Intracerebroventricular physostigmine facilitates heat loss mechanisms in running rats

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Rodrigues, Alex G., Nilo R. V. Lima, Cândido C. Coimbra, and Umeoko Marubayashi. Intracerebroventricular physostigmine facilitates heat loss mechanisms in running rats. J Appl Physiol 97: 333–338, 2004. First published March 19, 2004; 10.1152/japplphysiol.00742.2003.—The aim of this study was to evaluate the participation of central cholinergic transmission in the regulation of metabolic rate, core temperature, and heat storage in untrained rats submitted to exercise on a treadmill (20 m/min, 5% inclination) until fatigue. The animals were separated into experimental and control groups. The core temperature or metabolic rate was measured in the rats while they were exercising or at rest after injection of 2 μl of 5 × 10−3 M physostigmine (Phy) or 0.15 M NaCl solution (Sal) into the lateral cerebral ventricle. Metabolic rate was determined by the indirect calorimetry system, and colonic temperature was recorded as an index of core temperature. In resting animals, Phy induced only a small increase in metabolic rate compared with Sal injection, without having any effect on core temperature. During exercise, the Phy-treated animals showed a lower core heating rate (0.022 ± 0.003°C/min Phy vs. 0.033 ± 0.003°C/min Sal; P < 0.02), lower heat storage (285 ± 37 cal Phy vs. 436 ± 34 cal Sal; P < 0.02) and lower core temperature at fatigue point than the Sal-treated group (38.5 ± 0.1°C Phy vs. 39.0 ± 0.1°C Sal; P < 0.05). However, despite the lower core heating rate, heat storage, and core temperature at fatigue, the Phy-treated group showed a similar running time compared with the Sal-treated group. We conclude that the activation of the central cholinergic system during exercise increases heat dissipation and attenuates the exercise-induced increase in core temperature without affecting running performance.

body temperature; oxygen consumption; central cholinergic system; fatigue

According to the heat regulation hypothesis (52), high body temperature is the result rather than the cause of control, whereas metabolic heat production is an independent variable that is constantly adjusted to meet the changing need for energy and heat loss adjusted to match heat production. Heat loss has known physiological effectors that may be influenced and limited by other nonthermoregulatory restraints such as cardiovascular and respiratory control. Therefore, delay in its adjustment during exercise may limit heat loss, increase both heat storage and body core temperature, and induce fatigue.

Internal body temperature is considered to be a limiting factor during prolonged physical exercise (14, 16, 39). However, the mechanisms responsible for exercise fatigue related to increasing body temperature are still not well understood, including whether exercise temperature increase is due to a rise in set point levels or occurs passively through an unwanted accumulation of heat (2). Controversy also exists as to whether there is a critical absolute value of BT, heat storage rate, or both that determines the point of fatigue. Fuller et al. (14) found that rats exercising in high ambient temperatures reached fatigue at the same abdominal and brain temperatures (~40°C), even after preexercise body temperature was altered. These results are therefore consistent with the concept that a high body core temperature reduces the central nervous system drive for exercise performance (39, 40) and with the hypothesis that hyperthermia precipitates feelings of fatigue at a sublethal threshold and, in so doing, establishes a safety level against heat stroke, protecting the brain from thermal damage (5, 26).

On the other hand, high body heat storage is also associated with the termination of work in animals (14) and healthy humans (16, 35). 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. However, it is still unclear whether there is a specific hypothalamic controller for heat loss that interacts with other central controllers of circulation, metabolism, and respiration. In previous studies in primate species, it has been postulated that two independent cholinergic pathways originate in the anterior preoptic region and traverse the hypothalamus in a caudal direction to the posterior area (48, 50). One of these pathways apparently mediates the heat production system, whereas the other serves the heat loss mechanism, although a chemically mediated heat loss pathway has not been identified in the hypothalamus (20, 38). However, more recent studies have provided physiological and pharmacological evidence that activation of heat dissipation mechanisms is related to hypothalamic cholinergic activity. Acetylcholine and other cholinergic agonists such as carbamol, pilocarpine, and oxotremorine produce hypothermia when injected intracerebroventricularly into various species (7, 33, 34). Similarly, microinjection of acetylcholine directly into hypothalamic thermoregulatory centers causes comparable hypothermia (28, 34). However, there is little information concerning the relationships between activation of central cholinergic neurons, body temperature, and metabolic rate during exercise, despite indications that central cholinergic blockade drastically

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METHODS

Adult male Wistar rats weighing 250–300 g (11–12 wk old) were used in all experiments. Animals were housed individually under a 14:10-h light-dark cycle and had free access to water and rat chow. Under thionembutal anesthesia (30 mg/kg body wt ip), a brain guide cannula (22 gauge) was implanted into the lateral cerebral ventricle according to a previously described technique (30, 31, 46). All animals were allowed to recover for at least 1 wk before being utilized in the experiments. Because the experimental procedure did not allow us to measure oxygen consumption and colonic temperature in the same animal simultaneously, the animals were separated at random in eight groups: four to record oxygen consumption and another four to measure colonic temperature. Core temperature or metabolic rate was measured in rats exercising or at rest 2–4 d after a saline (5% NaCl) or physostigmine (3 M) injection. All experiments were carried out according to the regulations of the Ethical Committee for Care and Use of Laboratory Animals at the Federal University of Minas Gerais.

Experimental protocol. On the day of the experiment, the animals were allowed to rest for 1 h in the experimental room. An injection needle (30 gauge) 0.3 mm longer than the guide cannula and connected to a Hamilton syringe was introduced, via the cannula, into the right lateral cerebral ventricle. Each rat was tested only once with Sal or Phy. Beginning 1 wk before the experiments, the rats were familiarized with rectal probes and the experimental procedure by running them daily on a motorized level treadmill for 5 min at 15 m/min. The purpose of this preliminary exercise was to orient animals as to the direction to run so that they would not become entangled with the rectal probe leads during running. This period of habituation according to a previously described technique (30, 31, 46). All animals were allowed to recover for at least 1 wk before being utilized in the experiments. Because the experimental procedure did not allow us to measure oxygen consumption and colonic temperature in the same animal simultaneously, the animals were separated at random in eight groups: four to record oxygen consumption and another four to measure colonic temperature. Core temperature or metabolic rate was measured in rats exercising or at rest 2–4 d after a saline (5% NaCl) or physostigmine (3 M) injection. All experiments were carried out according to the regulations of the Ethical Committee for Care and Use of Laboratory Animals at the Federal University of Minas Gerais.

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All experiments were performed on a motor-driven metabolic treadmill chamber (Columbus Instruments, Columbus, OH) between 10:00 AM to 2:00 PM at 23 ± 2°C. For running rats, the intensity of exercise was 20 m/min on a 5% gradient. The point of fatigue was considered to be the time when animals were unable to keep pace with the treadmill (30, 31, 46). Time to fatigue (in min) and workload (in kgm) were taken as indexes of capacity for exercise.

Metabolic assessment. Metabolic rate was determined by an open-flow indirect calorimetry system (Oxymax v500, Columbus Instruments) that was calibrated before use with a certified mixture of gases (20.5% O₂ and 0.5% CO₂). The rat was placed in the metabolic treadmill chamber for 60 min to establish basal oxygen consumption over the next 30 min. Sal or Phy was then injected, and oxygen consumption (ml·kg⁻₁·min⁻¹) was continuously recorded online by using a computerized system (Oxymax apparatus, Columbus Instruments).

Core temperature. Colonic temperature was taken as core temperature index and measured with a telethermometer (Yellow Springs Instruments) and a thermistor probe (model 401, Yellow Springs Instruments). The fecal pellets were removed from the colon by using gentle external massage, and a lubricated rectal thermistor was inserted 4 cm past the anal sphincter and taped to the tail. The temperature values were recorded every 5 min during the rest or exercise and recovery periods.

Calculations. Core heating rates (HR; °C/min), i.e., rate of rise in core temperature, were calculated as HR = [ΔTₚ/running time interval, where ΔTₚ is change in body temperature. Body heat storage (HS) was calculated (17) as HS = (ΔTₚ)·c·m·C, where m is body weight in grams, and c is specific heat of the body tissues (0.826 cal·g⁻¹·°C⁻¹). Workload was calculated as workload = body weight (kg)·TTF·treadmill speed (m/min)·sine (treadmill inclination) (3, 31), where TTF is time to fatigue.

Statistical analysis. Data are expressed as means ± SE. Differences between groups were checked by analysis of variance followed by the Newman-Keuls test. The data were also compared using paired or unpaired Student’s t-tests as applicable.

RESULTS

As illustrated in Fig. 1A, intracerebroventricular injection of Phy into resting animals (n = 5) induced only a small and transient increase in metabolic rate relative to those injected with Sal. The effects of Phy injection on the oxygen consumption of resting rats were observed only at 10 min and peaked at 15 min after injection, returning to the basal value within 30 min of injection. Despite the increased oxygen consumption, no effect of Phy injection on core temperature of resting rats (n = 6) was observed (Fig. 1B). On the other hand, intracerebroventricular injection of Sal into the cerebral ventricles of
resting animals did not induce any change in the body temperature \((n = 7)\) or metabolic rate \((n = 5)\), which remained stable at 37.4°C and 19.1 ml·kg\(^{-1}\)·min\(^{-1}\), respectively, throughout the experimental period (Fig. 1, A and B).

As shown in Fig. 1C, exercise induced a marked and rapid increase in metabolic rates both in Sal- \((n = 8)\) or Phy-injected rats \((n = 8)\). The oxygen consumption in both groups was already significantly elevated 5 min after exercise started \((35.2 \pm 0.8 \text{ ml·kg}^{-1} \text{·min}^{-1})\) at 5 min vs. 16.1 ± 1.0 ml·kg\(^{-1}\)·min\(^{-1}\) resting value for Sal-treated rats, \(P < 0.01\); and \(35.1 \pm 2.0 \text{ ml·kg}^{-1} \text{·min}^{-1}\) at 5 min vs. 15.6 ± 1.2 ml·kg\(^{-1}\)·min\(^{-1}\) resting value for Phy-treated rats, \(P < 0.01\).

Oxygen consumption remained elevated in both groups of animals until the end of the experimental period \((P < 0.01)\). There were no differences in oxygen consumption between the two experimental groups (Phy- and Sal-treated animals) throughout the experimental period or at the fatigue point.

As illustrated in Fig. 1D, rats injected with Sal \((n = 7)\) or Phy \((n = 7)\) into the cerebral ventricle and submitted to treadmill exercise showed a rapid increase in colonic temperature, which was more intense during the first 30 min but remained constant thereafter, whereas metabolic rate showed no significant fluctuations until the fatigue point. However, intracerebroventricular injection of Phy attenuated the exercise-induced increase response in colonic temperature. The differences between the treatments were already observed at 10 min and remained different until fatigue. The highest difference between treatments occurred at 20 min after exercise had started: 38.6 ± 0.2°C Sal vs. 37.8 ± 0.2°C Phy \((P \leq 0.02)\). Until this point, the Sal group showed a higher core heat rate than the Phy-treated group \((0.062 \pm 0.001°C/\text{min Sal vs.} 0.030 \pm 0.001°C/\text{min Phy}; P < 0.01)\). From the 30th min after exercise had started until the fatigue point, the rates of increase in core temperature were reduced and stabilized at the same level for both groups \((0.017 \pm 0.003°C/\text{min})\). To compare the total thermal effects of exercise between treatments, heating rate and total heat storage were calculated and are illustrated in Fig. 2, A and B, respectively. It can be seen that both the

heating rate and heat storage of Phy-treated animals were \(~65\%\) \((P \leq 0.02)\) of that of the Sal-treated group.

As illustrated in Fig. 3, A and B, intracerebroventricular injection of Phy \((n = 17)\) did not induce any significant difference in exercise performance compared with Sal-treated rats \((n = 17)\) when measured by time to fatigue \((54.0 \pm 2.3\) min Phy vs. 52.5 ± 1.8 min Sal) or on the basis of total workload performed \((24.1 ± 1.3\) kgm Phy vs. 24.3 ± 1.1 kgm Sal).

### DISCUSSION

The present study provides evidence that stimulation of the central cholinergic system by Phy during exercise decreased core heat rate, heat storage, and core temperature, without affecting metabolic rate or the time to fatigue. These findings suggest that a central cholinergic pathway is involved in thermoregulatory cooling mechanisms during exercise.

Intracerebroventricular injection of Phy increased oxygen consumption (Fig. 1A) without affecting core temperature in normal resting rats (Fig. 1B). These data suggest that the increased heat production occurred simultaneously with the increased heat loss, maintaining core temperature unchanged. Lin et al. (33) showed that, in conscious rats, injection of Phy into the lateral cerebral ventricle produces hypothermic effects brought by both decreased metabolic heat production and increased cutaneous vasodilation. However, our data show that there was no difference in body temperature between Sal- or Phy-treated resting rats. It is possible that the hypothermic response induced by Phy in resting rats was compensated by an elevation in metabolic rate. We would like to point out that the dose used in our experiment was lower than those used by other researchers who observed a central hypothermic effect of Phy administration (33, 48–50).

Cholinoreceptor agonists such as acetylcholine produce hypothermia when injected intracerebroventricularly (7, 33, 34) or into hypothalamic thermoregulatory centers (28, 34). These pharmacological data, to-
together with results of immunohistochemical studies demonstrating the presence of intrinsic cholinergic neurons in the hypothalamus, suggest that cholinergic neurons are involved in thermoregulation (47).

However, during exercise, when heat generated by physical activity is much higher than that observed in resting rats after Phy treatment, mechanisms to maintain body temperature within safe limits may differ from those utilized at rest to lose the heat generated. In fact, besides the known central effect of Phy in promoting peripheral vasodilation (33) and increased sympathetic activity in the heart (18), the thermal and hemodynamic effect of exercise may also contribute to improvement of peripheral vasodilation and consequent heat loss. This hypothesis agrees with the fact that, in rats, nonevaporative heat loss occurs mainly through the tail, the blood vessels of which vasodilate when the preoptic area is warmed (27). In addition, dynamic exercise is known to result in increased blood pressure and central venous pressure, which accelerate the rise in skin blood flow, resulting in a sustained exercise elevation of heat loss (10). The only relevant results showing an involvement of central cholinergic transmission in exercise performance in rats (30) have shown that intracerebroventricular administration of atropine, a cholinoreceptor antagonist, drastically reduced the time to fatigue. Our present results involving cholinergic stimulation are compatible with this earlier report. The exact location and precise pathways involved in this cholinergic mediation of normal thermoregulation remain to be elucidated. However, hypothalamic regions expressing muscarinic receptors, such as the preoptic area or paraventricular nucleus, are possible sites for cholinergic influences on thermoregulation during exercise. It has been well established that the preoptic area and anterior hypothalamus is an integrative region for the maintenance of metabolic and thermal homeostasis (6, 8, 9, 11, 12, 32, 44, 45). Inputs from thermal and osmotic sensors are integrated there with the subsequent neural output, modulating autonomic and neuroendocrine pathways that influence thermoregulatory and osmoregulatory effector organs (13, 24, 36, 37). Recently, it has been shown that cholinergic synapses within the preoptic area and the paraventricular nucleus of the rat are important in temperature regulation (48, 50). However, these may be linked to serotonergic (18, 25, 49) or dopaminergic (21) pathways to exert the final thermoregulatory effects.

In the present experiments, Phy-treated rats showed the same baseline colonic temperature as control animals. However, within only 10 min of the onset of exercise, they presented a significantly lower rate of body temperature increase that was maintained until the end point of fatigue. Thus Phy-treated rats reached fatigue at a lower temperature than those treated with Sal. This finding suggests a stronger activation of heat dissipation mechanisms in Phy-treated rats, which is reflected by the marked reduction (61%) in heat storage. Analysis of metabolic rate records did not reveal any difference in oxygen consumption during exercise between the two experimental groups, confirming that the generation of heat was not disrupted by Phy. However, despite the protective effect that a lower body heat rate should have against thermal damage, Phy-treated animals did not extend their time to fatigue, and this was the same as that of Sal-treated animals. This was an unexpected finding, because exercise-induced increases in metabolic heat load provide a considerable challenge to temperature homeostasis and may ultimately impair physical work performance. On the other hand, it is reasonable to postulate that Phy treatment may raise vasodilation or lower the threshold for exercise-induced cutaneous vasodilation, increasing competition for blood flow between the skin, muscles, and brain, as well as reducing time to fatigue.

On the other hand, lowering body temperature provides a wider temperature span before the upper critical body temperature can be reached. It has been described to have beneficial effects that include thermoregulatory, circulatory, and performance benefits in humans and rats (1, 22, 29, 42). By creating a greater body heat storage capacity, precleaning delays the onset of heat dissipation mechanisms by lengthening the time required to reach sweat threshold (15, 23, 42). As a consequence of reducing or delaying the need to dissipate heat, precleaning may result in less competition for blood flow between the skin and working muscles during exercise at high temperatures, resulting in less cardiovascular strain. In contrast to the precleaning situation in which low body temperature delays the heat loss mechanism by vasodilation, our experiments showed an early activation of heat loss mechanisms in animals pretreated with Phy that decreased heat storage capacity. This early onset of heat dissipation mechanisms might have increased exercise-induced competition for blood flow between the skin and working muscles. Thus, even at lower body temperature, Phy did not allow the full benefit of a longer time to fatigue. It is important to point out that rats do not lose heat by sweating. Instead, cutaneous vasodilation redirects heat to the body surface and evaporative cooling is achieved by spreading saliva onto the skin and fur. However, this form of cooling is unlikely to be involved while the animal is running on the treadmill. An alternative mode of cooling is to redistribute blood flow to the tail. This increase in heat dissipation could be associated with either a decrease in set point or a direct action within the heat loss pathway. Our data do not allow distinction between these two possibilities.

It is known that part of the metabolic energy consumed during exercise is dissipated as heat and the other part is used to perform mechanical work. The balance between heat production and heat loss determines internal body temperature in homeothermic animals. To maintain a thermal balance, heat produced by exercising muscles should be counteracted by increased heat loss; otherwise activity results in greater body temperature. Furthermore, hyperthermia reduces physical performance in many mammalian species, including rodents (14, 39, 51). Several authors have suggested that “the will” or “the drive” to exercise might be diminished by hyperthermia (4, 16, 39). Another factor contributing to fatigue was presented by Gonzalez-Alonso et al. (16), who reported that time to exhaustion in a hot environment was inversely related to the initial level of body temperature and directly related to the rate of heat storage. Rodrigues et al. (43) demonstrated that heat storage rate was the main predictive factor of fatigue during thermal stress. However, Phy-treated rats showed a low heat storage rate and presented a lower body temperature than controls at the fatigue point. These results suggested that other factors besides heat storage rate or critical body temperature, such as the activation of heat loss mechanisms, might also contribute to fatigue in running rats.

We conclude that activation of the central cholinergic system during exercise increases heat dissipation mechanisms,
proteins against the exercise-induced increase in body temperature and allows the animal to reach fatigue at lower body temperature without affecting its performance.

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REFERENCES