Título Geral Do Projeto: Efeitos do treinamento físico sobre a obesidade induzida pela dieta hipercalórica e palatável.
Ref.CBB 1443/05 período 2006 a 2007

Coordenador: Cândido Celso Coimbra, Prof. Adjunto Doutor
Relatório Final

(Anexo 11)

Balthazar CH, Leite LRH, Coimbra CC. *Ergogenic effect of brain dopaminergic activity in rats.* Em preparação
Title: Ergogenic effect of brain dopaminergic activity in rats

Keywords: Exercise; Body temperature; Fatigue; Oxygen consumption; Mechanical efficiency; Running performance

Abstract: To assess the role of central dopaminergic (DA) systems in the metabolic rate, thermoregulatory mechanisms, and running performance, 2.0 μL of DA solution [5 x 10^{-3} M] (DA, n = 6) or 2 μL of 0.15 M NaCl (n = 6) was intracerebroventricularely (icv) injected in male Wistar rats immediately before animals started running in a progressive exercise protocol until fatigue (starting at 10 m.min^{-1}, 5% inclination). Oxygen consumption (VO2) and body temperature (Tb) were recorded at rest, during exercise, and following 30 min of recovery. Body heating rate (BHR), heat storage (HS), workload (W) and mechanical efficiency (ME) were calculated during exercise. Heat loss rate (HLR) was calculated during recovery period. DA treatment induced a marked increase in running performance and W (W: 10.7±2.5 kgm, DA, vs. 5.9±1.3 kgm, NaCl, p<0.05) compared to NaCl treatment. At fatigue point, DA treated rats attained greater VO2 max. and Tb levels than NaCl rats (VO2 max.: 44.9±4.5 mL O2.kg^{-1}.min^{-1}, DA, vs. 37.4±2.7 mL O2.kg^{-1}.min^{-1}, NaCl, p<0.05; Tb: 39.67±0.30 °C, DA, vs. 38.96±0.24 °C, NaCl, p<0.05). Despite higher VO2 max and Tb attained during exercise, DA treated rats reached VO2 basal values within the same recovery period and dissipated heat ~ two times faster than NaCl treated rats. BHR and ME were similar between treatment groups; nevertheless HS was greater in DA than NaCl treated animals (HS: 519.8±93.4 cal, DA, vs. 336.2±34.6 cal, NaCl, p<0.05). Our data demonstrate that DA icv injection has an ergogenic effect without changing ME, improving post-exercise recovery of metabolic rate and heat loss. The increased DA
availability in CNS improves tolerance to heat and to exercise-induced elevated metabolic rate. Finally, our results provide evidence that central dopaminergic systems have important effects on metabolic rate, thermoregulation, and CNS drive for exercise performance.

Suggested Reviewers: Romain Meesen PhD
Professor, Department of Human Physiology and Sports Medicine, Vrije Universiteit Brussel
rmeusen@vub.ac.be
Expert in exercise and central amines.

Luiz G Siqueira Branco PhD
Full Professor, Departamento de Morfologia, Estomatologia e Fisiologia, Faculdade de Odontologia de Ribeirão Preto, USP, 14040-904, Ribeirão Preto, São Paulo, Brazil
bco@forp.usp.br
Expert in thermal biology.

Hiroshi Hasegawa PhD
Professor, Laboratory of Exercise Physiology, Faculty of Integrated Arts and Sciences, Hiroshima University, Higashihiroshima, Japan
hasehiro@hiroshima-u.ac.jp
Expert in thermal biology.

David H Wasserman PhD
Professor, School of Medicine, Vanderbilt University
david.wasserman@mcmail.vanderbilt.edu
Expert in exercise physiology.

Michael R Drew PhD
Professor, Division of Integrative Neuroscience, Department of Psychiatry, College of Physi, Columbia University, New York
mrd28@columbia.edu
Expert in neuropharmacology and behavioral neuroscience.

Opposed Reviewers:
1. Introduction

The increase in body core temperature ($T_b$) that occurs in response to continuous or progressive exercise results from the temporary imbalance in the rates of metabolic heat production and heat dissipation during the early stage of exercise [Watson et al., 2005; Gieson, 1998]. The energy efficiency of the body becomes apparent during exercise when ~20–27% of the energy expended can be used for external work. The remaining adenosine triphosphate (ATP) production is used for homeostasis or dissipated as heat [Brooks et al., 1984]. During exercise, oxygen consumption (i.e., total energetic cost of physical work) is an important parameter that reflects both mechanical efficiency and running performance [Sonne and Galbo, 1980; Brooks and White, 1978]. Therefore, any modification in the rates of metabolic heat production and/or heat dissipation will influence the heat balance during exercise.

High $T_b$ is considered to be a limiting factor during prolonged physical exercise [González-Alonso et al., 1999; Fuller et al., 1998; Nielsen et al., 1993; Jessen, 1987; Caputa et al., 1986] and is associated with reduction in central nervous system (CNS) drive for exercise performance [Walters et al., 2000; Nielsen et al., 1990; Nielsen et al., 1997] that leads to the termination of work in animals [Fuller et al., 1998] and healthy humans [González-Alonso et al., 1999; MacDougall et al., 1974]. In addition, the hypothesis that hyperthermia precipitates feelings of fatigue at a sublethal threshold and thus, establishes a safe guard against heat stroke by protecting the brain from thermal damage is supported by various studies [Walters et al., 2000; Jessen, 1987; Caputa et al., 1986]. It has been demonstrated that dopamine (DA) and DA receptor agonists acting on the brain exert thermoregulatory effects that include changes in central temperature set-point, hypothermia, and anaplexia [Benaliouad et al., 2007; Drew et al., 2007; Yavich and Tanila, 2007; Bressan and Crippa, 2005; Oerther, 2000; MacDougall et al., 1974]. DA receptor agonists and antagonists have been used to investigate the role of DA in $T_b$ [Chaperon et al., 2003; Varty and Higgins, 1998; Nunes et al., 1991; Salmi et al., 1994; Salmi et al., 1993]. Several studies have observed that intracerebroventricular (icv) injection of DA blockers causes an increase in the $T_b$ of rats, indicating
that central DA plays a tonic role in reducing $T_b$ [Chaperon et al., 2003; Varty and Higgins, 1998; Nunes et al., 1991; Salmi et al., 1994; Salmi et al., 1993]. Moreover, in the case of D-methamphetamine-induced hyperthermia both SCH23390 and eticlopride (D1 and D2 DA receptor antagonists, respectively), reduce the depletion of striatal DA concentration, exerting a neuroprotective action and reducing the hyperthermic reaction, thus supporting the hypothesis that central DA is involved in reduction of $T_b$ [Broening et al., 2005]. Therefore, considering that fatigue is coincident or may be precipitated by high $T_b$ and/or heat storage, activation of a central mechanism that would increase heat loss and decrease core temperature might improve exercise performance.

Since hypothermia and increased heat dissipation may be neuroprotective, the activation of central dopaminergic systems may exert important effects on thermoregulation during exercise, influencing running performance. It is important to emphasize that there are other physiological functions modulated by central DA, such as arousal, reward, motivation, sympathetic nervous system activity, stress response, motor behavior and, addiction mechanisms that could, similarly, modify running performance [Benaliouad et al., 2007; Drew et al., 2007; Leblanc and Ducharme, 2007; Yavich and Tania, 2007; Bressan and Crippa, 2005; Meeusen, 2005; Nestler et al., 2001; Mannelli et al., 1999; Guerrera, 1999; Americ et al., 1984]. Taking into account that central DA metabolism is enhanced during exercise in animals [Nybo and Secher, 2004] and that central DA depletion has been linked to CNS fatigue [Davis, 2000; Davis and Bailey, 1997; Owasoyo et al., 1992; Chaoulloff, 1989], the aim of this current study is to assess the effects of central administration of DA on heat balance, energetic cost, and exercise tolerance in untrained rats submitted to progressive exercise until fatigued.
2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) were housed individually at a room temperature of 22 ± 2°C under 14-h light: 10-h dark cycles and had free access to water and rat chow. Following anesthesia, achieved using an association of Ketamin and Xylazin (2.0 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 [Rodrigues et al., 2004; Soares et al., 2004; Soares et al., 2003; Lima et al. 2001; Lima et al., 1998]. During the same surgical procedure, a TR3000 VM-FH temperature sensor (Mini Mitter, Sun River, OR) was implanted into the peritoneal cavity through a small incision in the linea-alba. Following the surgical procedure, the rats received a single dose of analgesic (Flunixin 0.11 mg/100 g body weight; IM) and antibiotic (Pentabiolóico®, Fort Dogde, Brazil, 0.2 ml; IM). All animals were allowed to recover for at least 1 week before being submitted to the test exercise protocol. The rats were familiarized to exercise on the metabolic motor-driven treadmill by running 5 min per day, at 5% inclination, during five consecutive days at a speed of 10 m·min⁻¹, on the first and second days, and at 11, 13 and 15 m·min⁻¹ on subsequent days. The purpose of this preliminary exercise was to teach the animals the direction to run and to avoid conditioned hyperthermia [Briese, 1998]. This protocol was approved by the Ethics Committee for the Care and Use of Laboratory Animals of the Federal University of Minas Gerais and was carried out in accordance with the regulations described in the Committee’s Guiding Principles Manual (protocol 057/05).

2.2. Exercise

Progressive work was performed on a metabolic motor-driven treadmill (Columbus Instruments, OH, USA) at a constant inclination of 5%. The rats started running at 10 m·min⁻¹ and treadmill speed was increased by 1 m·min⁻¹ every 3 min until fatigue. Fatigue was defined as the point at
which the animals were no longer able to keep pace with the treadmill [Rodrigues et al., 2004; Soares et al., 2004; Soares et al., 2003]. Time to fatigue (minutes) and workload (kgm) were considered indexes of running performance.

2.3. Experimental protocol

On the day of the test exercise, the animals were allowed to rest for 1 h on the treadmill 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 1 min prior to exercise, 2.0 μL of 0.15 M NaCl or 2.0 μL of DA solution [5 x 10⁻³ M] was injected into the right lateral ventricle. This dosage of DA was selected based on the results of our previous experiments that showed a clear dose-dependent response on resting oxygen consumption after DA icv injection. These previous experiments demonstrated a reduction of ~200% on resting metabolic rates in rats after injection of this DA selected dose [5 x 10⁻³ M].

According to the literature, DA generally produces inhibitory effects on most neurons in CNS but in high concentrations may produce excitatory effects, similarly to those produced by noradrenaline [Greenspan and Gardner, 2004; Katzung, 2001; Rang et al., 2001]. Rats were randomly assigned to groups receiving either NaCl or DA solution. An interval of at least three days was allowed for the animals to recover between test exercises.

Tₚ was measured by telemetry (Mini Mitter, Sun River, OR). Oxygen consumption (VO₂) was measured by open-flow indirect calorimeter (Columbus Instruments), calibrated before each use with a certified mixture of gases (20.5% O₂ and 0.5% CO₂). VO₂ (mL O₂ · kg⁻¹ · min⁻¹) and Tₚ (°C) were continuously recorded on-line by a computerized system (Oxymax Apparatus, Columbus Instruments for VO₂ and Mini Mitter, Sun River, OR for Tₚ registrations).

2.4. Calculations

Body heating rate (BHR; °C · min⁻¹), rate of increase in core temperature, was calculated as BHR = Δ Tₚ / (running time interval), where Δ Tₚ represents the change in core temperature (Tᵢ - Tᵢ), where Tᵢ represents core temperature at fatigue point and Tᵢ represents initial core temperature measured
prior to exercise. Heat storage was calculated [Gordon, 1993] as \( HS = (\Delta \ T_b) \cdot m \cdot c \), where "m" represents body weight in grams and "c" represents specific heat of the body tissues (0.826 cal g\(^{-1}\) \cdot C\(^{-1}\)). Heat loss rate (HLR, °C \cdot min\(^{-1}\)), rate of decrease in core temperature, was calculated as HLR = \( \Delta \ T_b / \text{(recovery time interval)} \), where \( \Delta \ T_b \) represents the change in core temperature \((T_t - T_f)\), \( T_t \) represents core temperature at fatigue point and \( T_f \) represents core temperature measured after recovery period. Workload (\( W \); kgm) was calculated as \( W = [\text{body weight (kg)}] \cdot [\text{TTF}] \cdot [\text{treadmill speed (m min}\(^{-1}\))] \cdot [\sin \theta \ (\text{treadmill inclination})] \) [Lima et al., 1998; Brooks et al., 1984; Brooks and White, 1978], where TTF is time to fatigue (minutes). Mechanical efficiency (ME; %) was calculated in two phases of progressive exercise intensity: 0-40% of VO\(_2\) max. and 60-100% of VO\(_2\) max. by the formula: \( ME = (\text{Wenergetic cost}) \cdot 100 \) [Lacerda et al., 2006; Soares et al., 2003; Brooks et al., 1984]

2.5. Statistical analysis

The data are reported as means ± standard error means (S.E.M.). Differences between groups and the effect of time were evaluated using ANOVA, followed by the Newman-Keuls test. The data were also compared using paired or unpaired Student’s t-tests, as applicable. All of the correlations presented in this study were assessed using Pearson’s correlation coefficient and the significance level was set at \( p < 0.05 \).
3. Results

As illustrated in Fig. 1A, icv injection of DA (DA treatment, n=6 rats) induced a marked increase in running performance and workload (W: 10.7±2.5 kgm, DA, vs. 5.9±1.3 kgm, NaCl, p<0.05) compared to NaCl treated rats (NaCl treatment, n = 6 rats).

Exercise induced a gradual increase in VO₂ in both treatment groups (DA and NaCl). This VO₂ increase is represented in similar data curves with the maximum metabolic rate (VO₂max) attained at fatigue point in both treatment groups (Fig. 1A). However, NaCl treated rats fatigued earlier than DA treated rats, while DA treatment induced a VO₂ max greater than NaCl treatment (VO₂max: 44.9±4.5 mL O₂ . kg⁻¹ . min⁻¹, DA, vs. 37.4±2.7 mL O₂ . kg⁻¹ . min⁻¹, NaCl p<0.05). After a 30 minute recovery period, despite the greater VO₂ max attained by DA treated rats, the metabolic rate of both treatment groups returned to basal values. Thus, DA treated rats attained VO₂ resting values more rapidly than NaCl treated rats (Fig. 1B).

Exercise also induced a gradual rise in T_b in both treatment groups (DA and NaCl). This T_b increase is represented in similar data curves with the maximum T_b attained at fatigue point in both treatment groups. However, at fatigue point, T_b was greater in the DA treated rats than in the NaCl treated rats (T_b: 39.67±0.30 ºC, DA, vs. 38.96±0.24 ºC, NaCl, p<0.05)(Fig. 2A). Despite greater T_b, DA treated rats were more tolerant to exercise resulting in a fatigue point that occurred 10 min later than in the NaCl treated rats (p<0.05; Figs. 1A and 2A). After a 30 minute recovery period, T_b in both treatment groups did not return to basal values (Fig. 2B), although, DA treated rats dissipated heat approximately two times faster than NaCl treated rats. This was especially true during the first 15 min of the recovery period as shown by HLR values presented in Figure 3. Already at 15th minute of recovery period, DA rats showed similar T_b values to NaCl rats.

To compare the overall thermal effects of exercise across treatment groups, BHR and HS were calculated and are shown in Figure 4. There were no differences in BHR between treatment groups; nevertheless HS values were greater in DA than NaCl treated animals (HS: 519.8±93.4 cal, DA, vs. 336.2±34.6 cal, NaCl, p<0.05).
ME values were similar between across treatment groups during both phases of exercise (ME at 0-40%: 19.3±4.0 %, DA vs. 20.2±3.9 %, NaCl; ME at 60-100%: 7.6±1.4 %, DA vs. 9.1±1.4 %, NaCl).

The correlations, presented in Figure 5, demonstrate that VO$_2$ max (r = 0.89, p<0.01, Fig. 5A) as well as $T_b$ at fatigue point (r = 0.74, p<0.01, Fig. 5B) were strongly correlated with total workload.
4. Discussion

The present study demonstrated that the increased DA availability in CNS during exercise has an
ergogenic effect. Furthermore, DA icv injection increased animal tolerance to exercise despite high
$T_b$ ($\sim 40^\circ C$) postponing fatigue point. On the other hand, DA treatment promotes a greater heat
dissipation rate during the recovery period than SAL treatment, showing an improved heat loss in
these DA treated animals. Therefore, our data suggest that central DA-mediated pathways are
involved not only in thermoregulatory heat loss, but also involved in the ability of SNC neurons to
detect, prevent and protect the brain against thermal damage caused by high body temperatures.
The balance between heat production and heat loss determines internal body temperature in
homeothermic animals. Increased $T_b$ during exercise is the consequence of an increase in
metabolic rate and the failure of heat loss to keep pace with heat production [Gleeson, 1998; Webb,
1995; Jessen, 1987]. Elevated internal body temperature and increased heat storage have been
considered limiting factors in exercise by reducing CNS drive for performance, precipitating feelings
of fatigue, thus protecting the brain from thermal damage [Fuller et al., 1998; Nielsen et al., 1997].
This hyperthermia and reduced physical performance relationship has been shown in many
mammalian species, including rodents [Walters et al., 2000; Fuller et al., 1998; Nielsen et al., 1993].
Various studies provide evidence that central DA plays an important role in thermoregulatory
mechanisms leading to heat loss and reduced $T_b$ by inducing a.) central temperature set-point
adjustments, b.) increasing heat dissipation through skin vasodilatation, and c.) decreasing metabolic
rate [Reitsamer et al., 2004; Owasoyo et al., 1992; Americ et al., 1984], thus influencing
performance and lengthening total exercise time [Hasegawa et al., 2007; Watson et al., 2005]. Our
data also show that central DA exerts an ergogenic effect without impairment in mechanical
efficiency during all progressive exercise phases.
The exact location and precise central DA pathways involved in the effects of icv injection of DA
observed in this study still require clarification. However, we hypothesize that icv injection of DA
perfuse to thermoregulatory and metabolic centers, situated in the hypothalamus, augmenting the
heat tolerance and/or changing the critical $T_b$ point that leads to fatigue. Previous studies also
indicate that the so-called critical $T_b$ can be bypassed when animals are running in the heat in the presence of high concentrations of catecholamines in the brain [Hasegawa et al., 2007].

The hypothalamus is where tuberoinfundibular and tuberohypophyseal dopaminergic systems are located, both of which are involved in modulation of SNS and hence metabolic rate [Leblanc and Ducharme, 2007; Greenspan and Gardner, 2004; Katzung, 2001; Rang et al., 2001; Gurrera, 1999; Mannelli et al., 1999; Americ et al., 1984].

The preoptic area/anterior hypothalamus (POA/AH) is thought to be the primary locus for body temperature regulation [Briese, 1998; Santos et al., 1991; Santos et al., 1990; Coimbra and Migliorini, 1988] due to the fact that it contains both warm-sensitive and cold-sensitive neurons that respond to small changes in temperature [Ishiwata et al., 2002; Zhang et al., 1997]. It has been established that the POA/AH is an integrative region for the maintenance of metabolic, vasomotor, and thermal homeostasis in resting conditions as well as during exercise [Hasegawa et al., 2005; Ferreira et al., 1999]. These data indicate that the POA/AH might be an important mediator of heat production during exercise. The icv injected DA could also perfuse to mesolimbic structures and activate a common reward circuit such as the mesolimbic dopaminergic system [Bressan and Crippa, 2005; Greenspan and Gardner, 2004; Katzung, 2001; Rang et al., 2001]. It is possible the mesolimbic dopaminergic system could contribute to the ergogenic effect of DA treatment. The actions of DA in these reward circuits have been linked to changes in motivation, reward, satisfaction, and comfort, as well as in effort perception [Benaliouad et al., 2007; Drew et al., 2007; Yavich and Tanila, 2007; Bressan and Crippa, 2005; Meeusen, 2005; Nestler et al., 2001].

In summary, the study reported herein demonstrates that icv injection of DA results in an ergogenic effect inducing a marked increase in running performance, workload output, and VO$_2$ max without changing mechanical efficiency. In addition, DA treatment improves post-exercise recovery of metabolic rate and heat loss. The increased DA availability in CNS effects tolerance to heat and to elevated metabolic rate induced by exercise. Finally, our results provide evidence that central dopaminergic systems have important effects on metabolic rate, thermoregulation, and CNS drive for exercise performance.
Acknowledgements

The authors thank CNPq, CAPES, FAPESP, and FAPEMIG for financial support. The technical assistance of André Luis Pimenta de Faria, Janine Costa Ivo and Patrícia Andrade Guimarães Mittre are also acknowledged.
References


Legends to figures:

Fig. 1. Effect of icv injection of 2 μL of dopamine solution [5x10⁻³ M] (DA) or 2 μL of 0.15 M NaCl (NaCl) on oxygen consumption (VO₂) during exercise (A), and recovery period (B). On the panel A, the values represent the animals that were still exercising and the number in the brackets represents the rats that were not fatigued. Data are expressed as mean ± S.E.M., n = 6 in each treatment. * p<0.05 compared with NaCl fatigue point; ** p<0.05 compared with NaCl; # p<0.05 compared with corresponding basal value.

Fig. 2. Effect of icv injection of 2 μL of dopamine solution [5x10⁻³ M] (DA) or 2 μL of 0.15 M NaCl (NaCl) on body temperature (Tₘ) during exercise (A), and recovery period (B). On the panel A, the values represent the animals that were still exercising and the number in the brackets represents the rats that were not fatigued. Data are expressed as mean ± S.E.M., n = 6 in each treatment. * p<0.05 compared with NaCl fatigue point; ** p<0.05 compared with NaCl; # p<0.05 compared with corresponding basal value.

Fig. 3. Effect of icv injection of 2 μL of dopamine solution [5x10⁻³ M] (DA) or 2 μL of 0.15 M NaCl (NaCl) on heat loss rate (HLR) during recovery period. Data are expressed as mean ± S.E.M., n = 6 in each treatment. * p<0.05 compared with NaCl.

Fig. 4. Effect of icv injection of 2 μL of dopamine solution [5x10⁻³ M] (DA) or 2 μL of 0.15 M NaCl (NaCl) on body heat rate (BHR) and on heat storage (HS). Data are expressed as mean ± S.E.M., n = 6 in each treatment. * p<0.05 compared with NaCl.

Fig. 5. Correlation between oxygen consumption at fatigue point (VO₂ max.) and total workload (W) (Panel A: r = 0.89, p<0.01), and between body temperature (Tₘ) at fatigue point) (Panel B: r = 0.74, p<0.01) in rats treated with 2 μL of dopamine solution [5x10⁻³ M] (DA) or 2 μL of 0.15 M NaCl (NaCl). Data are expressed as mean ± S.E.M.
Figure 1

Click here to download Figure: Figure 1.doc