Physical Exercise Attenuates the Cardiac Autonomic Deficit Induced by Nitric Oxide Synthesis Blockade

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Summary

Background: The nitric oxide (NO) synthesis blockade is characterized by an increase in the cardiac sympathetic activity and the physical training promotes the decrease in the sympathetic activity.

Objective: We investigated the effect of the NO synthesis blockade on the autonomic cardiovascular control in rats submitted to aerobic exercises during a 10-week period.

Methods: Male Wistar rats were divided in four groups: control rats, treated with chow food and water ad libitum for 10 weeks (CR); control rats, treated with N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) during the last week (CRL); rats trained during 10 weeks on an electrical treadmill (TR); rats trained for 10 weeks and treated with L-NAME during the last week (TRL). The autonomic cardiovascular control was investigated in all groups with the use of a double blockade with methylatropine and propranolol and analysis of variability.

Results: The CRL and TRL groups presented hypertension. The CRL group presented tachycardia and predominance of the sympathetic tonus in heart rate (HR) measurement after the pharmacological autonomic blockade. The TR group presented bradycardia and lower intrinsic HR when compared to the others. The evaluation of the HR variability showed lower absolute and normalized values in the low frequency (LF) band in the CRL group. On the other hand, the TRL presented an increase in the LF band in absolute values. The analysis of variability of the systemic arterial pressure (SAP) showed that the CRL and TRL groups presented higher values in the LF band.

Conclusion: The previous physical exercise prevented the deficit in the autonomic cardiac control induced by the treatment with L-NAME, but did not prevent the increase in the SAP variability. (Arq Bras Cardiol 2009;92():29-36)

Key words: Exercise; nitric oxide; hypertension.

Introduction

The analogs of L-arginine (L-NMMA) or N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), promote elevated and sustained arterial hypertension and have been extensively used as an experimental model of hypertension\textsuperscript{1}. Hypertension occurs because these analogs compete with the endothelial L-arginine, preventing the action of the endothelial nitric oxide synthase (eNOS) and resulting in the substantial decrease in the nitric oxide (NO) production\textsuperscript{2}. However, the increase in blood pressure (BP) is not only a consequence of the elimination of the vasodilating action of the NO, but also due to the elimination of its influence on the sites of cardiovascular autonomic control, promoting the increase in sympathetic activity. This statement is based on experimental animal studies that showed that the increase in the sympathetic activity after the blockade of the NO synthesis\textsuperscript{3,4}. It was also observed the predominance of the sympathetic autonomic component on the vagal component, associated with the decrease in the baroreflex sensitivity after the blockade of the NO synthesis\textsuperscript{5}. In humans, the increase in the sympathetic activity in pathological situations such as arterial hypertension, many times associated to endothelial dysfunction, with a consequent decrease in the eNOS activity, has been observed\textsuperscript{6-8}.

Many studies have demonstrated that the practice of physical exercises also influences the cardiovascular autonomic control. It has been shown that rats submitted to training with aerobic exercises presented a decrease in the basal heart rate (HR), intrinsic heart rate (IHR), increase in the vagal tonus and decrease in the cardiac sympathetic tonus.

Additionally, it was also observed an improvement in the baroreflex sensitivity, represented by the enhanced reflex tachycardic response to the induced hypotension\textsuperscript{9-11}. Associated to these effects, the physical exercise also seems to reduce the BP levels and increase the NO release levels and activity\textsuperscript{12}.

In the clinical context, it has also been demonstrated that the physical training improves the cardiovascular autonomic and endothelial vasodilating functions in a systemic way,
promoting beneficial cardiac and vascular effects\textsuperscript{11,14}. These benefits, among several causes, would also be related to a higher NO production\textsuperscript{15}.

Routine physical exercise practice has been used as systemic arterial hypertension (SAH) prevention and anti-hypertensive therapy, attenuating the BP levels and decreasing the risk factors for cardiovascular diseases, including the autonomic ones\textsuperscript{9}. However, the mechanisms involved in this process have yet to be fully elucidated. Therefore, the objective of the present study was to investigate the effect of previous physical exercise practice on the autonomic cardiac and vascular adaptations in rats submitted to the arterial hypertension model induced by the NO synthesis blockade. For that purpose, the present study used two different approaches: the pharmacological assessment of the autonomic tonus and the analysis of the variability of the HR and BP through the spectral analysis.

Methods

All experimental procedures involved in the present study were approved by the Ethics Committee for Animal Research from the School of Medicine of Ribeirão Preto of the University of São Paulo.

Animals

Male Wistar rats (150-180 g) were placed in individual boxes under controlled temperature conditions (21°C) and 12-hour dark/light cycles. The animals were divided in four experimental groups:

1. Control rats, treated with chow food and water ad libitum for 10 weeks (CR group; \(n = 14\)).
2. Control rats, submitted to treatment with \(N^\circ\)-nitro-L-arginine methyl ester (L-NAME) dissolved in the drinking water during the last (tenth) week (CRL group; \(n = 14\));
3. Group of rats trained during 10 weeks on an electrical treadmill (TR group; \(n = 14\));
4. Group of rats trained for 10 weeks and treated with L-NAME dissolved in the drinking water during the last week (TRL group; \(n = 14\)).

Physical training

The animals from the training groups were submitted to the aerobic exercise protocol through forced treadmill exercise (Insight model EP-131, Ribeirão Preto, SP, Brazil) during ten weeks. The treadmill had six individual stalls that were 15-cm high, 10-cm wide and 50-cm length. The training was carried out with the treadmill at 0° angle of inclination, velocity of 25 m/s, five days a week.

Experimental protocol

- **Surgical procedure** - On the sixth day of the last week, after being anesthetized with tribromoethanol (250 mg/kg, i.p.), all animals had catheters implanted in the femoral artery and vein (PE-50 welded to PE-10), filled with saline solution and heparin (500 IU/ml), which were exteriorized at the posterior neck region of the animal.

- **Blood Pressure (BP) and Heart Rate (HR) recording** - 24 hours after the surgical procedure, the BP and the HR were recorded through a digital system of biological signal acquisition, which consisted of a pressure transducer (ADInstruments, Australia - MLT0380) and a signal amplifier (ADInstruments, Australia - ML110) coupled to the computerized acquisition system (ADInstruments, Australia - PowerLab 8/30). During the experimental procedure, the systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP) and HR were derived from the pulsatile BP through the use of software (PowerLab - Chart5 - ADInstruments, Australia).

- **Sympathetic-vagal tonus and pacemaker intrinsic HR (IHR)** - All experimental groups received methylatropine (4 mg/kg) and propranolol (5 mg/kg), through a catheter in the femoral vein, to block the vagal and sympathetic influence on the heart. After the 40-minute basal recording period, methylatropine was injected (\(n = 7\)) and the BP, MAP and HR were recorded for 15 minutes to evaluate the effect of the vagal blockade on the HR. Subsequently, propranolol was injected in the same animal and the HR was recorded for 15 more minutes to determine the IHR. In another animal subgroup (\(n = 7\)), the sequence was inverted to propranolol/ methylatropine, following the same recording procedure (15/15 minutes) for each drug. The data obtained in the methylatropine/propranolol and propranolomethylatropine sequences were added (\(n = 14\), in each group) to obtain the basal HR (before the drug injections) and the IHR.

- **Variability of HR (HRV) and systolic arterial pressure (SAP)** - The analysis of the HR and SAP variability was carried out on the 40-minute basal recording obtained before the pharmacological blockade with methylatropine and propranolol. In order to do that, the points of inflexion of the arterial pulse were obtained, cycle by cycle, determining the systolic values of the BP. The temporal series of pulse intervals (PI) was generated from the pulsatile signal of the SAP, by measuring the time between the adjacent systolic peaks, beat by beat.

The temporal series generated from the IP and SAP were divided in continuous segments of 300 beats, with an overlap of 50% between the adjacent segments. After the calculation of the means and the variance of each segment, these were submitted to the spectral analysis through an autoregressive method, as described in the literature\textsuperscript{16–17}.

The modeling of the oscillatory components present in the stationary segments of the temporal series of SAP and IP was calculated based on the Levinson-Durbin recursion, with the model being chosen according to Akaike criteria\textsuperscript{18}. This procedure allows the automatic quantification of the frequency axis and power of each relevant oscillatory component, present in temporal series. The oscillatory components were classified as: very low frequency (VLF: 0.01-0.20 Hz), low frequency (LF: 0.20-0.75 Hz) and high frequency (HF: 0.75-2.50 Hz). The power of the oscillations of the LF and HF of the heart rate variability was also expressed in normalized units, obtained through the calculation of the ratio between the band power, LF or HF and by the total power after the subtraction of the VLF values. The normalization procedure was carried out to minimize the total power variations in the absolute values of the components of the LF and HF\textsuperscript{16,17}.  

The analysis of the HR and SAP variability was carried out on the 40-minute basal recording obtained before the pharmacological blockade with methylatropine and propranolol. In order to do that, the points of inflexion of the arterial pulse were obtained, cycle by cycle, determining the systolic values of the BP. The temporal series of pulse intervals (PI) was generated from the pulsatile signal of the SAP, by measuring the time between the adjacent systolic peaks, beat by beat.
Statistical analysis - The results are shown as MEANS ± SEM. The evaluation of the normal homoskedastic variables was carried out by using ANOVA (analysis of variance) associated to Tukey post-hoc Test. Statistical significance was set at p values < 5% (p < 0.05).

Results

Table 1 shows the hemodynamic values obtained regarding MAP and basal HR of all studied groups. The CRL and TRL groups were hypertensive when compared to the CR and TR groups. As for the basal HR, the CRL group presented tachycardia, whereas the TR group presented bradycardia, when compared to the others. The values of the pharmacological autonomic blockade with methylatropine and propranolol, shown in Figure 1 and Table 1, demonstrate the vagal to sympathetic predominance in the measurement of the basal HR in the CRL group, in opposition to the other groups that presented vagal predominance. A lower IHR was also observed in the TR group.

Figure 2 and table 2 show the mean of the results of the HRV analysis in all studied groups. The analysis of the HRV indicates that the TR group presented a higher total variance than the CR and CRL groups. It also shows that the CRL group presented lower absolute and normalized values in the low-frequency oscillations (LF: 0.2-0.75 Hz), when compared to the other groups. In turn, the TRL group presented higher oscillations in the LF band, although, only in absolute values. The latter also presented, in absolute values, higher HF oscillations when compared to the CRL group. Figure 3 and table 2 show the means of the results of the analysis of SAP variability in all groups. The analysis of the SAP variability showed that the groups treated with L-NAME, CRL and TRL, presented higher values in total variance and LF band.

| Table 1 - Basal values (mean ± SEM) of heart rate (HR), mean arterial pressure (MAP) and HR values after the pharmacological blockade of the autonomic, parasympathetic (methylatropine) and sympathetic (propranolol) in non-anesthetized rats |
|---------------------------------|-----------------|-------------|-----------------|-----------------|
|                                | Basal HR, bpm   | MAP, mmHg   |                  |                  |
| Control (N = 14)               | 366 ± 8         | 101 ± 2     |                  |                  |
| LCR (N = 14)                  | 393 ± 8*        | 144 ± 4*    |                  |                  |
| TR (N = 14)                   | 333 ± 5*+       | 94 ± 5+     |                  |                  |
| LTR (N = 14)                 | 363 ± 9+        | 137 ± 4*#   |                  |                  |
| Autonomic control             |                 |             |                  |                  |
| Intrinsic HR, bpm             | 389 ± 6         | 373 ± 6     | 359 ± 4*+       | 374 ± 4 #       |
| Methylatropine, bpm           | 465 ± 12        | 436 ± 12    | 428 ± 12*       | 453 ± 9         |
| Propranolol, bpm              | 339 ± 14        | 341 ± 7     | 322 ± 12        | 342 ± 8         |

All values are expressed as means ± SEM. *P < 0.05 vs control rats (CR); +P < 0.05 vs L-NAME control rats (LCR); #P < 0.05 vs trained rats (TR). CR - control rats; LCR - L-NAME control rats; TR - trained rats; LTR - L-NAME trained rats; SEM - standard error of the mean.
oscillations when compared to the untreated groups, CR and TR. Figure 4 shows the representative spectrums of HRV (pulse interval) and SAP of one animal from each group, which was carried out by the autoregressive method.

Discussion

The aerobic physical exercise has been broadly used as a non-pharmacological anti-hypertensive therapy, attenuating the BP levels and decreasing the risk of metabolic and cardiovascular diseases, many times associated to endothelial dysfunction and decrease in the eNOS activity\(^1\)\(^3\)\(^4\)\(^5\).

In the present study, the aerobic physical training on an electrical treadmill was not capable to inhibit or attenuate the BP increase due to the NO synthesis inhibition. In turn, some studies have shown that the aerobic training promotes the decrease in the BP values in animals chronically treated with L-NAME. This decrease would be associated to the increase in the eNOS activity and, consequently, increase in the NO availability\(^1\)\(^2\)\(^3\). The non-attenuation of the BP observed in the present study might be associated to methodological differences, such as time of treatment (7 days) and L-NAME dose (70 mg/kg), time (10 weeks) and intensity (25 m/s) of training. However, it is clear in our study that the physical training promoted adaptations, especially on the cardiac control.

The NO synthesis blockade with L-NAME also promotes important alterations in the cardiovascular autonomic control. Among them is the inversion in the cardiac autonomic balance, with the sympathetic predominance. Our results showed that the previous physical training, for a period of 10 weeks, prevents the inversion of the cardiac autonomic balance, as a consequence of the NO synthesis blockade for one week (tenth), preserving the vagal predominance. In turn, the physical training did not prevent the increase in the BP variability, characterized by the elevated oscillation in the LF band of the SAP.

The treatment of the non-trained animals with L-NAME (CRL) promoted marked tachycardia, which seems to be attributed to the elevated sympathetic influence, as the IHR did not differ from that of the non-treated control group (CR).
Table 2 - Spectral parameters of the pulse interval (PI) and systolic arterial pressure (SAP) calculated from the temporal series using the autoregressive spectral analysis

<table>
<thead>
<tr>
<th></th>
<th>Control (N = 14)</th>
<th>Trained (N = 14)</th>
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<tbody>
<tr>
<td></td>
<td>RC</td>
<td>RCL</td>
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<tr>
<td>Basal values</td>
<td></td>
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<tr>
<td>PI, ms</td>
<td>0.164 ± 0.004</td>
<td>0.153 ± 0.003*</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>115 ± 3</td>
<td>154 ± 4*</td>
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<tr>
<td>Spectral parameters; PI</td>
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<td></td>
</tr>
<tr>
<td>Variance, ms²</td>
<td>9.92 ± 1.11</td>
<td>9.76 ± 2.09*</td>
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<tr>
<td>LF, ms²</td>
<td>0.26 ± 0.07</td>
<td>0.10 ± 0.05*</td>
</tr>
<tr>
<td>LF, un</td>
<td>3.29 ± 0.59</td>
<td>1.45 ± 0.58*</td>
</tr>
<tr>
<td>HF, ms²</td>
<td>5.71 ± 0.56</td>
<td>4.45 ± 1.36*</td>
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<tr>
<td>HF, un</td>
<td>79.89± 3.34</td>
<td>70.20 ± 9.49</td>
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<tr>
<td>LF/HF</td>
<td>0.18 ± 0.022</td>
<td>0.19 ± 0.089</td>
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<tr>
<td>Spectral parameters; SAP</td>
<td></td>
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<tr>
<td>Variance, mmHg²</td>
<td>12.2 ± 1.57</td>
<td>22.7 ± 5.25*</td>
</tr>
<tr>
<td>LF, mmHg²</td>
<td>7.73 ± 1.96</td>
<td>20.1 ± 5.13*</td>
</tr>
<tr>
<td>HF, mmHg²</td>
<td>1.43 ± 0.35</td>
<td>1.35 ± 0.32</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SEM. *P < 0.05 vs control rats (CR); +P < 0.05 vs L-NAME control rats (LCR); #P < 0.05 vs trained rats (TR); “nu” - indicates normalized units. CR - control rats; LCR - L-NAME control rats; TR - trained rats; LTR - L-NAME trained rats; LF - low frequency; HF - high frequency.

Figure 3 - The bar charts show the total variance of the systolic arterial pressure (SAP). The spectral power of the low frequency (LF) and high frequency (HF) oscillations in absolute values (mmHg²) in control rats (CR), L-NAME control rats (LCR), trained rats (TR) and L-NAME trained rats (LTR). * P < 0.05 vs CR; + P < 0.05 vs LCR; # P < 0.05 vs TR.
The trained animals treated with L-NAME (TRL), in turn, did not present tachycardia, but bradycardia, as the one presented by the group of untreated trained animals (TR). These findings indicate that the previous physical training was effective in attenuating the tachycardia induced by the L-NAME treatment, as these animals presented basal HR and IHR similar to those of the control group. This is possibly due to the effect of the physical exercise in preventing the increase of the cardiac sympathetic influence, induced by the NO deficiency. It was also observed, in the group of untreated trained animals, marked basal bradycardia associated to the IHR decrease. This bradycardia seems to be caused by some mechanisms, perhaps even with the participation of the NO. Some studies have shown that the NO would increase the HR response to the vagal stimulation, in vitro as well as in vivo. This response would possibly involve a NO/cGMP pathway, facilitating the cardiac cholinergic transmission, which could also be co-responsible for the bradycardia after the aerobic physical training. A subsequent study demonstrated that the genetic transference of neuronal NOS (nNOS) within the atrial wall accompanied the vagal phenotype induced by the exercise, suggesting that the nNOS could be the key to the increase in the vagal cardiac function. However, in opposition to this theory, a study demonstrated that the alterations found in automaticity and in the conduction of the sinus node impulse caused by the training of endurance athletes would be attributed to the intrinsic physiology of the heart and not to the autonomic influences. Therefore, the basal bradycardia observed in the group of trained animals might be attributed mainly to the decrease in the pacemaker IHR, as we did not observe alterations in the autonomic behavior in this group.

Regarding the autonomic control, the present study showed, at the pharmacological evaluation and HRV analysis, that the previous physical training attenuates or even prevents the alterations in the cardiac autonomic balance induced by the NO synthesis blockade. At the pharmacological evaluation, the cardiac autonomic tonus maintained the predominance of the vagal component over the sympathetic autonomic component in the basal HR measurement, in opposition to the sympathetic predominance observed in non-trained animals treated with L-NAME. Regarding the assessment of the HRV, the previous physical training was also responsible for the attenuation of many of the alterations found after the NO synthesis blockade. In this case, specifically, it was observed that the LF oscillations were decreased in the control animals treated with L-NAME and that the previous physical training prevented this decrease. This paradox, in which the possible increase in the sympathetic activity, promoting tachycardia in the hypertensive animals treated with L-NAME, would be associated with the decrease in the LF oscillations in the HRV has been observed before, in rats as well as in other situations with humans that presented increase in the sympathetic activity, such as heavy physical exercise or severe heart failure. In these situations, when the physiological mechanisms are activated to their maximum capacity to maintain homeostasis, the cardiovascular system would not present a reserve to maintain its variability.

Indeed, the cause of the decrease in the LF oscillations in the HRV in this model of hypertension remains unknown; however, it has been suggested that the increase in the sympathetic activity, associated to the abnormalities in the central autonomic modulation, the deficiency in the arterial baroreflex regulation and the alterations in the cardiac sensitivity to the catecholamines might be responsible for the decrease in the LF oscillations in the HRV. The sympathetic predominance observed in the pharmacological analysis of the autonomic tonus and the decrease in the LF oscillations in the HRV seem to reflect the effects of the NO synthesis inhibition, mainly in the central nervous system. This statement is based on previous studies that demonstrated that the NO would act on central sites of cardiovascular autonomic control. These studies, carried out in different species, suggest that the NO would influence the processing of information from the baroreceptor afference, mainly in the solitary tract nucleus (STN) and in the rostral...
ventrolateral medulla (RVLM)\textsuperscript{26-29}.

A study carried out in anesthetized rats showed that the intravenous infusion of L-NAME decreased the neuronal discharge of NT\textsuperscript{29} and that unilateral microinjections of L-arginine, also in the STN, produced a marked depressor neuronal response, bradycardia and decrease in the activity of the renal sympathetic nerve\textsuperscript{27}.

In turn, microinjections of L-NAME or L-NMMA inside the RVLM of rats and cats promoted BP increase\textsuperscript{26,27}. When the baroreflex was assessed, it was demonstrated that not only the microinjection of L-NAME in central sites of cardiovascular control\textsuperscript{29}, but also its systemic administration prompted the decrease in the baroreflex sensitivity\textsuperscript{3}.

Our findings demonstrated that the previous physical exercise decreases the impairment in the cardiac autonomic control induced by the NO deficiency. The mechanisms responsible for this effect are still uncertain, however, it is unquestionable that physical exercises, and mainly the aerobic type, would induce adaptations in central and peripheral sites in the cardiovascular control, even promoting an increase in the eNOS and nNOS expression\textsuperscript{10-12}. In this case, it was demonstrated in central regions, such as the hypothalamus, STN and RVLM, that the adaptations induced by the physical exercise would be characterized by the decrease in the autonomic sympathetic drive, suggesting the participation of NO in such adaptations\textsuperscript{13-18}.

In turn, the assessment of the SAP variability demonstrated that the NO synthesis blockade with L-NAME promoted a marked increase in the LF oscillations, attributed to the sympathetic modulation, which was not prevented by the previous physical training. Among the possible consequences, the increase in the LF oscillations of the SAP would cause the activation of mechanosensitive and autocrine pathways, promoting concentric cardiac hypertrophy and a higher incidence of cardiovascular dysfunctions\textsuperscript{37,38}. As for the NO, it would have a tamponade action over the LF oscillations, in opposition to the vascular sympathetic modulation\textsuperscript{39}.

This action would be triggered by acute alterations in the BP values, increasing the shearing tension in the endothelial cells and, consequently, the release of NO. The oscillations induced by the sympathetic activation would occur at frequencies ranging from 0.2 to 0.6 Hz, thus belonging to the LF band, differently from the baroreflex control of BP variability, which would act at frequencies lower than 0.1 Hz\textsuperscript{40}. Therefore, our results clearly demonstrate the participation of the NO in the modulation of the LF oscillations of the SAP, however, we cannot affirm that the physical exercise does not have an effect on this modulation or whether the physical-exercise induced adaptive mechanisms in BP could be totally dependent on the NO action.

In summary, our results show that the previous physical training prevents alterations in the cardiac autonomic control induced by the NO synthesis blockade with L-NAME. However, the previous physical training did not prevent the increase in the LF oscillations in the SAP variability after the NO synthesis blockade.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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