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Nitric oxide pathway is an important modulator of heat loss in rats during exercise

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Abstract

To assess the role of nitric oxide (NO) in central thermoregulatory mechanisms during exercise, 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). Core (T_b) and skin tail (T_{tail}) temperatures were measured. Body heating rate (BHR), threshold T_b for tail vasodilation (TTbV), and workload (W) were calculated. During the first 11 min of exercise, there was a greater increase in T_b in the L-NAME group than in the SAL group (BRH = 0.17 ± 0.02 °C min^{-1}, L-NAME, versus 0.09 ± 0.01 °C min^{-1}, SAL, $p < 0.05$). Following the first 11 min until ∼40 min of exercise, T_b levels remained stable in both groups, but levels remained higher in the L-NAME group than in the SAL group (39.16 ± 0.04 °C, L-NAME, versus 38.33 ± 0.02 °C, SAL, $p < 0.01$). However, exercise went on to induce an additional rise in T_b in the SAL group prior to fatigue. These results suggest that the reduced W observed in L-NAME-treated rats (10.8 ± 2.0 kg m, L-NAME, versus 25.0 ± 2.1 kg m, SAL, $p < 0.01$) was related to the increased BHR in L-NAME-treated animals observed during the first 11 min of exercise ($r = 0.74$, $p < 0.01$) due to the change in TTbV (39.12 ± 0.24 °C, L-NAME, versus 38.27 ± 0.10 °C, SAL, $p < 0.05$). Finally, our data suggest that the central nitric oxide pathway modulates mechanisms of heat dissipation during exercise through an inhibitory mechanism.

Keywords: Exercise; Core temperature; Central fatigue; Heat storage; Body heating rate

1. Introduction

Internal body temperature is considered to be a limiting factor during prolonged physical exercise [7,12,16,23,33]. However, the mechanisms responsible for exercise fatigue related to increasing body temperature (T_b) are still not fully understood, and it is not known whether the increase in temperature during exercise is due to a rise in set-point levels or whether it occurs passively through an unwanted accumulation of heat [4,12,40,54,55]. Controversy also exists regarding whether there is a critical absolute value of T_b and/or heat storage (HS) that determines the point of fatigue. Fuller et al. [12] showed that rats exercising at high room temperatures reached fatigue at the same abdominal and brain temperatures (∼40 °C) even after pre-exercise body temperature was altered. Their results are therefore consistent with the concept that high body core temperature reduces the CNS drive for exercise performance [34,35,54], and with the hypothesis that hyperthermia precipitates feelings of fatigue at a sublethal threshold and establishes a safe guard against heat stroke, protecting the brain from thermal damage [7,23,54]. On the other hand, high body heat storage is also associated with the termination of work in animals [12] and healthy humans [16,27]. Therefore, considering that fatigue is coincident or may be precipitated by high core temperature and/or heat storage, activation of a central mechanism that would increase heat loss and decrease core temperature might improve exercise performance. It has been demonstrated that nitric oxide (NO) acting on the brain exerts thermoregulatory effects producing anapyrexia [18,29] and hypothermia [1,2,3,30,48,52]. L-Arginine (nitric oxide synthase—NOS substrate) analogues

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and NOS blockers such as N^{ω}-nitro-L-arginine methyl ester (L-NAME) have been used to investigate the role of NO in body temperature under resting conditions [1,10,30,51,52]. Several studies have observed that intracerebroventricular injection of L-NAME causes a slight increase in the T_b of rats, indicating that central NO plays a tonic role in reducing T_b [3,10,30,51,52]. Moreover, in the case of fever induced by interleukins (IL-1), lipopolysaccharide (LPS) or psychological stress, intracerebroventricular L-NAME enhanced stress fever, supporting the hypothesis that NO in the CNS leads to a reduction in T_b [10,30,39,42,50]. Since hypothermia and increased heat dissipation may be neuroprotective, activation of central oxide nitrergic transmission may exert important effects on thermoregulation during exercise, influencing running performance. It is important to emphasize that until now, there have been no reports in the literature relating brain nitric oxide and thermoregulation during exercise. Therefore, the aim of this study was to assess the effects of the central administration of the NO synthase inhibitor L-NAME on heat balance and threshold T_b for tail vasodilation (TT_bV) in untrained rats submitted to exercise until fatigue.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–340 g) were housed individually 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 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 using a previously described technique [25,26,40,46,47]. 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 during 4 consecutive days prior to the experiments. The purpose of this preliminary exercise was to show the animals in which direction to run without becoming entangled in the rectal-probe leads and preventing measurement of the conditioned hyperthermic response. All experiments were approved by the Ethics Committee for the Care and Use of Laboratory Animals of the Federal University of Minas Gerais and were carried out in accordance with the regulations described in the Committee's Guiding Principles Manual (protocol 012/05).

2.2. Exercise

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} [6,21]. Fatigue was defined as the point at which the animals were no longer able to keep pace with the treadmill [40,46,47]. Time to fatigue (minutes) and workload (kilogram meter) were considered indexes of exercise performance.

2.3. Experimental protocol

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 percentual, related to SAL group, was clearly L-NAME dose-dependent (Fig. 1). Furthermore, according to the literature, the effect of L-NAME was mediated entirely centrally because of the inability of low doses of L-NAME to cross the blood brain barrier [8,24,31,53]. 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 fatigue was reached. Colonic temperature was taken as core temperature (T_b) index and it was measured using a thermistor probe (model 401, Yellow Springs Instruments, USA). The lubricated thermistor probe was inserted 4 cm past the anal sphincter after fecal pellets had been removed from the colon by gentle, external massage. Skin tail temperature (T_{tail}) was measured using a probe, series 409-B (Yellow Springs Instruments), taped to the dorsal surface of

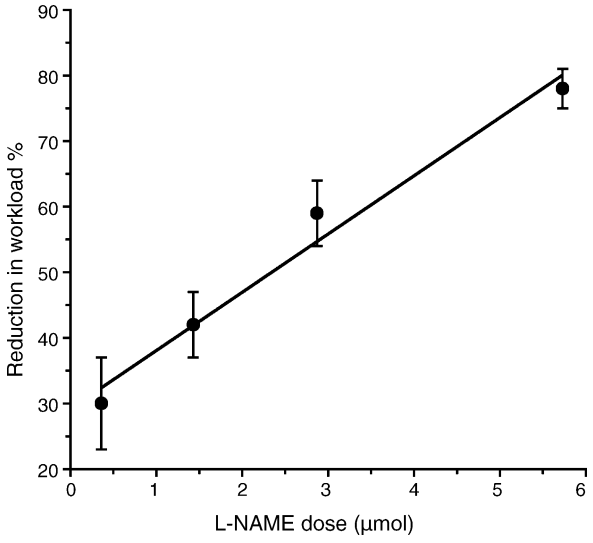

Fig. 1. Effect of intracerebroventricular injection of 2 μL of L-NAME (0.36 μmol, $n = 4$; 1.43 μmol, $n = 9$; 2.87 μmol, $n = 4$; 5.73 μmol, $n = 4$) on workload. Values are expressed as reduction in workload percentual related to SAL group. Data are mean ± S.E.M. $r = 0.988$, $p < 0.01$.

the skin, about 10 mm from the base of the tail. T_b and T_{tail} were used to determine the threshold T_b for tail vasodilation, i.e. the core temperature that corresponds to the moment at which T_{tail} clearly begins to increase (vasodilation).

T_b and T_{tail} were recorded at rest, every minute during the first 20 min of exercise, every 10 min from then until fatigue, and every 5 min thereafter during the 30 min of recovery.

2.4. Calculations

Body heating rate (BHR; $^\circ C\,min^{-1}$), i.e. rate of increase in core temperature, was calculated as BHS = ΔT_b/(running time interval), where ΔT_b is the change in core temperature ($T_f - T_i$), T_f: core temperature at fatigue point and T_i: initial core temperature measured prior to exercise. Heat storage was calculated [17] as HS = $(\Delta T_b)\cdot m\cdot c$, where "$m$" is the body weight in grams and "c" is the specific heat of the body tissues (0.826 cal $g^{-1}\,^\circ C^{-1}$). Workload (W; kg m) was calculated as W = [body weight (kg)]·[TTF]·[treadmill speed ($m\,min^{-1}$)]·[$\sin\theta$ (treadmill inclination)] [5,6,26], where TTF is time to fatigue (minutes).

2.5. Statistical analysis

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 association between BHR and W or TT_bV was assessed using Pearson' s correlation coefficient. Significance level was set at $p < 0.05$.

3. Results

As illustrated in Fig. 2, intracerebroventricular injection of L-NAME in untrained normal rats (L-NAME group, $n = 6$ rats) induced a marked decrease in time to fatigue and workload compared to saline-treated rats (SAL group, $n = 6$ rats). During the first 11 min of exercise, there was a greater increase in T_b in the L-NAME group (Fig. 3A). Following the first 11 min of exercise until ~40 min of exercise, T_b remained stable in both groups, but levels were higher in the L-NAME group than in the SAL group (39.16 ± 0.04 °C, L-NAME, versus 38.33 ± 0.02 °C, SAL, $p < 0.01$). However, exercise still induced an additional rise in T_b in the SAL group prior to fatigue. Although rats in the L-NAME group showed a more accentuated increase in T_b during the dynamic phase of exercise and voluntarily interrupted the exercise much earlier than the control rats, both groups showed a very similar core temperature at fatigue point (39.32 ± 0.13 °C, L-NAME, versus 39.17 ± 0.11 °C, SAL). On the other hand, during recovery period, L-NAME-treated rats took a longer time to dissipate the accumulated heat compared to SAL rats,

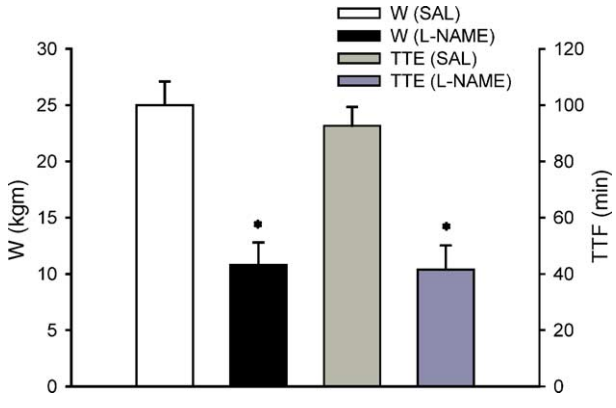

Fig. 2. Effect of intracerebroventricular injection of 2 μL of L-NAME (1.43 μmol; $n = 6$) or 0.15 M NaCl (SAL; $n = 6$) on time to fatigue (TTF) and workload (W) in untrained normal exercising rats. Data are expressed as mean ± S.E.M. Significantly different from the control group ($^*p < 0.05$).

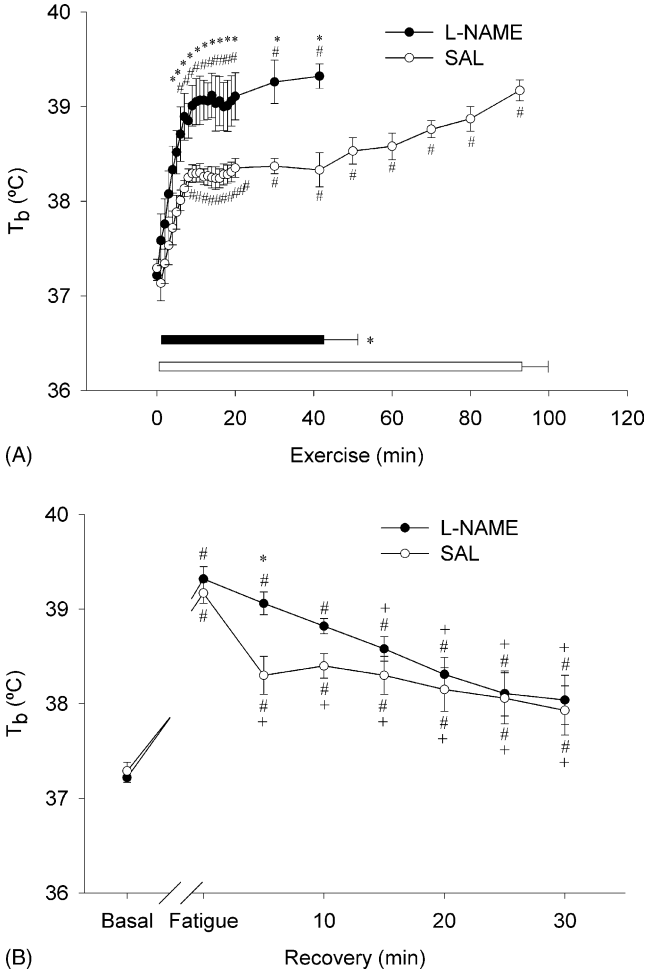

Fig. 3. Effect of intracerebroventricular injection of 2 μL of L-NAME (1.43 μmol) or 0.15 M NaCl (SAL) on core temperature (T_b) during exercise (A) and during recovery time (B). Time to fatigue is indicated in (A) by the horizontal bar at bottom of the graph: SAL (open bar) and L-NAME (solid bar). Values are expressed as mean ± S.E.M., $n = 6$ for each group. $^*p < 0.05$ compared with saline-treated group. $^\#p < 0.05$ compared with corresponding basal value. $^+p < 0.05$ compared with corresponding fatigue point.

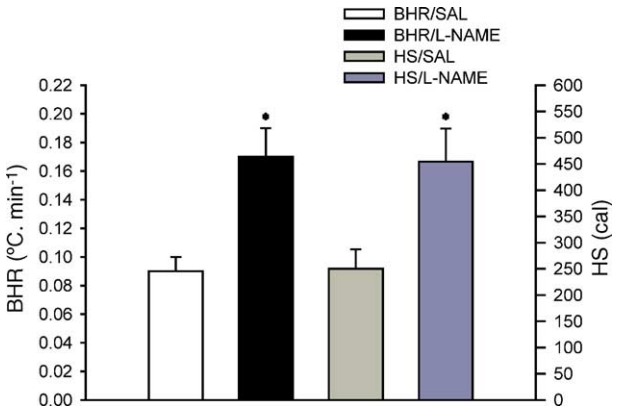

Fig. 4. Effect of intracerebroventricular injection of 2 μL of L-NAME (1.43 μmol) or 0.15 M NaCl (SAL) on body heating rate (BHR) and heat storage (HS) during the first 11 min of running. Values are expressed as mean ± S.E.M., $n = 6$ for each group. Significantly different from the control group ($^{*}p < 0.05$).

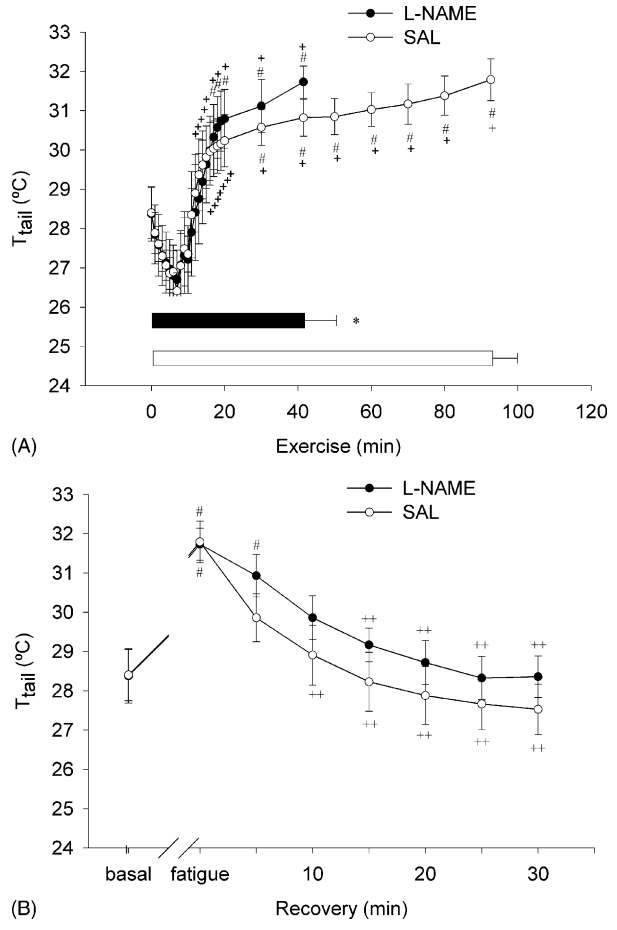

Fig. 5. Effect of intracerebroventricular injection of 2 μL of L-NAME (1.43 μmol) or 0.15 M NaCl (SAL) on skin tail temperature (T_{tail}) during exercise (A) and during recovery period (B). Time to fatigue is indicated in (A) by the horizontal bar at bottom of the graph: SAL (open bar) and L-NAME (solid bar). Values are expressed as mean ± S.E.M., $n = 6$ for each group. $^{*}p < 0.05$ compared with saline-treated group. $^{\#}p < 0.05$ compared with corresponding basal value. $^{+}p < 0.05$ compared with corresponding lower value (7 min). $^{++}p < 0.05$ compared with corresponding fatigue point.

displaying higher T_b during the first 5 min of the recovery period (Fig. 3B). To compare the total thermal effects of exercise in the two treatment groups, BHR and HS were calculated and are shown in Fig. 4. As may be seen, the BHR and HS of L-NAME-treated animals were, respectively, 53 and 55%, both higher ($p < 0.01$) than in SAL-treated group during the first 11 min of running (dynamic phase of exercise).

As illustrated in Fig. 5A, T_{tail} increased within 14–20 min of exercise in both groups, indicating that vasodilation had occurred and heat loss mechanisms had been activated. Thereafter, T_{tail} remained stable in both groups (SAL: 30.95 ± 0.06 °C; L-NAME: 31.17 ± 0.14 °C, the average measurement taken between 20 min and fatigue point). During the recovery period, T_{tail} gradually decreased, and differences in T_{tail} between groups were not apparent for up to 30 min (Fig. 5B). We also observed a close correlation between BHR and TT_bV (Fig. 6A, $r = 0.905$, $p < 0.01$), and between BHR and W (Fig. 6B, $r = 0.740$, $p < 0.05$).

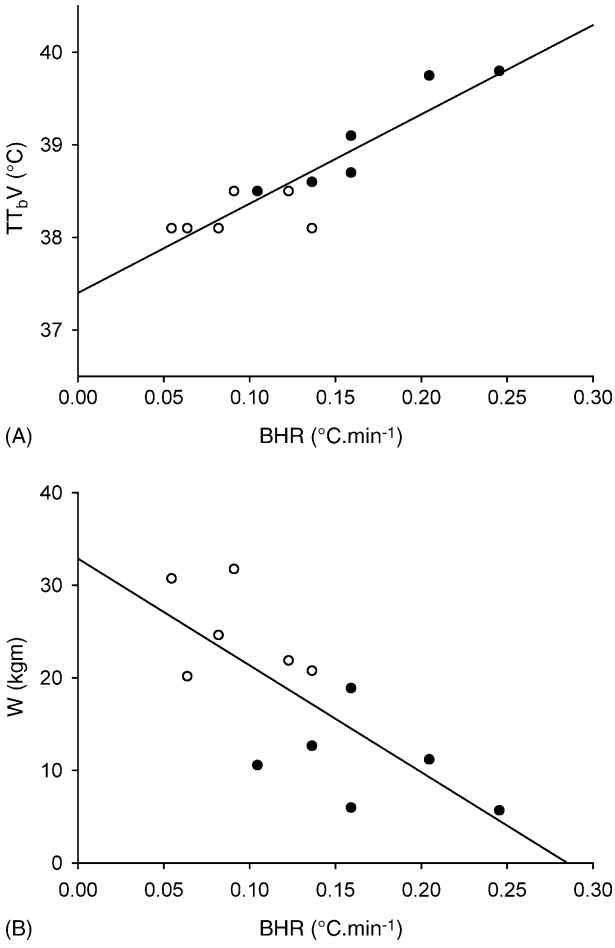

Fig. 6. Correlation between threshold T_b for tail vasodilation (TT_bV) and body heating rate (BHR) (A) and between total workload (W) and body heating rate (B) during the first 11 min of running in rats treated with 2 μL of L-NAME (1.43 μmol, filled circle) or 2 μL of 0.15 M NaCl (open circle). $n = 6$ for each group.

4. Discussion

In the present experiments, L-NAME-treated rats showed the same baseline T_b as control animals. However, within 11 min of the onset of exercise, they presented a significantly higher BHR that rapidly produced hyperthermia 0.8 °C higher than controls. This finding suggests a stronger inhibition of heat dissipation mechanisms in L-NAME-treated rats, which is reflected both by the marked increase (55%) in heat storage and the 0.85 °C increase in TT_bV observed in these animals. It is important to point out that the reduced exercise performance observed in L-NAME-treated rats was closely associated with BHR during the first 11 min of exercise. Our results provide evidence that central oxide nitrergic transmission exerts an important effect on thermoregulation during exercise by increasing heat dissipation through peripheral vasodilation and preventing high level of heat storage and excessive hyperthermia. To the best of our knowledge, this is the first study to examine the effect of central NO system on thermoregulation and our results may point towards a possible neuroprotective role in response to prolonged exercise.

The increase in body temperature that occurs in response to continuous exercise results from the temporary imbalance in the rates of heat production and dissipation during the early stage of exercise [14,23,55]. Vasoconstriction mediated by the sympathetic nervous system during this stage of exercise [19,28] impairs heat loss. Consequently, T_b increases rapidly until it reaches the threshold for peripheral thermal vasodilation, thereby improving heat dissipation. Thereafter, the rise in T_b plateaus at a high level and remains high until fatigue.

The dissipation of heat from the body is thought to be more important than the control of heat production in the regulation of body temperature during exercise [15,55]. In exercising rodents, tail skin vasodilation is an important route of heat loss from the body, as rats do not dissipate heat through sweat evaporation [17,45,56]. Therefore, it is reasonable to propose that inhibition of central NO by intracerebroventricular L-NAME reduces heat dissipation by increasing sympathetic outflow to superficial vascular beds in the tail, promoting vasoconstriction. The increased T_b threshold for tail thermal vasodilation induced by L-NAME treatment, which was strongly related to the BHR, supports this hypothesis. Therefore, the fact that the tail vasodilation threshold during exercise was induced at a higher T_b in the L-NAME-treated rats may have contributed to the increased BHR by delaying heat dissipation through skin tail.

Elevated internal body temperature and increased heat storage have been considered to be limiting factors that [7,12,16,33] reduce the CNS drive for exercise performance [34,35] and precipitate feelings of fatigue, thus protecting the brain from thermal damage. The brain nitric oxide pathway improving heat loss mechanisms may act by protecting the brain from excessive hyperthermia and improving physical performance.

Our results agree with the general idea that central NO plays a role in reducing the sympathetic tonus, and the increased tail thermal threshold to vasodilation induced by L-NAME treatment supports this hypothesis.

The findings of various studies involving intracerebroventricular administration of NO blockade [30,32,38,48], or administration within specific sites in the CNS [38,41], 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 suggest the specificity of NO actions on physiological temperature regulation, mainly inducing hypothermia and anapyrexia [1,2,3,13,16,29,50]. In addition, intracerebroventricular (icv) administration of L-NAME in anesthetized rats produces an increase in heart rate and arterial blood pressure [37] blocked by administration of the adrenergic beta blocker, atenolol. Conversely, administration of L-arginine icv increases NO synthesis within the CNS and produces a decrease in abdominal sympathetic nerve discharge in rats [36]. Furthermore, administration of L-NAME or L-NMMA, another inhibitor of nitric oxide synthase, into the PVN or medial preoptic area (MPOA) also produced an increase in blood pressure and heart rate [57] that was reversed by central administration of L-arginine. In summary, 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. Therefore, we hypothesized that infusion of L-NAME into the cerebral ventricle would perfuse the thermoregulatory centers situated in the hypothalamus, inhibiting the heat loss response and accelerating the BHR and HS during prolonged exercise. The POA/AH is thought to be the primary locus for body temperature regulation [4,9,22,43,44,56] due to the fact that it contains both warm-sensitive and cold-sensitive neurons that respond to small changes in temperature [22,56]. Moreover, lesions or pharmacologic blockade of the POA/AH have been shown to produce a severe impairment in thermoregulation [9,11,20,43,44]. It has been established that the POA/AH is an integrative region for the maintenance of metabolic, vasomotor, and thermal homeostasis [9,11,20,22,43,44,56]. It is important to point out that POA/AH cell groups project to the sympathetic outflow of the tail artery involved in heat loss in the rat [49], warming the preoptic area, and producing tail vasodilation in rats [22,56]. In addition, it has recently been shown that inhibition of the POA/AH by local infusion of tetrodotoxin impairs heat loss in running rats [20]. These results indicate that the POA/AH is an important mediator of heat loss as opposed to 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.

In summary, icv infusion of L-NAME induced a significant increase in BHR that rapidly produced hyperthermia 0.8 °C higher than in controls with a significant increase in TT_bV. In addition, treatment with L-NAME reduced exercise performance that was closely associated with BHR during the first 11 min of exercise. Therefore, our results provide the first evidence that central oxide nitrergic transmission has 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.

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