Physiol. Res. 59: 165-175, 2010

Physical Exercise-Induced Cardiovascular Adjustments Are Modulated by Muscarinic Cholinoceptors within the Ventromedial Hypothalamic Nucleus

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Received July 16, 2008
Accepted February 16, 2009
On-line June 19, 2009

Summary

The effects of blocking ventromedial hypothalamic nucleus (VMH) muscarinic cholinoceptors on cardiovascular responses were investigated in running rats. Animals were anesthetized with pentobarbital sodium and fitted with bilateral cannulae into the VMH. After recovering from surgery, the rats were familiarized to running on a treadmill. The animals then had a polyethylene catheter implanted into the left carotid artery to measure blood pressure. Tail skin temperature (T_{tail}), heart rate, and systolic, diastolic and mean arterial pressure were measured after bilateral injections of 0.2 µl of 5×10^{-9} mol methylatropine or 0.15 M NaCl solution into the hypothalamus. Cholinergic blockade of the VMH reduced time to fatigue by 31% and modified the temporal profile of cardiovascular and T_{tail} adjustments without altering their maximal responses. Mean arterial pressure peak was achieved earlier in methylatropine-treated rats, which also showed a 2-min delay in induction of tail skin vasodilation, suggesting a higher sympathetic tonus to peripheral vessels. In conclusion, muscarinic cholinoceptors within the VMH are involved in a neuronal pathway that controls exercise-induced cardiovascular adjustments. Furthermore, blocking of cholinergic transmission increases sympathetic outflow during the initial minutes of exercise, and this higher sympathetic activity may be responsible for the decreased performance.

Key words

Acetylcholine • Fatigue • Hypothalamus • Methylatropine

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Introduction

Exercise-induced increase in metabolic rate requires a higher blood flow in working muscles and cutaneous vessels to match metabolic demands and dissipate the heat produced. Thus, exercise promotes the redistribution of the blood flow among body tissues and resets the baroreflex, simultaneously raising mean arterial pressure and heart rate. These integrated cardiovascular adjustments provide adequate blood perfusion to all body tissues, thereby allowing animals to maintain physical activity. This cardiovascular control depends on both neural signals originating from the brain (central command) and feedback signals arising from the skeletal muscle, aorta and carotid arteries (Williamson *et al.* 2006).

Previous studies have shown that acetylcholine is involved in the central cardiovascular regulation and that the administration of cholinergic agonists in the brain raises mean arterial pressure in resting rats (Iitake *et al.*

1986, Valladão *et al.* 1990). Moreover, increased cholinergic transmission by intracerebroventricular physostigmine injection leads to higher pressor responses during exercise, suggesting that acetylcholine also modulates blood pressure when the organism is above its resting metabolic rate (Pires *et al.* 2007). However, no experiments have been performed using central injections of the non-selective muscarinic antagonist methylatropine, a step that is necessary to confirm that the cholinergic pathway is activated during exercise to control blood pressure.

It is also of interest to map the cholinergic neuronal pathways that control blood pressure; the involvement of the medial hypothalamus in cardiovascular regulation has already been reported (Valladão *et al.* 1990, Takenaka *et al.* 1996, Marsh *et al.* 2003, da Silva *et al.* 2003). Within the medial hypothalamic structures, the ventromedial nucleus (VMH) is a candidate for participating in this circuitry because it has efferent projections to many structures in the forebrain and brain stem that are involved in the central cardiovascular control mechanisms (Luiten and Room 1980, Canteras *et al.* 1994). This involvement is also supported by the results of an autoradiographic study that identified the existence of muscarinic receptors for acetylcholine in the VMH, particularly the M_3 subtype (Kow *et al.* 1995).

The VMH has been shown to modulate the sympathetic outflow to the periphery, including efferents to the adrenal glands, kidneys and smooth musculature of tail vessels (Morimoto *et al.* 1986, Yoshimatsu *et al.* 1987, Smith *et al.* 1998, Marsh *et al.* 2003). Additionally, bilateral VMH lesions impair the pressor responses elicited by stimulation of the anteroventral third ventricle (AV3V) (Bastos *et al.* 1997, do Vale *et al.* 1997), and functional studies support the participation of acetylcholine-receptive neurons of the VMH in liver glucose mobilization, a marker of sympathetic stimulation (Takahashi *et al.* 1998).

It is important to point out that the interaction between cardiovascular and thermoregulatory systems determines the cutaneous blood flow (O'Leary and Johnson 1989, Pires *et al.* 2007) and that tail skin vasodilation is the primary mechanism of heat loss in exercising rats (Wilson *et al.* 1978, Shellock and Rubin 1984). In previous experiments, we showed that methylatropine injection into the VMH delayed the activation of heat loss mechanisms, leading to a greater heat storage rate and a decreased exercise time to fatigue (Wanner *et al.* 2007). These results suggest that a delay in tail skin vasodilation may be related to an impaired sympathetic withdrawal on cutaneous vessels, resulting in a higher sympathetic vasoconstrictor tone and peripheral resistance.

Therefore, the purpose of the present study was to investigate the role of VMH muscarinic cholinoceptors in cardiovascular adjustments in running rats. We also investigated if the decreased exercise time to fatigue induced by blocking VMH cholinoceptors was related to changes in cardiovascular control.

Methods

Animals

Adult male Wistar rats weighing 250-350 g were used in all experiments. Animals were housed in individual cages under controlled light (0500-1900 hours) and temperature (24.5±1.6 ºC) conditions, with water and rat chow provided *ad libitum*. All experimental procedures were approved by the Ethics Committee of the Federal University of Minas Gerais for the Care and Use of Laboratory Animals (protocol 017/05) and were carried out in accordance with the regulations described in the Committee's Guiding Principles Manual.

Implantation of brain cannulae

Under anesthesia with pentobarbital sodium (50 mg/kg body weight i.p.), the animals were fixed to a stereotaxic apparatus, an incision was made in the skin covering the skull at the midline, and a 5 x 5 mm craniotomy was performed using a high-speed dental drill. Sterile stainless steel cannulae (20.0 mm in length, 0.3 mm OD, 0.1 mm ID) were stereotaxically implanted bilaterally, so that the tip of the cannula was aimed at the dorsal aspect of the VMH according to the coordinates described by Paxinos and Watson (2003). The coordinates used were as follows: AP, 2.5 mm posterior to bregma; ML, 0.6 mm lateral to midline; DV, 9.0 mm below dura mater. Both cannulae were anchored firmly to the skull with screws and fixed with acrylic cement. Sterile stainless steel obturators, 0.07 mm in diameter and of exactly the same length as the cannulae, were inserted into each cannula to prevent obstruction of the lumen.

Immediately after surgery, the rats received an intramuscular prophylactic dose of antibiotics (pentabiotic 24000 IU/kg body wt) and a subcutaneous injection of analgesic medication (flunixin meglumine, 1.1 mg/kg body wt). All animals were allowed to recover

for at least one week before the experiments.

The rats were gradually encouraged to exercise on a treadmill for small animals (Modular Treadmill, Columbus Instruments, OH, USA) by mild electrical stimulation (0.5 mA; 0.5 mV). The familiarization protocol consisted of running at a constant speed of 18 $m \cdot min^{-1}$ and 5 % inclination across 5 min for five consecutive days prior to the experiments (Lima *et al.* 1998, Wanner *et al.* 2007). The purpose of these familiarization exercise sessions was to show the animals in which direction to run without becoming entangled in the tail-skin probe thermocouple wires (Lacerda *et al.* 2005, Prímola-Gomes *et al.* 2007), allowing a steady performance on the treadmill.

Arterial cannulation

Following the last familiarization exercise session, the rats were implanted with a catheter to measure pulsatile arterial pressure. Under pentobarbital sodium anesthesia, a catheter (PE-10 connected to a PE-50, Becton Dickinson, Franklin Lakes, NJ, USA) filled with heparin in normal saline was inserted into the left common carotid artery. The free end of the PE-50 polyethylene tubing was tunneled subcutaneously and exteriorized at the cervical dorsal area.

After surgery, the animals received antibiotics and analgesic medication using the same doses and routes of administration as described above. The rats were given two days to recover from this surgery (Pires *et al.* 2007).

Exercise

Exercise was performed on a motor-driven treadmill, and its intensity (24 $m \cdot min^{-1}$ with a 5 % inclination) corresponded to an oxygen uptake of ~80 % of VO_{2max} (Sonne and Galbo 1980). Fatigue was defined as the point at which the animals were no longer able to keep pace with the treadmill for at least 10 s, even when being stimulated by mild electrical shock (Lima *et al.* 1998, Wanner *et al.* 2007). Time to fatigue (min) and workload (kgm) were considered the indexes of exercise performance.

Experimental protocol

On the day of the experiments, the arterial catheter was connected through a 30 cm length of PE-50 to a pressure transducer (Biopac Systems, Santa Barbara, CA, USA), coupled to an A/D Data Acquisition System (MP100, Biopac Systems). The animals were allowed to rest in their home cages for 60 min.

After the resting period, a 1 µl syringe (Hamilton, Reno, NV, USA) was connected by PE-10 tubing to the brain cannulae to inject the drugs. Rats were randomly assigned to two treatment trials, receiving bilateral injections of either 0.2 µl of 0.15 M NaCl solution (Sal) or 5 x 10^{-9} mol methylatropine (Matr; Sigma Chemical, St. Louis, MO, USA). This dose was selected on the basis of previous experiments, which showed that intra-ventromedial injection of 5 x 10^{-9} mol of methylatropine solution reduced time to fatigue by 37 % (Wanner *et al.* 2007). A thermocouple (series 409-B, Yellow Springs Instruments, Dayton, OH, USA) was taped to the tail, and the rats were then placed inside the treadmill. Ten minutes after Matr or Sal injections, the animals were submitted to exercise until fatigue.

Both experimental conditions were carried out at the same time of day with an interval of at least two days between exercise trials. The order of the trials was randomized, and care was taken to ensure that the experimenter who determined the point of fatigue remained blind to the solution injected during each experimental trial throughout the investigation. Ambient temperature in the experimental room was maintained at 23±1 ºC with relative humidity at 67±8 %.

Measurements and calculations

Tail skin temperature (T_{tail}) was taken as an index of cutaneous vasodilation (Yanagiya *et al.* 1999) and was measured using a thermocouple that was taped to the lateral surface of the skin, ~20 mm from the base of the tail. Mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and heart rate (HR) measurements were taken from pulsatile arterial pressure recordings with AcqKnowledge 3.7.0 (Biopac Systems). Workload (W), a performance index that takes the differences in body weight into account, was calculated as W = [body weight (kg)] · [time to fatigue] · [treadmill speed ($m \cdot min^{-1}$)] · [sinθ (treadmill inclination)] (Lima *et al.* 2001).

Verification of the position of brain cannulae

At the end of experiments, rats were deeply anesthetized with pentobarbital sodium (75 mg/kg body weight i.p.) and perfused with 0.9 % NaCl followed by 10 % formalin solution. The brain was removed and stored in formalin solution. After a few days, the brain tissues were frozen at –13 °C and cut into 50 µm slices using a cryostat microtome (Microm, Riverstone, NSW, USA). Brain slices were stained with a solution of cresyl

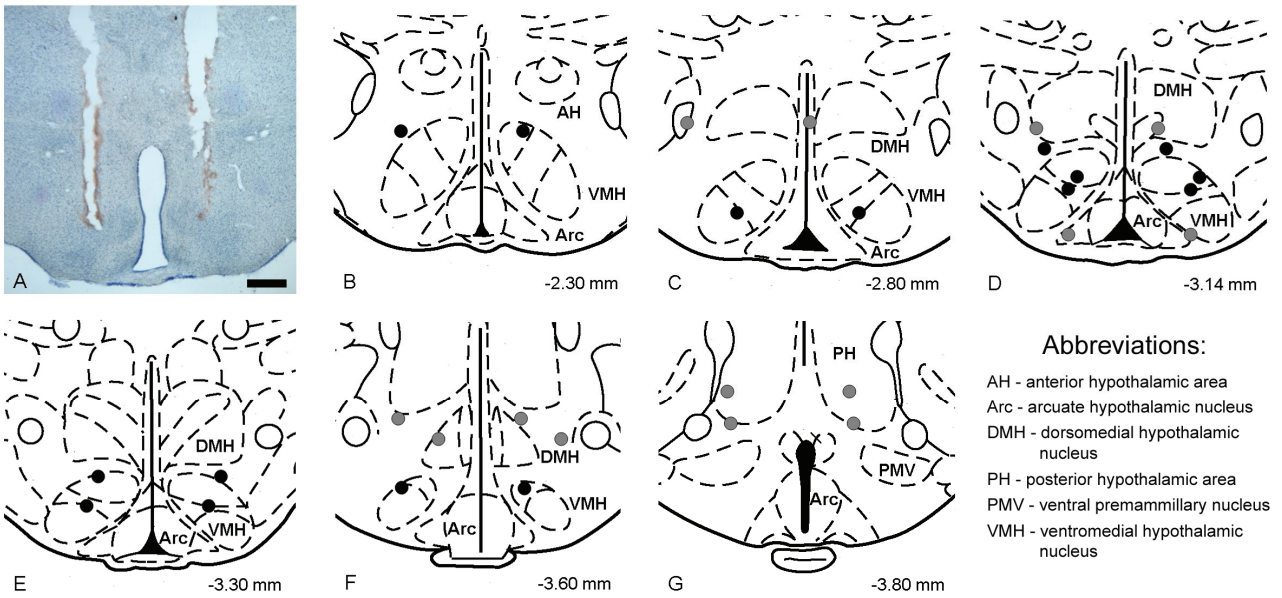

Fig. 1. Photomicrograph of a cresyl violet-stained coronal section from an animal that was inserted with bilateral cannulae in the VMH (A). The horizontal bar's length corresponds to 500 μm. Schematic representation showing the location of cannulae tips (B-G). Injection sites were localized in the ventromedial hypothalamic nucleus (VMH; black circles) and the peri-VMH region (gray circles).

violet and examined under a light microscope. The positions of the cannulae in the brain were confirmed by comparing them to the description in the Paxinos and Watson's atlas (2003).

Statistical analysis

Data are expressed as means ± S.E.M. Time to fatigue, workload performed and time elapsed until MAP peak were compared between treatments using paired or unpaired Student's t-test, as applicable. Differences in cardiovascular adjustments were compared across treatments and time points by two-way analysis of variance (ANOVA), with repeated measures when appropriate. The post hoc Student-Newman-Keuls test was used for multiple comparisons. The correlation between time to fatigue and the time elapsed until MAP peak was assessed using Pearson's correlation coefficient. The significance level was set at $P<0.05$.

Results

Brain cannulae placement

As shown in Figure 1, chronic cannulae were placed bilaterally into the VMH in eight rats. The cannulae were localized either in the dorsomedial or ventrolateral portion of the nuclei, with an anterior-posterior coordinate ranging from –2.3 to –3.6 mm.

In seven other rats, we failed to achieve the desired nuclei, and cannulae were localized at the third ventricle, in the posterior hypothalamus or unilaterally in the dorsomedial hypothalamic nucleus. These animals were then used as a control group (i.e. peri-VMH) with the objective of verifying whether methylatropine effects were specific to the VMH.

We failed to obtain blood pressure recordings in two animals treated with methylatropine; for this reason, the number of animals (n) is different in Figures 3-5. However, these rats were still submitted to the exercise protocol, and the other parameters (time to fatigue and T_{tail}) were recorded as usual.

Exercise performance

As expected, muscarinic blockade of the VMH reduced running time to fatigue by 31 % (15.1±1.2 min Matr vs. 21.7±1.7 min Sal; $p<0.01$). Workload, another index of exercise performance, was also decreased by intra-ventromedial injections of methylatropine (8.1±0.6 kgm Matr vs. 11.8±1.1 kgm Sal; $p<0.01$).

Tail skin temperature

Physical exercise induced tail skin vasodilation after a short latency period in both experimental trials (Fig. 2). In control rats, T_{tail} reached its nadir within 0.5 min after exercise had started, increased at the 4th min (24.91±0.38 ºC at 4 min vs. 24.32±0.19 ºC at 0 min; $p<0.05$), and then remained elevated until the fatigue point.

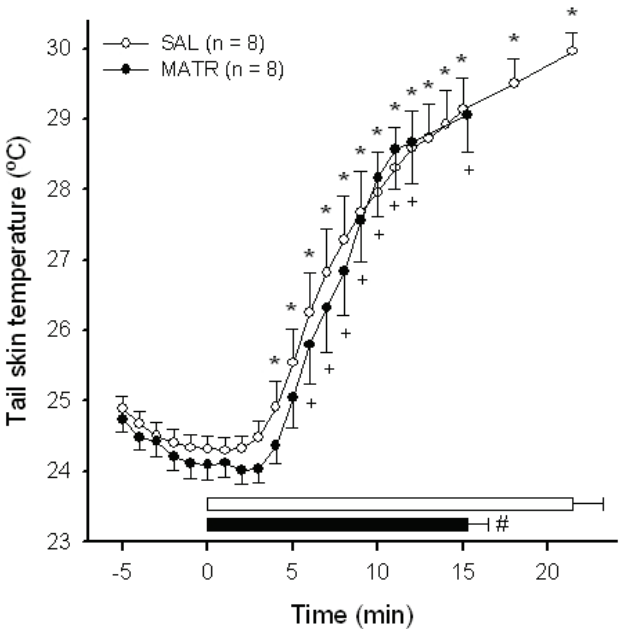

Fig. 2. Temporal profiles of exercise-induced changes in tail skin temperature (T_{tail}) after intraventromedial injection of either methylatropine (Matr; 5 x 10^{-9} mol) or 0.15 M NaCl (Sal). Values are means ± S.E.M. Time to fatigue is indicated by the horizontal bars at bottom. * $p<0.05$ compared with corresponding basal values (0 min) in Sal trial; + $p<0.05$ compared with corresponding basal values (0 min) in Matr trial; # $p<0.05$ compared with Sal trial.

However, in methylatropine-treated rats, T_{tail} reached its nadir within 2.5 min and increased only after 6 min of exercise (25.80±0.56 ºC at 6 min vs. 24.10± 0.21 ºC at 0 min; $p<0.05$), therefore, muscarinic blockade of the VMH delayed the exercise-induced increase in T_{tail} by 2 min. Methylatropine injections also decreased the mean value of T_{tail} during the initial 5 min of exercise (24.26±0.08 ºC Matr vs. 24.64±0.10 ºC Sal; $p<0.01$). At the fatigue point, no differences were observed between the two groups (29.06±0.53 ºC Matr vs. 29.96±0.27 ºC Sal).

Cardiovascular adjustments

As shown in Figure 3A, a slight increase of about 5 mmHg in MAP was observed after the microinjection procedure in both experimental trials (injections were performed 10 min before the animals were submitted to exercise). There were no differences between treatments at the onset of exercise, indicating that methylatropine had no effects during this period.

Exercise induced an additional increase in MAP that was observed in control rats within 1 min (118±4 mm Hg 1 min vs. 109±4 mm Hg 0 min; $p<0.05$); MAP then remained elevated until fatigue. In rats treated with methylatropine, treadmill running also increased MAP as soon as the exercise started (125±4 mm Hg 1 min vs. 113±5 mm Hg 0 min; $p<0.05$). Methylatropine injections also increased the mean MAP value during the initial 5 min of exercise (129±2 mm Hg Matr vs. 123±2 mm Hg Sal; $p<0.05$). No differences in MAP between treatments were observed at the fatigue point (122±5 mm Hg Matr vs. 122±5 mm Hg Sal). The physical exercise-induced alterations observed in SAP (Fig. 3B) were similar to those in MAP.

However, when the time elapsed from the beginning of exercise until MAP peak was evaluated, we observed that it was shorter in rats treated with methylatropine than in controls (2.7±0.3 min Matr vs. 5.6±1.1 min Sal; $p<0.05$) (Fig. 4A). Furthermore, MAP was adjusted as a function of time to fatigue percentage; it peaked at 20 % of time to fatigue and then remained elevated until the end of exercise, irrespective of the treatment (Fig. 4B). We also observed a significant positive correlation between the time elapsed until MAP peak and the time to fatigue ($r = 0.540$; $p<0.05$; Fig.5).

As illustrated in Figure 3C, physical exercise induced a transitory increase in DAP in control rats which was observed from the 3rd min until the 5th min of treadmill running (106±4 mm Hg 5 min vs. 95±6 mm Hg 0 min; $p<0.05$). On the other hand, muscarinic blockade of VMH induced faster and more prolonged DAP increases compared to control animals. DAP rose as soon as the exercise started (106±4 mm Hg 1 min vs. 94±5 mm Hg 0 min; $p<0.05$) and remained elevated until fatigue. Methylatropine injections also increased the mean value of DAP during the initial 5 min of exercise (112±2 mm Hg Matr vs. 106±2 mm Hg Sal; $p<0.05$).

Pre-exercise manipulation of the rats increased HR (426±10 bpm 0 min vs. 360±10 bpm –10 min; $p<0.001$; grouped data), and there were no differences between treatments at the onset of exercise. Running on the treadmill induced an additional increase in HR within 1 min of exercise in control rats (488±11 bpm 1 min vs. 425± 6 bpm 0 min; $p<0.05$) (Fig. 3D). In rats treated with methylatropine, treadmill running also increased HR as soon as the exercise started (495±8 bpm 1 min vs. 428±10 bpm 0 min; $p<0.05$). HR remained elevated throughout the exercise protocol, and no differences were observed between treatments, including the fatigue point (539±8 bpm Matr vs. 544±9 bpm Sal).

Peri-VMH group

When the muscarinic receptor blocker was

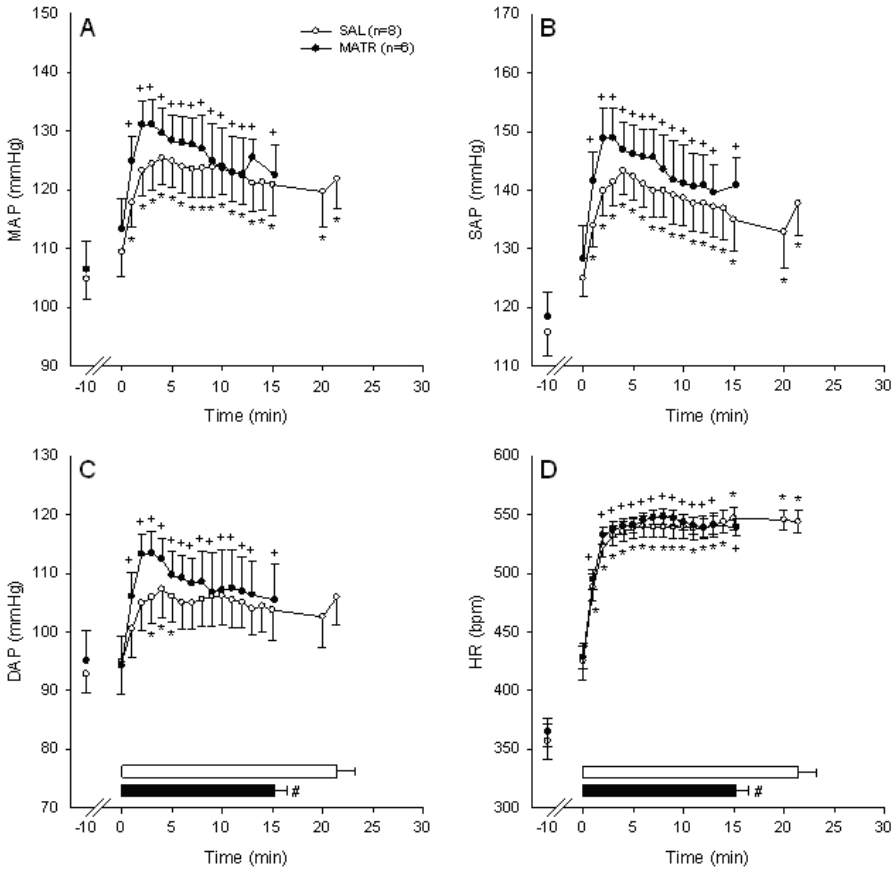

Fig. 3. Temporal profiles of exercise-induced changes in cardiovascular adjustments after intraventromedial injection of either methylatropine (Matr; 5 x 10^{-9} mol) or 0.15 M NaCl (Sal). Figure 3A presents mean arterial pressure; B: presents systolic arterial pressure; C: presents diastolic arterial pressure; and D: presents heart rate. Values are means ± S.E.M. Time to fatigue is indicated by the horizontal bars at bottom. * $p<0.05$ compared with corresponding basal values (0 min) in Sal trial; + $p<0.05$ compared with corresponding basal values (0 min) in Matr trial; # $p<0.05$ compared with Sal trial.

injected in other hypothalamic areas, no effects were observed in either time to fatigue (17.1±1.2 min Matr vs. 17.4±1.3 min Sal) or workload (9.5±0.6 kgm Matr vs. 9.6±0.8 kgm Sal). Furthermore, the blockade of cholinoceptors in the peri-VMH region did not change the exercise-induced increase in T_{tail}, blood pressure or heart rate. A trend toward a higher MAP during exercise was observed after methylatropine injections into the posterior hypothalamus (139 and 122 mm Hg Matr vs. 112 and 115 mm Hg Sal; at 5 min of exercise); however, these data are inconclusive due to the small number of experiments (n = 2).

Discussion

Our present data show that blocking the muscarinic cholinoceptors in the ventromedial hypothalamic nuclei caused that the exercise-induced increase in blood pressure occurred 3 min earlier. This result suggests an augmented sympathetic outflow during the initial minutes of exercise, which was associated with the lower performance of rats treated with methylatropine. The positive correlation observed between the time at which fatigue was reached and the time elapsed until the MAP peak reinforces this point of view. The muscarinic blockade of the VMH also delayed tail skin vasodilation and prolonged the increases in diastolic pressure until fatigue, where this may have induced higher peripheral resistance. However, it is important to point out that this higher methylatropine-induced sympathetic outflow seems to be specific to some peripheral organs, as indicated by the absence of changes in heart rate.

The involvement of the VMH in cardiovascular adjustments has already been described in resting rats using different experimental approaches, including electrolytic and ibotenic acid lesions (Bastos *et al.* 1997, do Vale *et al.* 1997), chemical manipulations (Marsh *et al.* 2003), and neuronal activity recordings (Hirasawa *et al.* 1996). Furthermore, acetylcholine is one of the neurotransmitters involved in this control, and stimulation of the ventromedial hypothalamus with cholinergic agonists increases blood pressure and heart rate (Valladão *et al.* 1990, 1992). Adding to the previous evidence showing VMH control of the cardiovascular system, this is the first study to demonstrate that VMH muscarinic cholinoceptors modulate blood pressure during physical exercise.

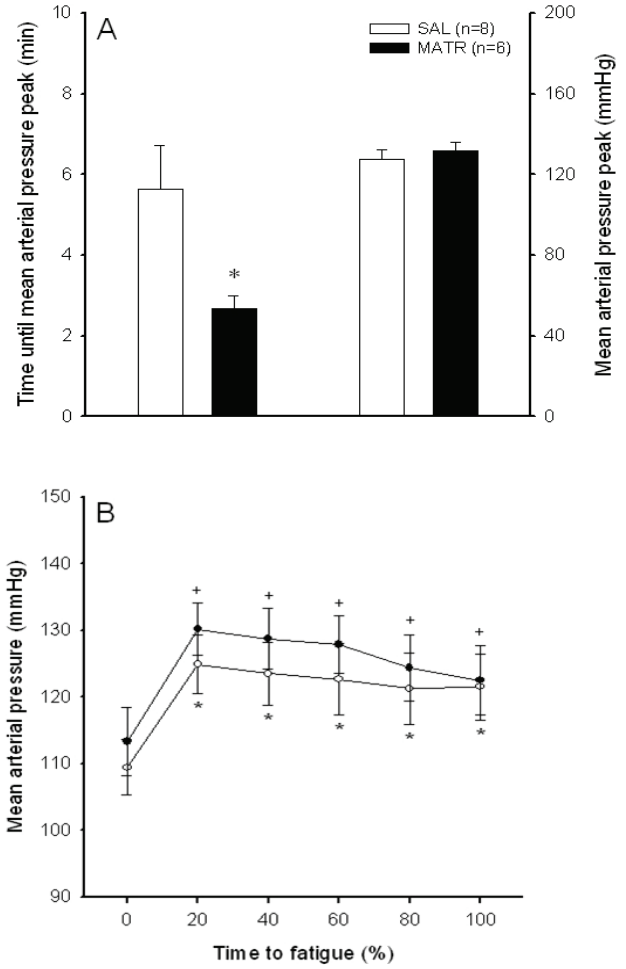

Fig. 4. Effects of bilateral injections of 0.2 µl of either Matr (5 x 10^{-9} mol) or 0.15 M NaCl into the VMH on the time elapsed until the peak pressure response during physical exercise (**A**) and the mean arterial pressure as a function of time to fatigue percentage (**B**).

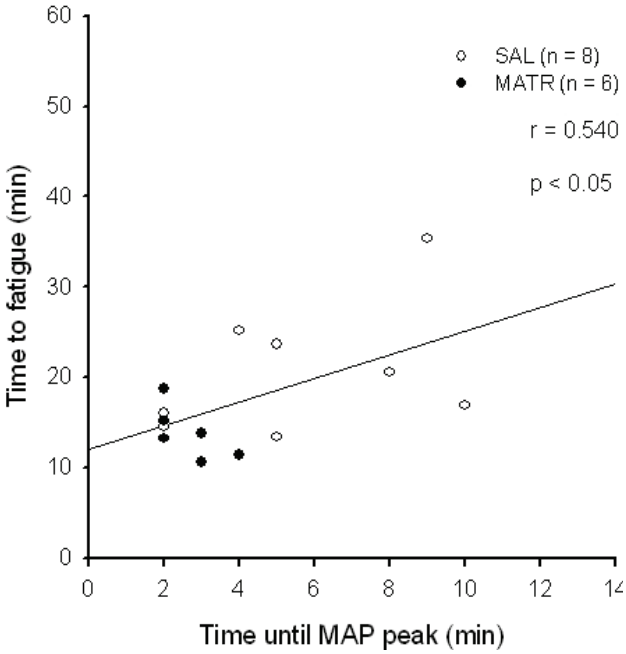

Fig. 5. Correlation between mean arterial pressure peak and time to fatigue in rats treated with 0.2 µl of either 0.15 M NaCl or Matr (5 x 10^{-9} mol) into the VMH.

There is anatomical and physiological evidence consistent with the hypothesis that acetylcholine within the VMH can regulate the cardiovascular system. Pathways linking the AV3V region to the primary cardiovascular control areas in the medulla are thought to pass through midline structures of the hypothalamus (Knuepfer *et al.* 1984, Bastos *et al.* 1997, do Vale *et al.* 1997). VMH may be one of these relay structures in the hypothalamus because it receives afferents from the medial preoptic area and projects to many nuclei in the forebrain and brain stem with connections to sympathetic preganglionic neurons, such as the dorsomedial and paraventricular hypothalamus, midbrain periaqueductal gray, and the nucleus of the solitary tract in the medulla (Luiten and Room 1980, Canteras *et al.* 1994).

Sympathetic outflows to individual organs can be differentially affected by central stimulation or autonomic reflexes. The existence of specific neuronal pathways controlling autonomic activity to different organs provides the structural substrate for their differential sympathetic regulation (Morrison 2001). In our experiments, methylatropine injection into the VMH did not modify the exercise-induced increase in heart rate (Fig. 3D), suggesting that the control of sympathetic outflow by the VMH may be site specific, as previously described by Marsh *et al.* (2003).

It is well known that central cholinergic stimulation increases mean arterial pressure in resting rats and that the administration of atropine, in doses that prevent or attenuate the cholinergic agonists' actions, has no effects on blood pressure (Buccafusco and Brezenoff 1979, Alves *et al.* 2007). In the present experiments, the methylatropine injections shortened the time to the MAP peak by approximately 3 min (Fig. 4A). However, the different temporal response in methylatropine-treated rats was not followed by changes in magnitude; the cholinergic blockade did not modify the maximal absolute values of blood pressure. Therefore, the cardiovascular effects induced by VMH muscarinic cholinoceptors stimulation in resting rats and those caused by blockade during exercise are qualitatively different, although this result may seem paradoxical when related to previous reports.

Another relevant point is that physical exercise changes the neural control of the cardiovascular system. The central command arising from the cortical areas and

the feedback signals arising from different sensors in the periphery, including the contracting muscles, are integrated at the central nervous system (CNS) to directly reset feed-forward stimuli to sympathetic and motor systems (Lambert *et al.* 2005). There is evidence that the VMH may constitute one of these relay areas in the CNS because this hypothalamic nucleus modulates substrate mobilization and hormonal secretion during exercise in a way that is completely opposite to the observed effect in resting rats (Vissing *et al.* 1989). Therefore, it is reasonable to suggest that exercise may activate an alternative cholinergic pathway that passes through the VMH and that is not tonically activated under resting conditions.

Blood pressure (Fig. 3) and core temperature (Wanner *et al.* 2007) adjustments seem to be very similar when both treatments – methylatropine and saline – are set as a function of percentage of time to fatigue (Fig. 4B). These observations suggest that acetylcholine in the VMH may participate in a finely-balanced mechanism that determines the sympathetic outflow for a given performed workload. In this case, the cardiovascular effects promoted by muscarinic blockades may be indirect and evoked by disrupted central afferent-efferent integration that overestimates the blood perfusion requirement of active tissues.

The anticipated blood pressure adjustments in running rats treated with methylatropine suggests that the VMH muscarinic cholinoceptors may exert inhibitory effects in a central pathway that stimulates sympathetic outflow during the initial minutes of exercise. The data concerning the control of cutaneous vasomotor tonus are consistent with this hypothesis because the bilateral blockade of VMH muscarinic cholinoceptors led to a delayed exercise-induced increase in skin temperature (Fig. 2). This delay suggests a higher sympathetic outflow to tail vessels because it is accepted that cutaneous vasodilation is mainly a consequence of tail sympathetic activity withdrawal (O'Leary *et al.* 1985, Yanagiya *et al.* 1999). Thus, when the organism senses that these responses are not compatible with prolonged and sustained effort, the feeling of fatigue increases and the rats cannot run at the predetermined intensity.

The present data also support previous experiments showing that impaired hypothalamic cholinergic neurotransmission limits exercise performance (Lima *et al.* 1998, 2001, Wanner *et al.* 2007). However, we cannot assume that acetylcholine is the only neurotransmitter involved in fatigue development because both the blockade of nitric oxide or angiotensin II neurotransmission (Lacerda *et al.* 2005, 2006a, 2006b, Leite *et al.* 2006, 2007) and exacerbating serotoninergic activity (Soares *et al.* 2003, 2004, 2007) decrease exercise performance by either increasing the heat storage rate or reducing mechanical efficiency. Physical exercise induces simultaneous and different adjustments in many physiological systems, and acute fatigue acts as a protective multifactorial mechanism (Noakes 2000). Therefore, it is likely that this sensation results from an interaction of several neurotransmitters and brain areas (Meeusen *et al.* 2006) that, together, signal that an effort can no longer be sustained without risks to body homeostasis, thus reducing muscular activation.

There is experimental evidence showing that elevated brain temperatures (Ansley *et al.* 2009), high heat storage rates (Rodrigues *et al.* 2003, Marino *et al.* 2004), reductions in either mechanical efficiency (Soares *et al.* 2003, Lacerda *et al.* 2006a, Leite *et al.* 2007) or muscle glycogen (Rauch *et al.* 2005), and other factors may signal for effort cessation. We have previously provided insights into this multifactorial characteristic of fatigue, using pharmacological manipulations of cholinergic neurotransmission. Intracerebroventricular physostigmine injection enhanced tail heat loss mechanisms and thus attenuated the exercise-induced increase in core body temperature. Despite the lower core temperature at the fatigue point, central cholinergic stimulation failed to increase exercise performance, suggesting that other physiological systems could be involved in the fatigue sensation under these experimental conditions (Rodrigues *et al.* 2004, 2008, Pires *et al.* 2007).

The present data corroborate the finding that the redistribution of blood flow between vascular beds is a limiting factor to prolonged physical effort. A higher sympathetic tone to skin vessels may shift a greater amount of blood from the periphery to central circulation and may contribute to a faster increase in MAP. This may explain the delay in activating the tail heat loss mechanism, which we have previously shown to occur at higher core temperatures in methylatropine-treated rats (Wanner *et al.* 2007). The delayed cutaneous vasodilation increases the heat storage rate, contributing to the reduced performance of these animals.

In conclusion, muscarinic cholinoceptors within the VMH are involved in neuronal circuitry that modulates exercise-induced blood pressure adjustments and heat loss mechanisms. Furthermore, our data suggest that a VMH muscarinic blockade increases sympathetic

outflow during the initial phase of exercise; this increase may be related to the reduced performance observed in rats treated with methylatropine.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The authors are indebted to the Coordenadoria de Apoio ao Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and Pró-Reitoria de Pesquisa da Universidade Federal de Minas Gerais for financial support. We also acknowledge the technical assistance of André Luis Pimenta de Faria and Maria Aparecida Vasconcelos Faria.

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