited to blood vessels from skeletal muscle. Higashi et al. (11) report that regular exercise in humans with chronic hypertension augmented NOS-dependent reactivity that appeared to be related to an increase in the release of nitric oxide. Finally, two studies have shown that exercise training improved NOS-dependent relaxation of the aorta in Type 2 diabetic rats (38) and forearm blood flow in Type 2 diabetic humans (30).

However, the precise mechanism by which exercise improved impaired NOS-dependent relaxation remains uncertain. In the present study, we found that exercise significantly alleviated impaired NOS-dependent dilatation of the basilar artery in diabetic rats. Thus the effects of exercise are not limited to large peripheral blood vessels and/or blood vessels contained within skeletal muscle but also involve the cerebral circulation. In addition, this effect of exercise occurred even though body weight of the diabetic rats remained significantly lower than in nondiabetic rats. Although we cannot rule out an effect of body weight on impaired responses of the basilar artery in diabetic rats, on the basis of the results of the present study, it does not appear that a lack of weight gain in the diabetic rats significantly contributes to impaired NOS-dependent vasoreactivity.
Further studies are needed to precisely determine the role of body weight on impaired responses of cerebral blood vessels in diabetic rats.

In summary, this is the first study that we are aware of to examine the effects of exercise on NOS-dependent reactivity of cerebral blood vessels. We found that exercise alleviated impaired NOS-dependent dilatation of the basilar artery in diabetic rats, but that it did not alter NOS-independent responses of the basilar artery. In addition, we found that the synthesis and release of nitric oxide accounts for dilatation of the basilar artery to acetylcholine in sedentary and exercised nondiabetic and diabetic rats. Furthermore, we found that exercise significantly increased eNOS protein in the basilar artery from nondiabetic and diabetic rats. Taken together, these findings suggest that exercise-induced activation of the nitric oxide biosynthetic pathway, and not activation of other compensatory vasodilator pathways, accounts for dilatation of the basilar artery in nondiabetic and diabetic rats. Thus we speculate that exercise may have potential therapeutic value for the treatment of diabetes-induced dysfunction of cerebral vessels and that it may be important in the prevention of diabetes-induced cerebrovascular abnormalities, possibly including stroke.

GRANTS

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