Brain Research Bulletin 108 (2014) 80–87

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Central blockade of nitric oxide transmission impairs exercise-induced neuronal activation in the PVN and reduces physical performance

Paulo M.A. Lima, Henrique P. Santiago, Raphael E. Szawka, Cândido C. Coimbra *

Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais 31270-901, Brazil

ARTICLE INFO

Article history:
Received 30 January 2014
Received in revised form 3 September 2014
Accepted 4 September 2014
Available online 16 September 2014

Keywords:
Treadmill exercise
L-NAME
c-Fos
Supraoptic nuclei
Thermoregulation
Fatigue

ABSTRACT

The blockade of central nitric oxide (NO) signaling modifies the thermoregulatory and metabolic adjustments that occur during exercise, thereby impairing physical performance. However, the brain areas involved in this response remain unknown. Nitrergic neurons are present in the hypothalamic areas that are activated during exercise and participate in autonomic and neuroendocrine responses, such as, the hypothalamic paraventricular nucleus (PVN) and the supraoptic nucleus (SON). To investigate whether brain NO signaling affects thermoregulation during exercise through the activation of hypothalamic neurons, rats underwent acute submaximal treadmill exercise (18 m min^{-1}, 5% inclination) until fatigue received an intracerebroventricular injection of 1.43 μmol Nω-nitro-L-arginine metil ester (L-NAME), a nitric oxide synthase inhibitor, or saline (SAL). Skin tail temperature (T_{sk}) and internal body temperature (T_i) were continuously recorded and c-Fos expression was determined in the PVN and the SON. L-NAME treatment reduced physical performance by 48%, which was positively correlated with tail vasodilation capacity, which was reduced by 28%, and negatively correlated with heat storage rate (HSR), which was increased by 38%. Physical exercise until fatigue increased the number of c-Fos-immunoreactive (ir) neurons in the PVN and the SON. L-NAME-treatment significantly reduced the exercise-induced c-Fos expression in the PVN, whereas it had no effect in the SON. Interestingly, the number of c-Fos-ir neurons in the PVN was closely correlated with physical performance and inversely associated with HSR. Thus, the inhibition of central NO attenuates neuronal activation induced by exercise in the PVN, impairs the autonomic regulation of heat dissipation, and anticipates the fatigue. Brain NO seems to play a role in exercise performance through the regulation of neuronal activation in the PVN, but not in the SON, although the SON neurons are also activated by running exercise. Moreover, this role in performance mediated by neuronal activation in the PVN can be related with the improvement of thermoregulatory adjustments that occur during exercise.

1. Introduction

Physical activity provides mechanical and metabolic stimuli sufficient to modify the energetic state of the organism, according to the type, intensity, and duration of exercise performed. The organism responds to these alterations, promoting very specific and varied adaptations that are necessary for the maintenance of body homeostasis (Coyle, 2000). Hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS) are known to react and to participate these adaptations (Leal-Cerro et al., 2003; Mastorakos et al., 2005). Accordingly, several studies have been shown that, among the hypothalamic components, the paraventricular and supraoptic nucleus of the hypothalamus (PVN and SON, respectively), are activated during physical exercise (Barna et al., 2012; Nunez et al., 2012; Saito and Soya, 2004; Soya et al., 2007; Yanagita et al., 2007); however, the mechanisms involved in these responses still remain unknown.

Brain nitrergic signaling exerts important autonomic effects and metabolic adjustments during exercise. Previous studies showed that decreasing central nitric oxide (NO) availability with intracerebroventricular L-NAME (Nω-nitro-L-arginine metil ester, a competitive inhibitor of nitric oxide synthase – NOS) administration impairs heat dissipation, reduces mechanical efficiency, and shifts the balance of substrate utilization during exercise, thereby reducing physical performance (Lacerda et al., 2005, 2006a,b).

* Corresponding author at: 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. Tel.: +55 31 3409 2929; fax: +55 31 3409 2924.
E-mail address: coimbrac@icb.ufmg.br (C.C. Coimbra).

http://dx.doi.org/10.1016/j.brainresbull.2014.09.002
0361-9230/

However, the brain areas involved in the regulation of these responses are unknown.

Nitric oxide synthase (NOS), which is an enzyme responsible for NO synthesis, is present in neurons in the PVN and the SON of the hypothalamus (Campese et al., 2007; Vanhatalo and Soinila, 1995; Vincent and Kimura, 1992), an important integrative center of homeostasis control, regulating autonomic and neuroendocrine functions, including the thermoregulatory and metabolic controls (Nagashima et al., 2000; Swanson and Sawchenko, 1980).

Thus, taking into account that (1) central NO signaling modulates the thermoregulatory adjustments and physical performance during exercise; (2) the hypothalamus participates in thermal control and is activated during physical exercise; and (3) nitrergic neurons are present in hypothalamic neurons; the aim of this study was to investigate whether the activation of PVN and SON neurons mediated by acute submaximal running exercise depends on NO transmission. Furthermore, we also investigated whether the exercise-induced activation of PVN and SON neurons was related to running performance and thermoregulatory adjustments during exercise.

2. Materials and methods

2.1. Ethics statement

All experimental procedures were approved by the Ethics Committee for the Care and Use of Laboratory Animals of the Federal University of Minas Gerais (protocol no. 045/2011) and were conducted in accordance with the regulations described in the Committee's Guiding Principles Manual.

2.2. Animals

Male Wistar rats ($n = 16$) weighing 240–330 g were housed in individual cages at a room temperature of $23 \pm 2\,^{\circ}C$ under 14-h light/10-h dark cycles and had free access to water and rat chow.

The rats were familiarized to exercise on the motor-driven treadmill by running at a speed of $15\,m\,min^{-1}$ at 5% inclination for 5 min per day on 4 consecutive days prior to the surgical procedures. The purpose of this preliminary exercise was to show the animals in which direction to run and to prevent the measurement of the increase in body temperature because of manipulation and exposure to an unknown environment. Electrical stimulation was determined according to each animal's tolerability, and it produced a discomfort without injury.

On the fifth day, following anesthesia with a mixture of ketamine ($80\,mg\,kg^{-1}$ bw; *ip*) and xylazine ($10\,mg\,kg^{-1}$ bw; *ip*), the rats were 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 according to the following stereotaxic coordinates of the De Groot atlas (De Groot, 1959): anteroposterior: −1.5 mm; lateral: −2.5 mm; and vertical: −3.0 mm, above the base of the skull. The contact of the tip of the cannula with the ventricular space was indicated by a pressure drop in a saline-filled manometer attached to the cannula (Antunes-Rodrigues and McCann, 1970). Cannula were anchored firmly to the skull with jeweler's screws and fixed with acrylic cement, and it was protected with a protective cap. During the same surgical procedure, a temperature sensor (ER-4000; G2 E-Mitter; 15.5 mm × 6.5 mm, 1.1 mg; Mini Mitter Company Inc., OR, USA) was implanted into the peritoneal cavity through a small incision in the linea alba. This sensor was used to measure the intraperitoneal temperature (T_i) using telemetry. It was inserted and sutured to the abdominal muscle to prevent its movement inside the cavity, keeping it fixed near the muscle. Following implantation, the abdominal muscle and skin were sutured.

Immediately after surgery, the rats received a single dose of an analgesic (flunixin meglumine $1.0\,mg\,kg^{-1}$ *bw*) and antibiotic mixture (pentabiotic; $48{,}000\,IU\,kg^{-1}$ *bw*).

All animals were allowed to recover for at least 1 week before being submitted to the exercise protocol. All experiments were performed at a room temperature of $23 \pm 1\,^{\circ}C$, between 7:00 and 12:00.

2.3. Experimental protocols

On the day of the experiments, rats were weighed and transferred from their home cage to the motor-driven treadmill. All rats had recovered from surgery and exceeded the preoperative weight.

Initially, 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, and a thermocouple (series 409-B, Yellow Springs Instruments, Dayton, OH, EUA) was taped to the lateral surface of the skin, 1 cm from the base of the tail to measure the skin tail temperature (T_{sk}). The rats were left undisturbed for 60 min until the T_i achieved resting and stable values between 37.0 and $37.5\,^{\circ}C$ for at least 20 min. Immediately prior to exercise, in a randomized and paired test, 2.0 μL of 1.43 μmol Nω-nitro-L-arginine metil ester (L-NAME, Merck Sharpe & Dohme, Campinas, Brazil; group L-NAME-EXE, $n = 10$) or 0.15 M NaCl (group SAL-EXE, $n = 10$) were injected into the right lateral ventricle. The dose of L-NAME was based on the results of our previous experiments, which showed a dose-dependent reduction in workload percentual related to the SAL group (Lacerda et al., 2005). After the intracerebroventricular (i.c.v.) injections, the animals were submitted to constant-intensity running exercise until their reached fatigue. The intensity of exercise ($18\,m\,min^{-1}$ and 5% inclination) corresponded to an oxygen uptake of ∼66% of VO_{2max} (Brooks and White, 1978; Lacerda et al., 2006a; Sonne and Galbo, 1980). Fatigue was defined as the point at which the animals were no longer able to keep pace with the treadmill and stayed on the shock grid for 10 s. An interval of at least two days was allowed for the animal to recover between the tests.

Ninety minutes after the determination of fatigue during the second exercise trial, the animals were deeply anesthetized with ketamine and xylazine and were transcardially perfused with 40 mL of heparinized 0.01 M phosphate-buffered saline (PBS), followed by 400 mL of 4% paraformaldehyde (PFA) in 0.2 M phosphate-buffered (pH 7.4). The brains were removed for subsequent immunohistochemical analysis.

Intraperitoneal temperature was measured every 5 s and was used as the internal body temperature value. Tail skin temperature was measured every 30 s and was considered as an index of cutaneous heat loss (Young and Dawson, 1982). Time to fatigue (TTF, minutes) and workload (W, kgm) were considered indices of exercise performance.

A group of resting rats receiving an i.c.v. injection of 0.15 M NaCl (group SAL-REST, $n = 6$) was used as a control group. To confirm whether the exercise protocol increases neuronal activation in the PVN and the SON, these animals were subjected to the same conditions of familiarization, surgical procedures, and parts of the exercise protocol. However, after the T_i achieved resting and stable values and the saline was injected, the rats remained at rest on the treadmill for an additional 30 min, were returned to their cages and were perfused 90 min after this rest period.

2.4. Immunohistochemistry

After perfusion, the brains were removed and post-fixed in 4% PFA for 2 h at $4\,^{\circ}C$. Thereafter, the brains were stored in cold 30%