A single bout of moderate-intensity exercise increases vascular NO bioavailability and attenuates adrenergic receptor-dependent and -independent vasoconstrictor response in rat aorta

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Abstract

The present study investigated the effect of one bout of moderate-intensity exercise on the adrenergic receptor-dependent and -independent vasoconstrictor response in rat aortas, and the role of nitric oxide (NO) bioavailability on these vasomotor responses. One group of rats was submitted to a 60 min of exercise at approximately 60% of maximal exercise capacity on a treadmill (exercise group) and the other one was placed in the treadmill without running (control group). Immediately after this period, both groups were euthanized and the thoracic aorta was removed to evaluate the vasoconstrictor response to norepinephrine and potassium chloride, and to evaluate the vascular nitrite and nitrate concentration. One bout of exercise attenuated the maximal contractile response to both norepinephrine and potassium chloride compared to control group. These differences on vascular reactivity were not observed in endothelium-denuded aortic rings and aortic rings pre-incubated with a nitric oxide synthesis inhibitor. Additionally, exercise group increased NO bioavailability (nitrite and nitrate concentration) as compared to control group. These results demonstrate that one bout of moderate-intensity exercise is able to attenuate adrenergic receptor-dependent and -independent vasoconstrictor response in rat aorta, mainly by increasing vascular NO bioavailability.

Key words: exercise, nitric oxide, norepinephrine, potassium chloride, vasoconstrictor response

Introduction

Several studies (Stepp and Frisbee, 2002; Ajay and Mustafa, 2006; Čačányiová et al., 2006; Koida et al., 2006; Lesniewske et al., 2008) have demonstrated that the arterial vasoconstrictor
response to different adrenergic agonists is increased in the majority of circulation system pathologies and cardiovascular risk factors, where this disturbance on vasomotor response might be related to a decreased nitric oxide (NO) bioavailability (Ajay and Mustafa, 2006; Lesniewske et al., 2008), an important chemical compound synthesized by vascular endothelium, or to an exacerbated sympathetic activity (Alves et al., 2007). Furthermore, other studies (Chen et al., 1994; Delp and Laughlin, 1997; Clarkson et al., 1999; Minami et al., 2002; Kobayashi et al., 2003; Meyer et al., 2006) have revealed that aerobic exercise training is very efficient for prevention and for non-pharmacological treatment of these cardiovascular diseases, improving endothelial function and attenuating adrenergic vasoconstrictor response in both healthy individuals and individuals who have this vasomotor response altered.

This effect of exercise training observed on arterial vasomotion is very consistent in the scientific community. However, the underlying mechanisms in this adaptive response are not completely understood. Some studies suggest that the main factor involved in this improvement of vasomotor function is the increased vascular shear stress resulting from increased blood flow observed during the bouts of aerobic exercise (Cheng et al., 2003), which leads to this chronic beneficial effect of exercise training on arterial vasomotion (Laughlin, 1995; Delp and O'Leary, 2004). Thus, it is extremely important to understand the acute effect of aerobic exercise on the arterial vasomotor response and the main mechanisms involved in the modulation of this response.

However, in spite of this related relevance, the acute effects of exercise on the vascular system are not consistent, mainly on arterial vasoconstrictor response. Some reports have shown that there is an attenuation of adrenergic receptor-dependent vasoconstriction after a single bout of exercise (Howard et al., 1992; Izawa et al., 1996; Ruble et al., 2002), while other studies have not found this attenuation response (Delorey et al., 2007), or have observed it only after some weeks of exercise training (Oltman et al., 1992; Spier et al., 1999). With regard to the acute effect of exercise on adrenergic receptor-independent vasoconstrictor response, only a small number of studies were conducted, where only a tendency for an attenuation of vasoconstrictor response to potassium chloride (KCl) has been observed (Howard et al., 1992; Spier et al., 1999).

Taking into consideration that aerobic exercise is characterized by increasing shear stress (Cheng et al., 2003) and circulating catecholamine levels (Wang and Cheng, 1999), and that both of them are stimulus able to increase endothelial NO synthase (eNOS) activity and expression, which is the principal enzyme responsible for NO production by vascular endothelium (Corson et al., 1996; Gürdal et al., 2005; Harrison et al., 2006), we hypothesized that a single bout of moderate-intensity exercise would increase NO bioavailability, which would modulate negatively the response to both adrenergic receptor-dependent and independent vasoconstrictor response. As a result, in the present study we found that a single bout of moderate-intensity exercise is able to attenuate both adrenergic receptor-dependent and independent vasoconstrictor response in rat aorta, mainly by increasing vascular NO bioavailability.
Methods

Animals and exercise protocol

This study was approved by the Ethics Committee in Research of the School of Physical Education and Sport of the University of São Paulo, Brazil. Twenty-eight male Wistar rats were housed in the animal care facility in the Faculty of Medicine of the University of São Paulo in a room maintained at 22–23°C with 12:12 h light-dark cycle, and were randomly assigned to either the control (n=14) or exercise (n=14) group after one week of familiarization period (treadmill, 10 min/day, 5–10 m/min). Rats from both control and exercise groups were individually submitted to a progressive exercise test (Brooks and White, 1978) to determine their maximum exercise capacity. The maximum exercise test was performed on a treadmill using an incremental speed protocol (5 m/min every 5 min) until exhaustion. At least forty-eight hours after exercise test, the rats of the exercise group were submitted to a treadmill exercise bout (60 min at approximately 60% of individual maximum running speed). The rats of the control group were placed on the treadmill without running during exercise bout.

Preparation of vessel segments

Immediately after exercising, the rats were euthanized and the thoracic aorta was excised, cleaned of connective and/or adipose tissue and cut into rings (4 mm long). Two rings were promptly applied to evaluate in vitro vasomotor responses and the remaining rings were frozen for posterior measurement of nitrite and nitrate concentrations. Every vasomotor protocol was simultaneously performed on exercising and resting rats.

The aortic rings utilized to determine vascular responsiveness were carefully submerged in bath organ chambers containing oxygenated (95% O₂ and 5% CO₂) Krebs solution which had the following composition (mM): NaCl 115, KCL 4.7, MgSO₄ 1.2, KH₂PO₄ 1.5, NaHCO₃ 25, CaCl₂ 2.5, glucose 11.1 mM, (37°C, pH 7.4). Rings were mounted on a force transducer (BIOPAC, U.S.A.), using an initial passive tension of 2.0 g, corresponding to the previously determined maximal contractile response evoked by norepinephrine, and were equilibrated for a 60-min period.

Vascular reactivity studies

To investigate the acute effect of exercise on adrenergic receptor-dependent and -independent vasoconstrictor response, cumulative concentration-response curves to norepinephrine (NE: 10⁻¹⁰ to 10⁻⁴ M) and potassium chloride (KCl: 10 to 100 mM) were respectively performed, and compared between control and exercise groups. In order to verify the potential role of endothelium and NO on these vasoconstrictor responses, both NE and KCl concentration-response curves were constructed for endothelium-denuded rings and for rings pre-incubated with N-nitro-L-arginine methyl ester (L-NAME, 10⁻⁴ M) for 30 min, respectively.

Data are expressed as g/mg dry tissue mass to avoid errors related to morphological differences among rings. The agonist concentration that evokes 50% of the maximal response (EC₅₀) and the maximal effect (E_max) were calculated.
Vascular nitrite and nitrate concentrations measurement

In order to evaluate the NO bioavailability, the vascular nitrite and nitrate concentrations were measured. Vascular homogenates were prepared under liquid N$_2$ and centrifuged at 5,000 rpm for 5 min, and 10-μL aliquots of the supernatant were injected into Sievers chemiluminescence analyzer (model 280; Sievers Instruments, Inc., Boulder, CO, USA) with VCl$_3$ and HCl (at 95°C) as reductants, as previously described (Leite et al., 2003). NO results were normalized for protein concentration.

Data analysis

Results are presented as means ± SEM. The maximum effect ($E_{\text{max}}$ values) and the potency, concentration that evokes 50% of the maximal response ($EC_{50}$ values), were estimated by an iterative nonlinear regression analysis of each individual concentration-response curve using the GraphPad Prism Software (San Diego, CA, U.S.A.). The data were analyzed statistically by unpaired Student's $t$-test or two-way analyses of variance (ANOVA) followed by Bonferroni's post hoc test. $P<0.05$ was considered significant.

Results

Body mass and maximum exercise capacity

Body mass at initial time was similar in control (348.0 ± 10.1 g) and exercise (350.5 ± 11.6 g) groups. No changes were found in maximum exercise capacity between control group (26.8 ± 1.0 m/min) and exercise group (27.1 ± 1.1 m/min).

Vasoconstrictor responses

Norepinephrine (10$^{-10}$ to 10$^{-4}$ M) produced a concentration-dependent contraction response in isolated aortic rings. The maximal effect for norepinephrine was significantly reduced in intact aortic rings from exercise group as compared to control group (Fig. 1A and Table 1). However, no changes were observed in the $EC_{50}$ values.

Neither the maximal responses nor the potency values were different between control and exercise groups in endothelium-denuded rings (Fig. 1B and Table 1) and in rings pre-incubated with L-NAME (Fig. 1C and Table 1).

Vasoconstriction to potassium chloride (10 to 100 mM) also produced a concentration-dependent response in isolated aortic rings. Similar to norepinephrine, the $E_{\text{max}}$ values for potassium chloride were significantly reduced in intact aortic rings from exercise group as compared to control group (Fig. 2A and Table 1), and no changes were observed in the potency values.

In endothelium-denuded rings (Fig. 2B and Table 1) and in rings pre-incubated with L-NAME (Fig. 2C and Table 1), the maximal responses and the $EC_{50}$ values were similar between control and exercise groups.

Vascular nitrite and nitrate concentrations

The vascular nitrite and nitrate concentrations were markedly increased in rats that were
submitted to a moderate exercise bout \((0.17 \pm 0.04 \text{ and } 16.8 \pm 4.4 \text{ nmol/mg protein, respectively})\) as compared to control group \((0.06 \pm 0.02 \text{ and } 6.5 \pm 1.2 \text{ nmol/mg protein, respectively})\). These data, illustrated in Fig. 3, show that one bout of aerobic exercise is able to increase vascular NO bioavailability.

**Discussion**

The present study is the first to evaluate the acute effect of moderate-intensity exercise on
Table 1. Maximum effect ($E_{\text{max}}$) and potency (EC$_{50}$) values obtained from concentration-response curves to norepinephrine (NE) and potassium chloride (KCl) performed in endothelium-intact aortic rings (+ end), endothelium-denuded aortic rings (− end) and aortic rings pre-incubated with L-NAME (+ L-NAME) from control and exercise groups

<table>
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<tr>
<th></th>
<th>Control</th>
<th>Exercise</th>
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<tbody>
<tr>
<td><strong>Norepinephrine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ end</td>
<td>$E_{\text{max}}$</td>
<td>2.99 ± 0.05</td>
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<tr>
<td></td>
<td>EC$_{50}$</td>
<td>6.78 ± 0.04</td>
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<tr>
<td>− end</td>
<td>$E_{\text{max}}$</td>
<td>3.21 ± 0.06</td>
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<td></td>
<td>EC$_{50}$</td>
<td>7.69 ± 0.07</td>
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<tr>
<td>+ L-NAME</td>
<td>$E_{\text{max}}$</td>
<td>3.19 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>EC$_{50}$</td>
<td>7.33 ± 0.11</td>
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<tr>
<td><strong>Potassium chloride</strong></td>
<td></td>
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<tr>
<td>+ end</td>
<td>$E_{\text{max}}$</td>
<td>2.12 ± 0.06</td>
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<tr>
<td></td>
<td>EC$_{50}$</td>
<td>26.70 ± 1.16</td>
</tr>
<tr>
<td>− end</td>
<td>$E_{\text{max}}$</td>
<td>2.31 ± 0.07</td>
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<tr>
<td></td>
<td>EC$_{50}$</td>
<td>23.97 ± 1.47</td>
</tr>
<tr>
<td>+ L-NAME</td>
<td>$E_{\text{max}}$</td>
<td>2.47 ± 0.09</td>
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<tr>
<td></td>
<td>EC$_{50}$</td>
<td>24.41 ± 1.89</td>
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$E_{\text{max}}$ is expressed as g/mg dry weight. EC$_{50}$ values to NE are represented as negative logarithm of the molar concentration to produce 50% of the maximal response. EC$_{50}$ values to KCl are represented as mM concentration to produce 50% of the maximal response. Data are presented as mean ± SEM. *, $P<0.05$ compared to control group.

vasoconstrictor response to norepinephrine and potassium chloride, in a parallel manner, focusing on the participation of the powerful endothelium-dependent vasodilator (NO) in these responses. Our findings show that one bout of moderate-intensity exercise is able to attenuate vasoconstrictor response to NE and KCl in rat aorta, and also to increase vascular nitrite and nitrate concentrations.

While some studies have already shown that there is an improvement on endothelium-dependent vasodilator response after a bout of exercise (Cheng et al., 1999; Jen et al., 2002; Goto et al., 2007), there is still a controversy about the acute effect of exercise on vasoconstrictor response to norepinephrine. In the present study, we observed an important decrease on adrenergic vasoconstriction after a bout of moderate-intensity exercise. This finding is consistent with results from previous studies (Howard et al., 1992; Izawa et al., 1996; Ruble et al., 2002), but disagrees with other studies that did not find changes in this response (DeLorey et al., 2007; Spier et al., 1999). The reason for these discrepancies could be related to the differences among the intensity of exercise, the animal specimens, and the blood vessels in the arterial tree.

However, in spite of the disagreement among findings with regard to the acute effect of exercise on vasoconstriction response to norepinephrine, it seems to be a consensus that one of the chronic beneficial effects of aerobic exercise is the attenuation of adrenergic vasoconstriction (Oltman et al., 1992; Chen et al., 1994; Spier et al., 1999; McAllister et al., 2005), which presents
Fig. 2. Concentration-response curves to potassium chloride (KCl) in endothelium-intact aortic rings (A), endothelium-denuded aortic rings (B) and aortic rings pre-incubated with L-NAME (C) from control (○) and exercise (●) groups. Data are presented as mean ± SEM. *, P<0.05 compared to control group. The number of animals in each group is showed between parentheses.

important clinical relevance, since this response is increased in the majority of circulation system pathologies and cardiovascular risk factors (Stepp and Frisbee, 2002; Ajay and Mustafa, 2006; Čačányiová et al., 2006; Koida et al., 2006; Lesniewske et al., 2008).

Another important finding of this study was that vasoconstrictor response to KCl was also attenuated in exercised rats as compared to control group. This result shows that the acute attenuating effect of exercise on the arterial vasoconstriction is not completely adrenergic receptor-dependent. To our knowledge, this is the first study that has demonstrated that vasoconstrictor response to potassium chloride is decreased after a bout of moderate-intensity exercise. In a previous report, in parallel with the attenuated vasoconstrictor response to
phenylephrine observed in exercised rabbits, Howard et al. (1992) found only a tendency for an attenuation of vasoconstrictor response to KCl, which is similar to the response reported by Spier et al. (1999). The intensity of exercise performed in these studies (until exhaustion) might be the main reason for this discrepancy. In the present study, the rats were submitted to a moderate-intensity exercise protocol (60 min at approximately 60% of individual maximum running speed), which is within the suggested aerobic exercise training zone used in fitness and cardiac rehabilitation programs in both humans (Fletcher et al., 1996) and experimental animals (Ramires and Ji, 2001).

With regard to the participation of vascular endothelium in the attenuated vasoconstrictor response after exercise, we observed that this vessel layer is involved in this response, since the remotion of the endothelium abolished the difference in vascular reactivity, to both NE and KCl, between the groups. Although we did not evaluate the time-course of the endothelial participation on the attenuation of vasoconstrictor response after a bout of exercise, Haram et al. (2006) have already demonstrated that a single exercise session improves endothelial function for about 48 h in sedentary rats.

Another important finding of this study was that the attenuation of vasoconstrictor response after exercise to both NE and KCl was not observed on the concentration-response curves constructed in aortic rings pre-incubated with a nitric oxide synthesis inhibitor (L-NAME). This finding demonstrates that NO is directly involved in this acute effect of exercise on vasmotor control.

Other studies have also reported an important role of nitric oxide in promoting beneficial changes in the modulation of the vasmotor tonus after a bout of exercise (Endo et al., 1994; Gilligan et al., 1994), where this response might be related to an increased NO bioavailability, which has already been observed in exercised humans by the measurement of plasma nitrogen oxide content (Allen et al., 2006). However, this is the first study that demonstrates an increased concentration of the end products of nitric oxide metabolism (nitrite and nitrate) in aorta homogenate after a bout of moderate-intensity exercise, which permits to establish a better relationship between this vasodilator compound and the changes on vasmotor response.
This increased NO bioavailability observed seems to be an important clinical relevance, since NO is a key mediator of artery vasomotor function, and it also has multiple anti-atherogenic properties, which include inhibition of monocyte, leukocyte, and platelet adhesion to the vessel wall, inhibition of platelet aggregation, antioxidant properties, and inhibition of smooth muscle proliferation (Vane et al., 1990).

One of the main reasons that could explain this increased vascular NO bioavailability might be an enhanced eNOS activity after exercise, since exercise is characterized by increasing both shear stress (Cheng et al., 2003) and circulating catecholamine levels (Wang and Cheng, 1999). It has already been observed that the acute exposure of endothelial cells and rat aorta to both increased shear stress (Corson et al., 1996; Harrison et al., 2006) and high levels of adrenergic agonists (Gürdal et al., 2005) is able to enhance the activity and expression of this enzyme. Furthermore, exercise is also able to increase the endothelial calcium influx and the calcium-dependent nitric oxide release (Jen et al., 2002).

Although we did not evaluate directly the eNOS activity in our experimental model, we suggest that it might be increased after the bout of exercise performed, since the pre-incubation with L-NAME shows a higher increase in the developed tension by the exercise group compared with control group on the concentration-response curves to NE (31% vs. 7%, respectively) and KCl (29% vs. 16%, respectively).

Other factors that might be involved in the increased vascular NO bioavailability observed in the present study are mechanisms related to redox signaling, since superoxide anion, which is also produced by blood vessels, is one of the main factors responsible for nitric oxide inactivation (Dickhout et al., 2005). Although we do not know if the synthesis of NO is increased or the inactivation of this compound is decreased, our results show that the vascular NO bioavailability is enhanced in the aorta of exercised rats.

In conclusion, the present study shows that a single bout of moderate-intensity exercise attenuates both adrenergic receptor-dependent and -independent vasoconstrictor response in rat aorta, mainly by increasing vascular NO bioavailability.

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