Phenylephrine-induced vasoconstriction of the rat superior mesenteric artery is decreased after repeated swimming

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Abstract

This study was performed to determine the effect of forced swimming on the vascular responsiveness of the rat superior mesenteric artery to phenylephrine, focusing on the involvement of locally produced substances. Repeated but not single sessions of forced swimming exercise reduced the vasoconstrictor potency of phenylephrine in the studied arteries, regardless of the presence of intact endothelium. No significant changes were observed in the maximal response to phenylephrine. Treatment with indomethacin (1 μ M) did not affect the exercise-induced reduction in vascular responsiveness to phenylephrine. However, the reduction of vascular reactivity to phenylephrine due to repeated exercise was no longer observed after treatment with N^G-monomethyl-L-arginine (L-NMMA, 100 μ M). The results suggest that repeated exercise reduces vasomotor responses to phenylephrine in rat superior mesenteric arteries through a non-endothelial nitric oxide (NO)-related mechanism.

Key words: endothelium, mesenteric artery, nitric oxide, phenylephrine, prostanoids

Introduction

It has been reported that norepinephrine or phenylephrine-induced vasoconstrictions result from α_1 -adrenoceptor stimulation, which is modulated by locally produced vasoconstrictor and vasodilator substances such as endothelium-derived nitric oxide (NO), prostanoids as well as endothelium-derived hyperpolarizing mechanisms (Asbun-Bojalil *et al.*, 2002; Riksen *et al.*, 2003; Srivastava *et al.*, 1998; Vinet *et al.*, 1991). This local modulation of sympathetic agonist-induced vasoconstriction may be influenced by physical exercise. After exercise training, reductions in the vasomotor activity of norepinephrine have been reported to occur in several arteries, such as

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rabbit aorta (Chen *et al.*, 1994), pig coronary artery (Parker *et al.*, 1994), rat abdominal aorta (Delp *et al.*, 1993; Spier *et al.*, 1999), and rat thoracic aorta (Izawa *et al.*, 1996). Repeated swimming exercise also reduces contractile responses to phenylephrine in the isolated rat aorta (Jansakul, 1995). However, no significant reduction in norepinephrine vasomotor activity in the rat abdominal aorta following running exercise training also has been shown (Edwards *et al.*, 1985). These discrepancies may result from differences among the vascular territories used in these studies. In fact, it was demonstrated that the phenylephrine-induced vasoconstriction of rat abdominal and thoracic aorta might be differently influenced by local modulator mechanisms (Asbun-Bojalil *et al.*, 2002).

Finally, important changes in blood flow distribution may occur during physical exercise (Laughlin and Armstrong, 1982; Laughlin and Armstrong, 1983). Blood flow distribution involves vascular actions of α_1 -agonists, which are modulated by locally produced mediators. Although several studies have provided information about these local mechanisms, a definitive understanding of the latter remains elusive. Thus, the aim of the present study was to investigate the effects of forced swimming on the phenylephrine-induced vasoconstriction of rat superior mesenteric arteries, focusing on local regulatory mechanisms.

Methods

Animals

Animals were carried for and treated according to the Guide for the Care and Use of Laboratory Animals, National Academy of Sciences (1996). Male Wistar rats (300–330 g) were used in the experiments. Rats were housed in plastic cages (50 × 40 × 20 cm), 5 animals per cage in an animal room under a 12 h light-dark cycle beginning at 7:00 h, at room temperature (25°C). The animals received food and water *ad libitum*.

Drugs

The following drugs were used: 1-[p-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid (indomethacin), N^G-monomethyl-L-arginine (L-NMMA) and L-phenylephrine hydrochloride (purchased from Sigma Chemical Co., USA) and 2,2,2-tribromoethanol (purchased from Acros Organics, USA).

Swimming training protocol

The swimming protocol used was previously described by Chies *et al.* (2003). Rats were individually submitted to forced swimming in a cylindrical polyethylene tank containing water at room temperature (24–27°C) for 30 min, carrying a metal ring corresponding to about 2% of their body weight on their tails. The cylindrical polyethylene tank was 45 cm in diameter and 40 cm deep. The training consisted of submitting the animals to the same procedure of daily forced swimming sessions for 5 weeks.

Organ bath studies

Immediately after the single swimming session or the last swimming session, the animals

were anesthetized with tribromoethanol (250 mg/kg, *i.p.*) and exsanguinated. Next, segments of superior mesenteric arteries were carefully removed, cleared of all fat and connective tissues and cut into 3–4 mm long rings. Each ring was suspended in a 20 ml organ bath using two stainless steel stirrup hooks placed vertically and passed through its lumen. The lower hook was fixed and the upper one connected to an isometric force recording transducer. Contractions were recorded using a digital acquisition system (Letica, Spain). Each organ bath contained Krebs solution of the following composition (mM): NaCl 130; KCl 4.7; CaCl₂ 2.5; KH₂PO₄ 1.2; MgSO₄ 1.2; EDTA 0.03; NaHCO₃ 15 and glucose 5.5. The nutritive solution, pH 7.4, was kept at 37°C and continuously bubbled with a 95% CO₂ and 5% O₂ mixture. Prior to the addition of drugs, rings were equilibrated for 60 min under a resting tension of 7.5 mN. After the experiments, the rings were dehydrated and weighed.

To remove the endothelium, we modified the chemical method proposed by Lee and Man (2003). These authors perfused porcine coronary arteries for 30 s with 0.5% Triton X100 at the rate of 30 ml/min. In the present study, rings were incubated with 0.5% Triton X100 solution for 10 s. The effectiveness of this procedure was confirmed by the lack of dilator responses to acetylcholine 3×10^{-5} M in each artery ring pre-contracted with phenylephrine 3×10^{-6} M. Preparations that after incubation presented any reduction in the response to phenylephrine 3×10^{-6} M in comparison to their responses before Triton X100 exposition, were discarded. These preparations were used to determine the endothelial involvement in the vascular responsiveness to phenylephrine during exercise.

Cumulative concentration-response curves were generated by adding phenylephrine (10^{-10} M to 10^{-4} M) or KCl (10^{-3} M to 1.2×10^{-2} M) to each ring. Data concerning phenylephrine- and KCl-induced responses were expressed as g/mg dry tissue mass. From these concentration-responses curves we determined the negative logarithm of the concentration that evoked 50% of the maximal response (pD₂), indicative of potency. We also analyzed the maximal response evoked by phenylephrine (Rmax). The involvement of cyclooxygenase (COX) and nitric oxide synthase (NOS) products in the contractile effects of phenylephrine was evaluated by comparing the effects of phenylephrine on rings pre-incubated with indomethacin (10^{-6} M) or L-NMMA (10^{-4} M) for 20 min with those observed in untreated rings.

Statistical analysis

Data are reported as mean $\pm$ standard error of the mean (SEM). pD₂ and Rmax values were first compared by multiple analysis of variance (MANOVA). When differences were found between treatments, one-way ANOVA was applied, followed by the Student Newman-Keuls post-test. *P* values less than 0.05 were considered to be statistically significant.

Results

The concentration-response curves to phenylephrine obtained in rat superior mesenteric arteries were significantly shifted to the right, with reduction in pD₂ but not in Rmax, after repeated but not after a single session of forced swimming (Fig. 1A; Table 1). The effects of repeated swimming on the concentration-response curves to phenylephrine were also observed

Fig. 1. Concentration-response curves for phenylephrine determined in rat superior mesenteric arteries, with (A) or without endothelium (B), obtained from control animals (CT) or animals submitted to single (SS) or repeated swimming (RS) sessions. Points represent the mean $\pm$ SEM of 7–10 independent determinations.

Table 1 Values of pD_2 and R_{max} in response to phenylephrine determined in superior mesenteric arteries with endothelium obtained from controls rats or rats submitted to either single or repeated swimming sessions

	pD_2			R_{max}		
	control	single stress	repeated stress	control	single stress	repeated stress
Saline	8.10 ± 0.28 (8)	8.57 ± 0.16 (8)	$7.03 \pm 0.18^*$ (8)	1.12 ± 0.15 (8)	1.47 ± 0.15 (8)	0.95 ± 0.12 (8)
Indomethacin 10^{-6} M	$6.86 \pm 0.30^\dagger$ (8)	$6.91 \pm 0.34^\dagger$ (8)	7.01 ± 0.17 (8)	1.04 ± 0.12 (8)	0.91 ± 0.17 (8)	1.12 ± 0.17 (8)
L-NMMA 10^{-4} M	7.86 ± 0.06 (7)	7.80 ± 0.34 (8)	$7.86 \pm 0.14^\dagger$ (8)	1.66 ± 0.22 (7)	1.34 ± 0.13 (8)	$1.96 \pm 0.19^\dagger$ (8)

Values are expressed as mean $\pm$ SEM. Number of experiments is indicated within parenthesis. *, $P < 0.05$, compared to animals not submitted to swimming (control) and † , $P < 0.05$, compared to saline-treated control (multiple analysis of variance followed by analysis of variance and the Newman-Keuls post-test).

in preparations without endothelium (Fig. 1B, Table 2).

In preparations of rat superior mesenteric arteries obtained from controls and from animals submitted to a single swimming session, phenylephrine potency was reduced following treatment with indomethacin, resulting in a significant pD_2 reduction. This reduction in potency was not observed in preparations obtained from animals submitted to repeated forced swimming (Fig. 2A, Table 1). This effect of indomethacin on the vascular responsiveness to phenylephrine was also observed in arteries without endothelium (Fig. 2B, Table 2).

Single or repeated swimming sessions did not shift the concentration-response curves to phenylephrine to the right when obtained in rat superior mesenteric arteries treated with L-NMMA (Fig. 2C). Consequently, no significant changes of pD_2 and R_{max} values were observed in these preparations (Table 1). Single or repeated swimming sessions also did not shift the concentration-response curves to phenylephrine to the right nor did they changed pD_2 and R_{max} values in preparations without endothelium treated with L-NMMA (Fig. 2D, Table 2). On the other hand, in preparations obtained from animals submitted to repeated swimming it was

Fig. 2. Concentration-response curves for phenylephrine determined in rat superior mesenteric arteries, with (A, C) or without endothelium (B, D), obtained from control animals (CT) or animals submitted to repeated swimming sessions (RS), either untreated or treated with indomethacin 10^{-6} M (INDO) (A and B) or L-NMMA 10^{-4} M (L-N) (C and D). Points represent the mean $\pm$ SEM of 7–10 independent determinations.

Table 2 Values of pD_2 and R_{max} in response to phenylephrine determined in superior mesenteric arteries without endothelium obtained from controls rats or rats submitted to either single or repeated swimming sessions

	pD_2			R_{max}		
	control	single stress	repeated stress	control	single stress	repeated stress
Saline	8.43 ± 0.14 (8)	8.34 ± 0.22 (8)	$7.13 \pm 0.10^*$ (8)	1.04 ± 0.19 (8)	1.11 ± 0.23 (8)	1.13 ± 0.13 (8)
Indomethacin 10^{-6} M	$7.32 \pm 0.16^\dagger$ (8)	$7.31 \pm 0.21^\dagger$ (8)	7.11 ± 0.15 (8)	1.35 ± 0.11 (8)	1.36 ± 0.14 (8)	1.42 ± 0.17 (8)
L-NMMA 10^{-4} M	7.71 ± 0.06 (7)	7.83 ± 0.15 (9)	$7.87 \pm 0.14^\dagger$ (8)	1.75 ± 0.23 (7)	1.49 ± 0.14 (8)	$2.11 \pm 0.19^\dagger$ (8)

Values are expressed as mean $\pm$ SEM. Number of experiments is indicated within parenthesis. *, $P < 0.05$, compared to animals not submitted to swimming (control) and † , $P < 0.05$, compared to saline treated control (multiple analysis of variance followed by analysis of variance and the Newman-Keuls post-test).

observed that the treatment with L-NMMA increased the values of pD_2 in comparison with saline-treated controls (Tables 1 and 2). Moreover, it was observed that treatment with L-NMMA increased the values of R_{max} , showing statistically significant difference in preparations obtained from animals submitted to repeated swimming (Tables 1 and 2).

Discussion

The present study demonstrates that repeated swimming may change the action of phenylephrine in superior mesenteric arteries, reducing its vasoconstrictor potency. This phenomenon may be a part of a whole exercise-related adaptation in response to important changes in blood flow distribution that occurs (Laughlin and Armstrong, 1982; Laughlin and Armstrong, 1983). Possibly, the described exercise-related adaptation may attenuate the vasoconstrictor effects of catecholamines on the mesenteric territory in exercise-exposed animals. Moreover, this exercise-related adaptation seems to be gradual since no change was observed following a single swimming session.

Similar results were reported with norepinephrine in several arteries, such as rabbit aorta (Chen *et al.*, 1994), pig coronary artery (Parker *et al.*, 1994), rat abdominal aorta (Delp *et al.*, 1993; Spier *et al.*, 1999), and rat thoracic aorta (Izawa *et al.*, 1996). Repeated swimming exercise also reduces contractile activity to phenylephrine in isolated rat aorta (Jansakul, 1995).

Considering that phenylephrine stimulates α_1 -adrenoceptors, the observed exercise-induced reduction of vascular responsiveness may involve alterations of these receptors. On the other hand, previous studies have suggested that the vascular endothelium is a source of substances that modulate vascular tonus and mediate the actions of different vasoconstrictor or dilator agonists (Furchgott and Zawadzki, 1980; Gryglewski *et al.*, 1986; Palmer *et al.*, 1987; Ignarro *et al.*, 1987). Vanhoutte (1996) proposed the existence of an imbalance between vasoconstrictor and vasodilator mechanisms. Moreover, some authors suggested that endothelial NO regulates the vasomotor actions of α_1 -adrenergic agonists, promoting a physiological antagonism (Bullock *et al.*, 1986; Vinet *et al.*, 1991).

Occurrence of repeated exercise-induced reductions in vascular responsiveness to norepinephrine were described in rat aorta (Delp *et al.*, 1993; Jansakul, 1995; Spier *et al.*, 1999), rabbit aorta (Chen *et al.*, 1994) and pig coronary artery (Parker *et al.*, 1994), but were not observed in endothelium-denuded arteries, supporting the idea of an endothelial role in the exercise-induced reduction of vascular responsiveness.

The results of the present study have shown that repeated swimming-induced reduction in vascular responsiveness to phenylephrine occurs also in endothelium-denuded arteries, suggesting that non endothelial mechanisms may have a pivotal participation in the exercise-induced reduction of vascular responsiveness to phenylephrine. These results are in agreement with previous studies which described a non-endothelium-dependent decrease in rat aorta responsiveness to norepinephrine following treadmill running exercise (Izawa *et al.*, 1996).

Treatment with indomethacin, a nonspecific COX inhibitor, decreased the vasoconstrictor potency of phenylephrine on arteries obtained from control rats. This suggests that, in the presence of indomethacin, the synergistic interaction between locally produced vasoconstrictor prostanoids and phenylephrine may be blocked, resulting in a consequent reduction of the effects of phenylephrine. Moreover, the effects of indomethacin on the vascular responsiveness to phenylephrine were observed both in preparations with and without endothelium, supporting previous studies that demonstrated a synergistic participation of non-endothelial vasoconstrictor prostanoids in the contractile actions of norepinephrine in rat mesenteric arteries (Peredo, 2001;

Peredo, 2003) and aorta (Lamb *et al.*, 1994) and of phenylephrine in rat aorta (Connolly *et al.*, 1998).

In the presence of indomethacin, no repeated exercise-induced reduction in phenylephrine vasoconstrictor potency or R_{max} was observed in either intact or endothelium-denuded rat superior mesenteric arteries. In this situation, preparations obtained from control animals or animals submitted to a single swimming session presented lower vascular responsiveness, which was similar to that of the group submitted to repeated swimming. On the other hand, treatment with L-NMMA prevented the repeated swimming-induced reduction in phenylephrine vasomotor activity in either endothelium-intact or endothelium-denuded arteries. Furthermore, the treatment with L-NMMA increased the values of pD_2 obtained in both intact and endothelium-denuded arteries, as result of inactivation of the NO-related mechanism in these preparations. It suggests that the repeated swimming-induced reduction in phenylephrine potency is consequent to an enhancement in the NO production. A similar exercise-induced reduction in vascular responses to phenylephrine, which was also prevented by a NOS inhibitor, was observed in the endothelium-denuded mesenteric arterial bed (Jansakul and Hirurpan, 1999).

NO-related mechanisms have been proposed to explain exercise-induced and endothelium-mediated reduction in vascular responsiveness to sympathomimetic agonists (Chen *et al.*, 1994; Delp *et al.*, 1993; Jansakul, 1995; Parker *et al.*, 1994; Spier *et al.*, 1999). In fact, it has been described that exercise-induced blood flow changes may alter endothelial function due the shear stress enhancement (Miller *et al.*, 1986; Miller and Vanhoutte, 1988). Moreover, a shear stress-induced increase in either NOS mRNA expression (Sessa *et al.*, 1994; Uematsu *et al.*, 1995; Xiao *et al.*, 1997) or NOS protein synthesis (Delp and Laughlin, 1997) has also been reported. On the other hand, there has been growing evidence of NO production in vascular smooth muscle cells (Moncada *et al.*, 1991; Rees *et al.*, 1990; Schini and Vanhoutte, 1991). Moreover, Nichols *et al.* (1994), using NOS histochemistry and endothelial cell immunohistochemistry, demonstrated that NO could be synthesized both in endothelial and smooth muscle cells of rat and human arterioles.

In addition to shear stress, other factors may stimulate the production of NO during exercise. Forced swimming has been considered as an important emotional stress stimulus since it is able to increase plasma levels of corticosterone and catecholamines (Cox *et al.*, 1985; Scheurink *et al.*, 1989; Watanabe *et al.*, 1991). These hormonal changes tend to be reduced after chronic exposure to swimming (Cox *et al.*, 1985). We have previously demonstrated that plasma corticosterone levels were increased significantly after acute or repeated exposure to forced swimming, corroborating the stressogenic component of forced swimming (Chies *et al.*, 2003). It is possible that these hormonal changes enhanced NO release after 5 weeks of daily repeated swimming.

It has been proposed that immobilization in a cold environment, an emotional stress model, reduces the rat aorta responsiveness to norepinephrine. This phenomenon has not been observed in denuded arteries or in the presence of a NOS inhibitor (Cordellini and Vassilieff, 1998; Navarro-Oliveira *et al.*, 2000). The reduction observed in the responses of endothelium-denuded arteries contrasts with the previously presented data. Problems with the endothelium

removal could explain such differences. However, the lack of acetylcholine-induced relaxation observed in all studied endothelium-denuded arteries as well as the L-NMMA-induced enhancement of Rmax values observed in either intact or endothelium-denuded preparations contradict this hypothesis.

The remaining question is whether repeated swimming reduces the phenylephrine-induced vasomotor activity exclusively through the increase of NO or also involves reduction in vasoconstrictor prostanoid production. In fact, it has been proposed in skeletal muscle arteries (Varin *et al.*, 1999) and in pig pulmonary arteries (Johnson *et al.*, 2000) that exercise may increase NO production in parallel to a reduction of vasoconstrictor prostanoids. Moreover, it has been described in denuded rat mesenteric arteries that the locally produced NO may inhibit noradrenaline-stimulated production of vasoconstrictor prostanoids (Peredo, 2003). In this way, the present results do not discard a possible reduction of vasoconstrictor prostanoids in the repeated swimming-induced reduction of the vasomotor activity of phenylephrine. They suggest that a non endothelial NO-related mechanism play a pivotal role in such vasomotor activity reduction since no swimming effect was observed in L-NMMA treated preparations.

The vascular responses to KCl did not change following repeated swimming exercise (data not shown), suggesting that changes in calcium-activated contractile mechanisms are not involved in the decreased vascular responsiveness observed in the present study.

In conclusion, our results indicate that repeated but not single sessions of forced swimming reduce the vasomotor responses to phenylephrine in rat superior mesenteric arteries. Because this reduction was also observed in endothelium-denuded arteries and was absent in the presence of L-NMMA, a non-endothelial nitrergic mechanism may, at least in part, mediate this phenomenon.

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