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Nitric oxide synthesis blockade reduced the baroreflex sensitivity in trained rats

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ABSTRACT

Objective: The present study has investigated the effect of blockade of nitric oxide synthesis on cardiovascular autonomic adaptations induced by aerobic physical training using different approaches: 1) double blockade with methylatropine and propranolol; 2) systolic arterial pressure (SAP) and heart rate variability (HRV) by means of spectral analysis; and 3) baroreflex sensitivity.
Methods: Male Wistar rats were divided into four groups: sedentary rats (SR); sedentary rats treated with N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) for one week (SRL); rats trained for eight weeks (TR); and rats trained for eight weeks and treated with L-NAME in the last week (TRL).
Results: Hypertension and tachycardia were observed in SRL group. Previous physical training attenuated the hypertension in L-NAME-treated rats. Bradycardia was seen in TR and TRL groups, although such a condition was more prominent in the latter. All trained rats had lower intrinsic heart rates. Pharmacological evaluation of cardiac autonomic tonus showed sympathetic predominance in SRL group, differently than other groups. Spectral analysis of HRV showed smaller low frequency oscillations (LF: 0.2–0.75 Hz) in SRL group compared to other groups. Rats treated with L-NAME presented greater LF oscillations in the SAP compared to non-treated rats, but oscillations were found to be smaller in TRL group. Nitric oxide synthesis inhibition with L-NAME reduced the baroreflex sensitivity in sedentary and trained animals.
Conclusion: Our results showed that nitric oxide synthesis blockade impaired the cardiovascular autonomic adaptations induced by previous aerobic physical training in rats that might be, at least in part, ascribed to a decreased baroreflex sensitivity.

1. Introduction

Nitric oxide (NO) plays an important role in the control of vascular tonus and arterial pressure (AP) (Furchgott and Zawadzki, 1980). Endogenous reduction of NO synthesis is related to several physiopathological disorders or associated conditions, such as increase in vascular tonus, increase in platelet adhesion, decrease in endothelial-dependent vasodilatation, hypercholesterolemia, diabetes, and arteriosclerosis (Moncada and Higgs, 1991).

In fact, nitric oxide plays an important role in regulating peripheral blood circulation (Palmer et al., 1987; Klimaschewski et al., 1992; Toda et al., 1993). However, there are studies demonstrating the NO involvement in the regulation of cardiac and vascular autonomic control by means of modulation, particularly the central sites of cardiovascular autonomic neural integration (Matsuda et al., 1995; Kantzides and Badoer, 2005; Martins-Pinge et al., 2007). We have reported in a previous study that NO synthesis blockade with N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) promoted an increase in cardiac sympathetic influence as well as a decrease in baroreflex sensitivity (Souza et al., 2001).

On the other hand, aerobic training physics in either experimental animals or human beings can promote important adaptations for heart and cardiovascular autonomic control, often including reduction in mean AP. Aerobic physical exercises are also usually employed for preventive purposes and combined with therapies aimed at controlling chronic-degenerative diseases in order to attenuate the effects of cardiovascular risk factors (Arakawa, 1993; Kelley and McClellan, 1994; Tsatsoulis and Fountoulakis, 2006; Warburton et al., 2006). The mechanisms involved in cardiovascular adaptations induced by physical exercises are not fully understood. It has been suggested the NO participation, either directly by improving the endothelial function, or indirectly by influencing cardiac autonomic adaptations characterized by increased cardiac vagal tonus and decreased cardiovascular sympathetic tonus (Danson and Paterson, 2003; Kuru et al., 2002; Laterza et al., 2007).

Therefore, the objective of the present study was to investigate the effect of NO synthesis blockade on cardiovascular autonomic adaptations induced by previous physical training in conscious rats. In order to do so, we have used different approaches: 1) pharmacological

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evaluation of cardiac autonomic tonus by means of double blockade using methylatropine and propranolol; 2) evaluation of heart rate variability and systolic arterial pressure in terms of frequency using spectral analysis; and 3) evaluation of baroreflex cardiac sensitivity.

2. Methods

All experimental procedures involved in this study were approved by the ethics committee for animal experiments of the University of São Paulo, Ribeirão Preto School of Medicine.

2.1. Animals

Male Wistar rats (150–180 g) were kept in individual cages under conditions of controlled temperature (21 °C) and a dark/light cycle of 12 h. The animals were divided into four experimental groups: 1) Sedentary rats treated with feed and water *ad libitum* for 8 weeks (SR Group; $n=14$). 2) Sedentary rats treated with feed and water *ad libitum* for 8 weeks and L-NAME dissolved in drinking water (70 mg/kg) during the last week (SRL Group; $n=14$). 3) Trained rats submitted to swimming exercise for 8 weeks (TR Group; $n=14$). 4) Trained rats submitted to swimming exercise for 8 weeks and treated with L-NAME during the last week (TRL Group; $n=14$). The inhibition of NO synthesis during seven days was chosen because in previous study has shown that this model promotes important hypertension and damage in cardiovascular autonomic control (Souza et al., 2001).

2.2. Physical training

The 8-week swimming training program was conducted in a glass aquarium (100 cm long, 80 cm wide and 80 cm high) with heated water (30 °C). Before beginning the training program, the animals were submitted to a two-week adaptation program, that is, the water exercises lasted 10 min initially and then were gradually increased to 45 min. After the adaptation program, the animals were trained for 1 h a day, 6 days a week. The sedentary animals were submitted to water stress for 2 min a day during the training program.

2.3. Experimental protocol

On the sixth day of the last week, under tribromoethanol anaesthesia (250 mg/kg, i.p.), the animals were instrumented with femoral venous and arterial catheters (PE-50 fused to PE-10) filled with heparinised saline (500 IU/mL) and exteriorized through the animal's back. Twenty-four hours after the surgical procedures, AP was measured in conscious rats kept in a quiet environment. AP was recorded with a pressure transducer (ADInstruments — MLT0380) and the amplified signal (ADInstruments — ML110) was fed to a computer acquisition system (ADInstruments — PowerLab 8/30). Mean arterial pressure (MAP) and heart rate (HR) were calculated from arterial pulse pressure.

2.4. Sympatho-vagal tonus and intrinsic heart rate

Methylatropine (4 mg/kg) and propranolol (5 mg/kg) were used to block the parasympathetic and sympathetic influences on HR in SR ($n=14$), SRL ($n=14$), TR ($n=14$), and TRL ($n=14$) groups. After the basal period (30 min), half of the rats were injected with methylatropine, and the HR was recorded during the next 15 min to evaluate the parasympathetic effect on HR. Propranolol was then injected and HR was recorded for another 12 min to determine the intrinsic heart rate. The other half of the rats received the same drugs but in a reversed sequence (propranolol/methylatropine) during the same period of time (12/15 min) in order to assess the sympathetic effect on HR and also to determine the intrinsic heart rate. Data from the methylatropine/propranolol (SR, $n=07$; SRL, $n=07$; TR, $n=07$; TRL, $n=07$) and propranolol/methylatropine (SR, $n=07$; SRL, $n=07$; TR, $n=07$; TRL, $n=07$) sequences were pooled to provide basal HR (before any blockade) and intrinsic heart rate.

2.5. Power spectral analysis of heart rate and arterial pressure variability

The baseline AP and HR recorded during a 30-min period were processed by a customised computer software which applies an algorithm to detect cycle-to-cycle inflection points in the pulsatile AP signal, thus determining beat-by-beat values of systolic and diastolic pressures. Beat-by-beat pulse interval series from pulsatile AP signal were also generated by measuring the length of time between adjacent systolic waves. From the baseline 30-min recording period, the time series of pulse interval (PI) and systolic arterial pressure (SAP) were divided into contiguous segments of 300 beats, overlapped by half. After calculating mean value and variance of each segment, they were submitted to a model-based autoregressive spectral analysis as described elsewhere (Malliani et al., 1991; Rubini et al., 1993; Task Force, 1996). Briefly, a modelling of the oscillatory components presented in stationary segments of beat-by-beat time series of PI and SAP was calculated based on Levinson–Durbin recursion, with the model order chosen according to Akaike's criterion (Malliani et al., 1991). This procedure allows an automatic quantification of the centre frequency and power of each relevant oscillatory component present in the time series. The oscillatory components were labelled as very low (VLF: 0.01–0.20 Hz), low (LF: 0.20–0.75 Hz) or high frequency (HF: 0.75–2.50 Hz). The power of LF and HF components of PI variability was also expressed in normalised units, obtained by calculating the percentage of the LF and HF variability with respect to the total power after subtracting the power of the VLF component (frequencies <0.20 Hz). The normalisation procedure tends to minimise the effect of the changes in total power on the absolute values of LF and HF variabilities (Malliani et al., 1991; Rubini et al., 1993; Task Force, 1996).

2.6. Baroreflex sensitivity

Baroreflex sensitivity was determined by the method of Head and Mccarty (1987). Changes in MAP were elicited by alternating bolus injections of phenylephrine (0.1 to 16.0 μg/kg) and sodium nitroprusside (0.1 to 32.0 μg/kg). MAP and HR were measured before and immediately after injection of phenylephrine (or sodium nitroprusside) when the arterial pressure achieved a new steady-state level. The two parameters were then allowed to return to baseline, after which the next injection was given. A total of at least six increases and six decreases in MAP of different degrees were elicited in each rat.

Table 1
Baseline values (mean ± SEM) of heart rate (HR, mean arterial pressure (AP) and autonomic pharmacological blockade in sedentary and trained conscious rats after nitric oxide blockade with L-NAME.

	Sedentary		Trained	
	SR ($n=14$)	SRL ($n=14$)	TR ($n=14$)	TRL ($n=14$)
Baseline values				
Heart Rate, bpm	346 ± 5	403 ± 15*	314 ± 6^{*+}	290 ± 8$^{*+\#}$
Mean AP, mmHg	98 ± 3	162 ± 4*	102 ± 2	133 ± 4$^{*+\#}$
Tonic autonomic control				
Methylatropine, bpm	458 ± 10	434 ± 8	414 ± 12*	399 ± 11^{*+}
Propranolol, bpm	331 ± 9	356 ± 10	290 ± 8^{*+}	269 ± 9^{*+}
Intrinsic Heart Rate, bpm	392 ± 6	379 ± 7	332 ± 4^{*+}	323 ± 9^{*+}

Sedentary rats (SR); sedentary rats L-NAME (SRL); trained rats (TR) and trained rats L-NAME (TRL). Values are mean ± SEM. $^{*}P<0.05$ compared to SR; $^{+}P<0.05$ compared to SRL; $^{\#}P<0.05$ compared to TR.