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Evidence that brain nitric oxide inhibition increases metabolic cost of exercise, reducing running performance in rats

Ana Cristina R. Lacerda, Umeko Marubayashi, Cláudio H. Balthazar, Cândido C. Coimbra *

Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte, Minas Gerais, Brazil

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Abstract

To assess the role of nitric oxide (NO) in the metabolic rate and running performance of rats submitted to exercise on a treadmill, 1.43 μmol (2 μL) of N^{ω}-nitro-L-arginine methyl ester (L-NAME, $n = 6$), a NO synthase inhibitor, or 2 μL of 0.15 M NaCl (SAL, $n = 6$) was injected into the lateral cerebral ventricle of male Wistar rats immediately before the animals started running (18 m min^{-1}, 5% inclination). Oxygen consumption (VO_2) was measured at rest, during the exercise until fatigue and thereafter during the 30 min of recovery using the indirect calorimetry system. Mechanical efficiency (ME) was also calculated during the running period. During the first 11 min of exercise, there was a similar increase in VO_2 while ME remained the same in both groups. Thereafter, VO_2 remained stable in the SAL group but continued to increase and remained higher in the L-NAME group until fatigue. The L-NAME-treated rats also showed a sharper decrease in ME than controls. In addition, there was a significant reduction in workload performance by L-NAME-treated animals compared to SAL-treated animals. This suggests that central blockage of nitric oxide increases metabolic cost during exercise, reduces mechanical efficiency and decreases running performance in rats.

Keywords: Exercise; Oxygen consumption; Mechanical efficiency; Running performance

The increase in body temperature that occurs in response to continuous exercise results from the temporary imbalance in the rates of metabolic heat production and heat dissipation during the early stage of exercise [10,34]. The energy efficiency of the body becomes apparent during exercise, when ~20–27% of the energy expended can be used for external work, whereas the remaining adenosine triphosphate (ATP) production is used for homeostasis or dissipated as heat [3]. During exercise, oxygen consumption (i.e., total energetic cost of the physical work) is an important parameter in physical work that reflects both mechanical efficiency and running performance until fatigue [4,31]. Therefore, the impairment in the rates of metabolic heat production and/or heat dissipation will influence the caloric balance during exercise. In our previous study, intracerebroventricular (i.c.v.) infusion of N^{ω}-nitro-L-arginine methyl ester (L-NAME)—nitric oxide synthase (NOS) blocker, induced a significant increase in body heating rate (i.e., rate of increase in core temperature) that rapidly produced hyperthermia 0.8 °C with a significant increase in threshold body temperature for tail vasodilation [15]. Therefore, central oxide nitrergic transmission exerts important effects on thermoregulation during exercise by increasing heat dissipation through peripheral vasodilation, preventing high levels of heat storage and protecting the brain against excessive hyperthermia [15]. The findings of various studies involving i.c.v. administration of NOS blockers [18,23,28] or administration within specific sites in the central nervous system (CNS) [23,25], are all in general agreement with the view that the central nitric oxide (NO) system is inhibitory to overall sympathetic outflow. However, it is not known whether, besides its central inhibitory effect on sympathetic outflow, the NO system may also exert an effect on metabolic heat production during exercise. If it does, it could interfere in the metabolic cost of exercise. Therefore, the aim of this study was to assess the effects of the central administration of the NOS inhibitor L-NAME on the metabolic cost of untrained rats submitted to exercise until fatigue.

Male Wistar rats (250–340 g) were individually housed under 14 h light/10 h dark cycles and had free access to water and rat chow. Following anesthesia achieved using 2,2,2-tribromoethanol (300 mg/kg body weight, i.p.), the rats were

* Corresponding author. Tel.: +55 31 34992936; fax: +55 31 34992924.
E-mail address: coimbrac@icb.ufmg.br (C.C. Coimbra).

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fixed to a stereotaxic apparatus (David Kopf Instruments, M-900, Tujunga, CA, USA) and a guide cannula (22 G) was implanted into the right lateral cerebral ventricle using a previously described technique [16,17,24,29,30]. All animals were allowed to recover for at least 1 week before being submitted to the experiments. The rats were familiarized to exercise on the motor-driven treadmill by running at a speed of 18 m min^{-1} at 5% inclination for 5 min per day for the 4 consecutive days prior to the experiments. The purpose of this preliminary exercise was to show the animals in which direction to run. All experiments were approved by the Ethics Committee of the Federal University of Minas Gerais for the Care and Use of Laboratory Animals and were carried out in accordance with the regulations described in the Committee's Guiding Principles Manual (protocol 012/05).

Exercise was performed on a motor-driven treadmill (Columbus Instruments, OH, USA, Modular Treadmill) between 13:00 and 17:00 h at a room temperature of 21 ± 2 °C. The intensity of exercise (18 m min^{-1} and 5% inclination) corresponded to an oxygen uptake of ~66% of VO_{2max} [4,12]. Fatigue was defined as the point at which the animals were no longer able to keep pace with the treadmill [15,24,29,30]. Time to fatigue (minutes) and workload (kg m) were considered indexes of running performance.

On the day of the experiment, the animals were allowed to rest for 1 h in the rodent treadmill chamber before being submitted to the test. A needle (30 G) protruding 0.3 mm from the tip of the guide cannula was introduced into the right lateral cerebral ventricle by connecting it to a Hamilton syringe. Immediately prior to exercise, 2.0 μL of 0.15 M NaCl (n = 6) or 2.0 μL of L-NAME (1.43 μmol, n = 6) was injected into the right lateral ventricle. The dose of brain L-NAME was based on the results of our previous experiments that showed that the response of reduction in workload was clearly L-NAME dose-dependent [15]. Thus, the choose dose (1.43 μmol; i.c.v.) of L-NAME reduced in ~42% the workload of treated animal [15]. According to the literature the effects of L-NAME, induced by this dose, are mediated entirely centrally because of the inability of low doses of L-NAME to cross the blood–brain barrier [2,5,7,14,19,33]. Rats were randomly assigned to groups receiving either saline or L-NAME solution. Immediately after the injections, the animals were submitted to running exercise until reaching fatigue. Oxygen consumption (VO_2) was measured by an open-flow indirect calorimeter (Columbus Instruments) that was calibrated before each use with a certified mixture of gases (20.5% O_2 and 0.5% CO_2). VO_2 (ml kg^{-1} min^{-1}) was continuously recorded on-line at rest, every minute during exercise until fatigue and thereafter during the 30 min of recovery, using a computerized system (Oxymax Apparatus, Columbus Instruments).

Workload (W; kgm) was calculated as W = body weight (kg) × TTF × treadmill speed (m min^{-1}) × sine θ (treadmill inclination) [3,4,17], where TTF is time to fatigue (minutes). Mechanical efficiency (ME; %) was calculated by the formula: ME = (W/energetic cost) × 100 [3,29].

The data are reported as mean ± S.E.M. Differences between groups and the effect of time were evaluated using the analysis of variance (ANOVA) test followed by the Newman–Keuls test. The data were also compared using paired or unpaired Student's t-test, as applicable. The relationship between workload and rate of increase in VO_2, as well as the relationship between workload and percentual of decrease in ME observed during the steady state of exercise were assessed by Pearson's correlation coefficient.

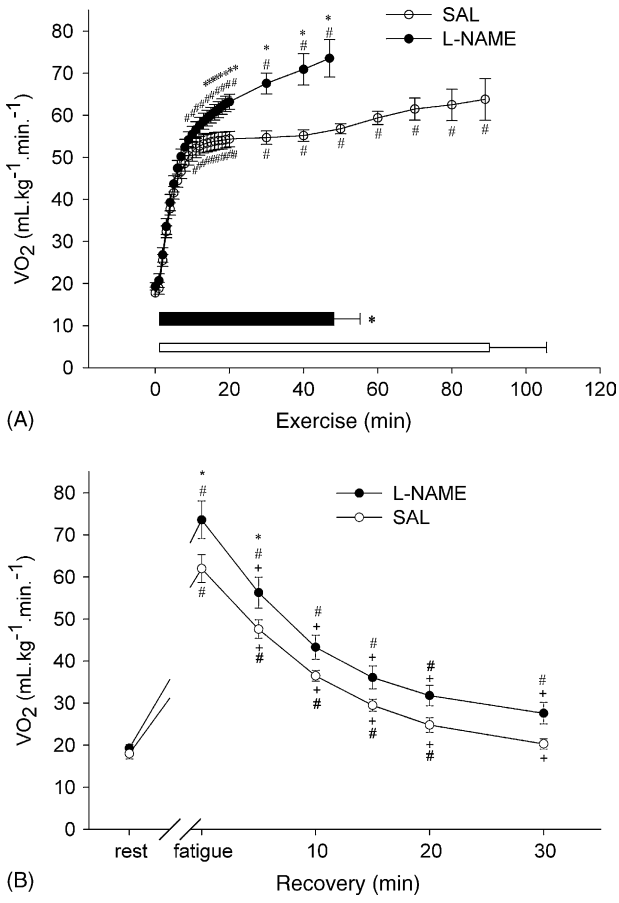

Fig. 1. Effect of i.c.v. injection of L-NAME (1.43 μmol/2 μL) or 0.15 M NaCl (2 μL, SAL) on oxygen consumption (VO_2) during exercise (A) and during recovery period (B). Values are expressed as mean ± S.E.M., n = 6 each group. Time to fatigue is indicated in (A) by the horizontal bar at the bottom of the graph: SAL (open bar) and L-NAME (filled bar). $^{*}P < 0.05$ compared with saline-treated group. $^{\#}P < 0.05$ compared with corresponding basal value. $^{+}P < 0.05$ compared with corresponding fatigue point.

Injection (i.c.v.) of L-NAME in untrained normal rats (L-NAME group, n = 6) resulted in a marked decrease in time to fatigue (47.07 ± 7.13 min, L-NAME versus 89.15 ± 15.40 min, SAL; $P < 0.05$) and workload (12.81 ± 2.21 kg m, L-NAME versus 23.11 ± 3.89 kg m, SAL; $P < 0.05$) compared to saline-treated rats (SAL group, n = 6). There was a similar increase in VO_2 in both groups during the first 11 min of exercise (Fig. 1A). However, after 12 min of exercise until fatigue, VO_2 remained stable in the SAL group but continued to increase until stabilizing at a higher level in the L-NAME group (66.8 ± 0.7 ml O_2 kg^{-1} min^{-1}, L-NAME versus 57.1 ± 0.3 ml O_2 kg^{-1} min^{-1}, SAL; $P < 0.05$), the highest level being attained at fatigue point (73.6 ± 4.5 ml O_2 kg^{-1} min^{-1}, L-NAME versus 62.0 ± 3.3 ml O_2 kg^{-1} min^{-1}, SAL; $P < 0.05$). During recovery period, L-NAME-treated rats still displayed higher VO_2 during the first

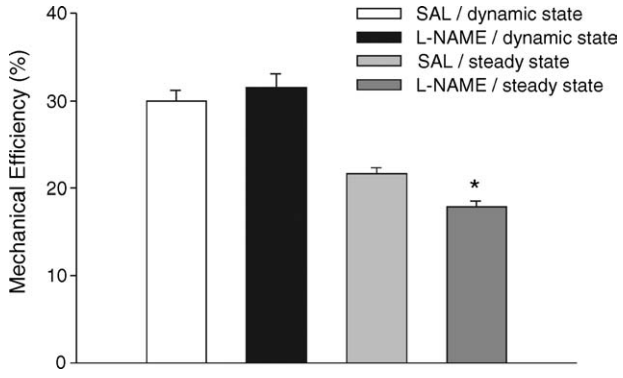

Fig. 2. Effect of i.c.v. injection of L-NAME (1.43 μmol/2 μL) or 0.15 M NaCl (2 μL, SAL) on mechanical efficiency (ME) during the first 11 min of running (dynamic state) and after 12 min of running until fatigue (steady state). Values are expressed as mean ± S.E.M., $n = 6$ each group. *Significantly different from the control group ($P < 0.05$).

5 min of the recovery period and took longer to return to the basal values observed during the rest period prior to exercise (Fig. 1B). To compare the metabolic cost of exercise in the two treatment groups, ME was calculated during the dynamic state of metabolic adjustment of exercise (first 11 min of exercise) and during the steady state of exercise (after 12 min of exercise until fatigue) and is shown in Fig. 2. The ME was similar between groups during the dynamic state (32 ± 1%, L-NAME versus 30 ± 1%, SAL; $P < 0.05$); however, L-NAME-treated animals showed a lower ME than the SAL-treated group during the steady state of exercise (18 ± 1%, L-NAME versus 22 ± 1%, SAL; $P < 0.05$). The rate of increase in VO_2 ($r = 0.73$; $P < 0.01$), as well as the percentual of decrease in ME ($r = 0.60$; $P < 0.05$) observed during the steady state of exercise was closely related to the workload.

The results of the present study show that inhibition of the nitric oxide (NO) pathway in the CNS by i.c.v. injection of L-NAME interferes with the metabolic rate adjustment induced by exercise. The L-NAME-treated rats showed a ∼15% higher metabolic cost than controls during the steady state of exercise. This was closely associated with the decrease in the mechanical efficiency observed in L-NAME-treated rats, leading to a decrease in running performance. These results are consistent with the hypothesis that central oxide nitrergic transmission plays an important role in the control of metabolic rate adjustment during exercise, controlling metabolic heat production as shown by the augmented metabolic rate in L-NAME-treated rats. The increased oxygen consumption associated with the reduced mechanical efficiency may be decreased the run-time to fatigue. These data agree with our previous study and offer further evidence that the control of heat balance is involved in the NO central system during exercise [15]. To the best of our knowledge, this is the first study to describe the role of brain nitric oxide in metabolic cost of exercise modulating heat production.

Increased body temperature during exercise is the consequence of an increase in metabolic rate and the failure of heat loss to keep pace with heat production. Elevated internal body temperature and increased heat storage have been considered limiting factors that reduce the CNS drive for exercise performance and precipitate feelings of fatigue, protecting the brain from thermal damage [9,20]. Thus, the brain nitric oxide pathway activation in normal rats may modulate metabolic heat production protecting the brain from excessive hyperthermia and improving physical performance.

Our results are in agreement with the general idea that central NO plays a role in reducing sympathetic tonus. The increased metabolic rate and reduced mechanical efficiency induced by L-NAME treatment support this hypothesis.

The findings of various studies involving i.c.v. administration of NOS blockade [18,23,28], or administration within specific sites in the CNS [23,25], are all in general agreement with the view that the central NO system is inhibitory to overall sympathetic outflow. At the central level, the currently available data suggests the specificity of NO actions on physiological temperature regulation, mainly inducing hypothermia and anapyrexia [1,32]. In addition, i.c.v. administration of L-NAME to anesthetized rats produces an increase in heart rate and arterial blood pressure [22] blocked by administration of the adrenergic beta blocker, atenolol. Conversely, administration of L-arginine i.c.v. increases NO synthesis within the CNS and produces a decrease in abdominal sympathetic nerve discharge in rats [21]. Furthermore, administration of L-NAME or L-NMMA, another inhibitor of NOS, into the PVN or medial preoptic area (MPOA) also produced an increase in blood pressure and heart rate [35] that was reversed by central administration of L-arginine. In other words, the results of the central administration of modulators on the NO pathways within the cerebral ventricles consistently support the concept of tonic restraint of central sympathetic outflow by NO.

The exact location and precise pathways involved in the nitrergic mediation of normal thermoregulation during exercise still require clarification. However, hypothalamic regions expressing NOS, such as the preoptic area or paraventricular nucleus, are possible sites at which NO may influence thermoregulation during exercise. It has been established that the POA/AH is an integrative region for the maintenance of metabolic, vasomotor and thermal homeostasis [6,8,11,13,26,27]. These results indicate that the POA/AH is an important mediator of heat production during exercise and might be one possible site for L-NAME action. However, further research is necessary to identify the exact location of nitrergic mediation involved in normal thermoregulation during exercise.

We conclude that central NO plays an important role in metabolic adjustment during exercise and that central blockage of nitric oxide synthase by i.c.v. injection of L-NAME in rats increases metabolic cost during exercise (∼15%), reduces mechanical efficiency (∼18%) and decreases running performance (∼53% run-time to fatigue and ∼55% workload).

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