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Pharmacology, Biochemistry and Behavior 78 (2004) 255–261

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Intracerebroventricular tryptophan increases heating and heat storage rate in exercising rats

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Received 9 September 2003; received in revised form 23 March 2004; accepted 24 March 2004
Available online 6 May 2004

Abstract

The role of increased hypothalamic tryptophan (TRP) availability on thermoregulation and rates of core temperature increase and heat storage (HS) during exercise was studied in normal untrained rats running until fatigue. The rats were each anesthetized with 2.5% tribromoethanol (1.0 ml kg^{-1} ip) and fitted with a chronic guiding cannula attached to the right lateral cerebral ventricle 1 week prior to the experiments. Immediately before exercise, they were randomly injected through these cannulae with 2.0 μl of 0.15 M NaCl (SAL; $n=6$) or 20.3 μM L-TRP solution ($n=7$). Exercise consisted of running on a treadmill at 18 m min^{-1} and 5% inclination until fatigue. Body temperature was recorded before and during exercise with a thermistor probe implanted into the peritoneal area. Rates of core temperature increase (HR, °C min^{-1}) and heat storage (HSR, cal min^{-1}) were calculated. TRP-treated rats showed a rapid increase in body temperature which was faster than that observed in the saline-treated group during the exercise period. The TRP group also showed a higher rate of core temperature increase and HS. TRP-treated rats that presented higher HR and HSR also fatigued much earlier than saline-treated animals (16.8 ± 1.1 min TRP vs. 40 ± 3 min SAL). This suggests that the reduced running performance observed in TRP-treated rats is related to increased HR and HSR induced by intracerebroventricular injection of TRP in these animals.

Keywords: Tryptophan; Body temperature; Exercise; Heat storage rate; Fatigue

1. Introduction

Prolonged physical exercise has been demonstrated to increase the availability of tryptophan (TRP) to the brain (Farris et al., 1998; Newsholme and Blomstrand, 1996; Chaouloff et al., 1989). Several experimental approaches have been used to test if it is also related to fatigue. According to Newsholme et al. (1992), fatigue during prolonged exercise may be influenced by the activity of the brain serotonergic system and this is commonly referred to as the "central fatigue hypothesis." Its major premise is that elevated central TRP availability increases serotonin (5-HT) activity during prolonged exercise, which may cause fatigue by increasing lethargy and loss of central drive/motivation (Newsholme et al., 1992). However, central 5-HT activity can also affect many physiological responses, such as pain tolerance (Prieto-Gomez et al., 1989), motor activity (Gerin and Privat, 1998), thermoregulation (Imeri et al., 2000; Lin et al., 1998; Fregly et al., 1996; Myers, 1981) and activity of the hypothalamo–pituitary–adrenal axis (Chaouloff, 2000; Korte et al., 1991). Thus, alterations in one or many of the physiological responses mediated by serotonergic systems may decrease work capacity during exercise. It is well established that during exercise from moderate to high intensities, particularly in hyperthermic environments, core temperature rises and it can reach dangerous levels and exercise will be terminated due to the symptoms of fatigue (Rodrigues et al., 2003; Fuller et al., 1998; Gleeson, 1998; González-Alonso et al., 1999; Nielsen et al., 1993). As core temperature rises, there is a corresponding decrease in working capacity in many mammalian species, including rodents (Rodrigues et al., 2003; Walters et al., 2000; Fuller et al., 1998). This loss of

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0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.03.015

motivation to continue exercise is thought to be a reflex inhibition (Brück and Olschewski, 1987) probably arising in hypothalamus or in brain stem, and may involve serotonergic pathways that project to higher centres (Bridge et al., 2003).

We recently showed that intracerebroventricular injection of L-TRP induces a decrease in running time to fatigue, associated with a marked reduction in mechanical efficiency and a higher metabolic rate in rats submitted to submaximal exercise (Soares et al., 2003). We hypothesized that the thermogenic responses observed in exercising rats treated with TRP could produce alterations in the thermoregulation function, such as hyperthermia.

Based on these findings, this study was designed to verify the effect of increased TRP availability to the central nervous system (CNS) on thermoregulatory responses to submaximal exercise at fatigue in untrained rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 270 ± 20 g were used. Animals were housed individually under a 14:10-h light/dark cycle and had free access to water and rat chow. Intraperitoneal temperature sensors and brain guide cannulae (22 G) were implanted under intraperitoneal anesthesia with 1.0 ml kg^{-1} of 2.5% tribromoethanol (Aldrich, Milwaukee, WI). A VitalView Mini-Mitter TR3000 XM-FM (Sun River, Oregon, USA) temperature sensor was implanted into the peritoneal cavity through a small incision in the linea alba, after calibration to a precision of 0.01 °C. After telemeter implantation, the animals were placed in a stereotaxic apparatus (David Kopf 900) and brain guide cannulae were implanted according to a previously described technique (Soares et al., 2003; Lima et al., 2001, 1998). All animals were allowed to recover for 1 week before being used in the experiments. The animals were familiarized to exercise in the motor-driven treadmill by running them daily at a constant speed of 18 m min^{-1} and 5% inclination for 5 min for 5 days before the experiments. All experiments were carried out according to guidelines established by the Federal University of Minas Gerais Ethical Committee for care and use of laboratory animals.

2.2. Temperature recording

Core temperature was monitored throughout the experimental period and recorded every 15 s. Temperature measurements started as soon the animals were placed in the treadmill chamber. Rats were killed after the studies were completed, their radiotelemeters removed and the implant sites checked for signs of infection, inflammation or other tissue pathology.

2.3. Exercise

Experiments were performed on a motor-driven treadmill from 1000 to 1400 h at room temperature (23 ± 2 °C). The intensity of exercise was 18 m min^{-1} and 5% inclination, corresponding to an oxygen uptake of 66% of $VO_{2\ max}$ (Lima, 2000; Brooks and White, 1978). Exercise time to fatigue (min) was taken as an index of maximal capacity for exercise. Fatigue was defined as the point when the animals were unable to keep pace with the treadmill.

2.4. Experimental protocol

On the day of the experiment, the animals were allowed to rest on the treadmill chamber (Columbus Instruments, Ohio, USA, Modular Treadmill, series 96002-2) for 1 h in the experimental room before running test. A 30-G needle protruding 0.3 mm from the tip of the guide cannula was introduced into the right lateral ventricle by connecting to a Hamilton syringe. Immediately before the exercise, 2.0 μl of 0.15 M NaCl ($n = 6$) or 2.0 μl of 20.3 μM of L-TRP solution ($n = 7$; Sigma, St. Louis, MO) was injected into the right lateral cerebral ventricle of each animal. Immediately after the injections, the animals were submitted to a regime of running until fatigue.

2.5. Calculations and statistical analysis

Thermal index (TI) was used for data analysis. It was calculated as integrated areas under the temperature curves during 14 min of exercise (°C 14 min; Steiner and Branco, 2000) because it corresponded to the time that all TRP-treated rats were running. Rate of core temperature increase (HR, °C min^{-1}) was calculated as $HR = \Delta Tc/TTF$. Heat storage rate (HSR, cal min^{-1}) was calculated as $HSR = (\Delta Tc/TTF)\ mc$, where $\Delta Tc = (TF - Ti)$; m = body weight in grams; c = specific heat of the tissues (0.826 cal $g^{-1}.°C^{-1}$); Ti = initial body temperature before exercise; TF = body temperature at fatigue point; TTF = time to fatigue. Heat storage (HS, cal) = HSR TTF (Gordon, 1993).

A two-way analysis of variance (ANOVA) was used for determining differences between time and treatment and also the interaction between them to evaluate the differences in changes of body temperature. Significant interactions observed by ANOVA were further evaluated by the Newman–Keuls post hoc analysis to locate significant differences between means. The data were also compared using paired or unpaired Student's t tests as applicable. Linear regression analysis was used to evaluate the correlation between TTF and HR and HSR. The significance level was set at $P \leq .05$. Data are expressed as mean values ± S.E.M.

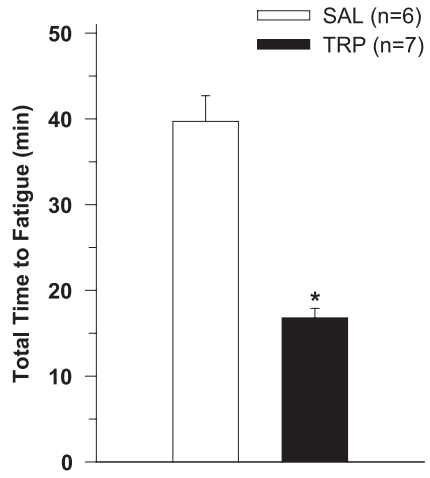

Fig. 1. Effect of intracerebroventricular injection of TRP (20.3 μM) or 0.15 M NaCl on time to fatigue (min) in exercising rats. Data are expressed as mean ± S.E.M. * Significantly different from the control group ($P \leq .0002$).

2.6. Results

As illustrated in the Fig. 1, TRP-treated group ($n = 7$) showed the expected decrease in running time to fatigue (approximately 60%, $P \leq .0002$), the magnitude of which was quite similar to our previous findings (Soares et al., 2003). The two-way ANOVA showed difference along time ($F = 8.64$; $P < .0001$), between treatments ($F = 42.81$; $P < .0001$) and also Time × Treatment interaction ($F = 1.77$; $P = .047$). Exercise-induced hyperthemia was observed in both experimental groups, as illustrated in Fig. 2A. However TRP-treated rats showed a faster increase in BT which was already observed at 8 min after the beginning of exercise (approximately 2% increase over basal value, $P \leq .002$) and attained a maximal plateau close to the fatigue point. As shown in Fig. 2A, TRP-treated rats presented significantly higher body temperatures than saline-treated animals from 9 min after exercise started. These remained higher than in the saline-treated group until they fatigued at 16.8 min. In contrast to the control TRP-treated rats, a gradual increase in body temperature was observed in the group, which was different from basal value at 9 min of exercise (approximately 1%). However, the body temperature of the latter attained maximal value at 15 min after exercise had started and maintained this level until fatigue. Although body temperature at fatigue point in TRP-treated animals was 1.23 °C above the basal level ($P \leq .009$) and was significantly different from saline-treated rats at this time (40 min), the two groups showed similar body temperature at fatigue (38.32 ± 0.12 °C TRP vs. 38.03 ± 0.26 °C SAL). Analysis of TI also revealed a significant difference ($P \leq .05$) between treatments, TRP-treated rats showing a TI approximately 40% higher than the SAL group.

As shown in Fig. 3A, treatment with TRP produced a higher rate of core temperature increase than saline (0.08 ± 0.01 °C min^{-1} TRP vs. 0.02 ± 0.01 °C min^{-1} SAL, $P \leq .002$). It can be seen from Fig. 3B that the rate of core temperature increase was inversely correlated with running time to fatigue in both groups. As with HS, HSR was higher for TRP-treated rats (17.8 ± 2.8 cal min^{-1} TRP vs. 4.7 ± 1.6 cal min^{-1} SAL, $P \leq .002$, Fig. 4A). Although they showed a reduced running time to fatigue (Fig. 1) the HS observed in TRP-treated animals during exercise until fatigue was much higher than in saline-

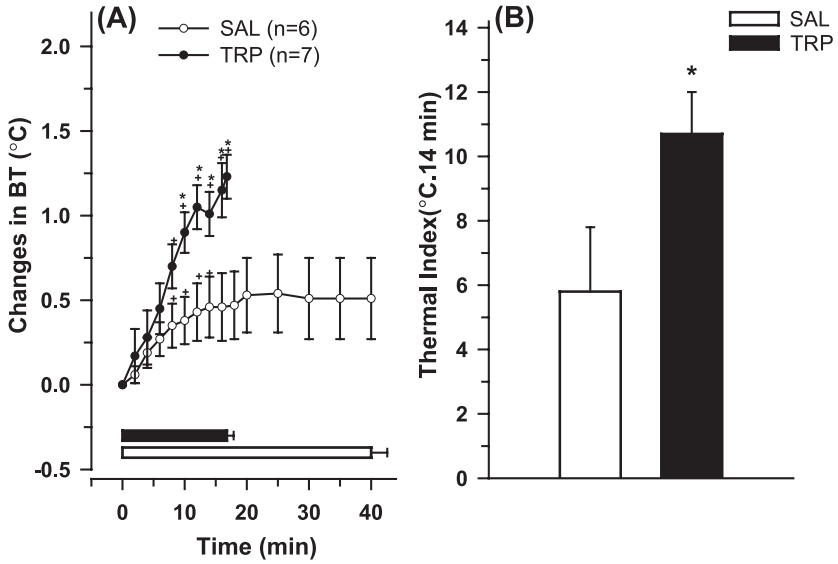

Fig. 2. (A) Effects of intracerebroventricular tryptophan (20.3 μM) or 0.15 M NaCl on changes in body temperature (BT, °C) in exercising rats. Baseline of BT: TRP 37.1 ± 0.12 °C; SAL 37.5 ± 0.18 °C. Values are mean ± S.E.M. in °C of deviation from preinjection levels. Time to fatigue (min) is indicated by the horizontal bar at the bottom of the graph: SAL (open) and TRP (filled). $^{+}P \leq .05$ from basal level; $^{*}P \leq .05$ from saline-treated rats. (B) TI (°C 14 min) after intracerebroventricular injections of TRP (20.3 μM) or 0.15 M NaCl in exercising rats. Data are expressed as mean ± S.E.M. * Significantly different from the control group ($P \leq .05$).

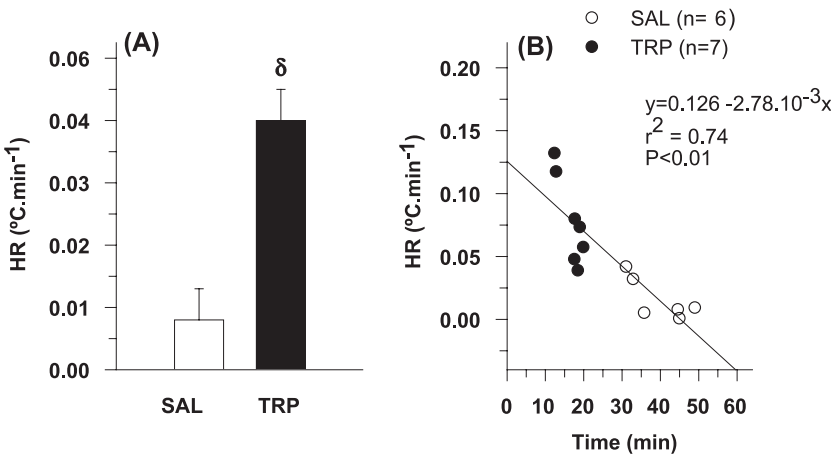

Fig. 3. (A) Mean value of rate of core temperature increase (HR, °C min^{-1}) after intracerebroventricular injections of TRP (20.3 μM) or 0.15 M NaCl in exercising rats. Data are expressed as mean ± S.E.M. $^{\delta}P \leq .002$ from control group. (B) Correlation between rate of core temperature increase (HR, °C min^{-1}) as function of time to fatigue (min) after intracerebroventricular injection of TRP (20.3 μM) or 0.15 M NaCl in exercising rats.

treated animals (282.2 ± 27.4 cal TRP vs. 134.5 ± 48.9 cal SAL, $P \leq .05$).

3. Discussion

The results of the present study provide further evidence that an increase in hypothalamic TRP is related to fatigue and confirms our previous observations that TRP injections into the cerebral ventricle drastically reduces (by 60–70%) running time to fatigue in rats submitted to submaximal exercise (Soares et al., 2003). The present investigation also provides new evidence that central TRP affects thermoregulation and exercise performance by elevating the rate of core temperature increase, HSR and HS until fatigue (Figs. 2A, 3A and 4A). In addition, our results showed that the rate of core temperature increase and HSR during exercise are inversely correlated with time to fatigue (Figs. 3B and 4B); that is, TRP-treated rats that presented higher rates of core temperature increase and HSR fatigued much earlier than the saline-treated group. These results are in agreement with the concept that increased body temperature and HSR during exercise reduce physical performance both in animals (Rodrigues et al., 2003) and healthy humans (González-Alonso et al., 1999; Galloway and Maugham, 1997). To the best of our knowledge, this is the first study to describe an effect of intracerebroventricular injection of TRP on core temperature, rate of core temperature increase and HSR in exercising rats until fatigue. This effect of central TRP seems to be at least one of the mechanisms by which increased TRP availability during exercise would reduce time to fatigue. This hypothesis is corroborated by the results of our recent study showing that intracerebroventricular TRP injection also increases metabolic rate and

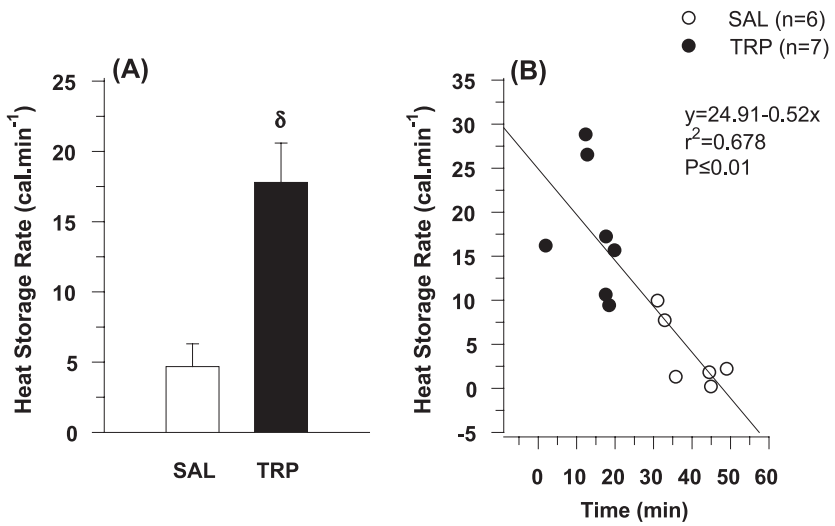

Fig. 4. (A) Mean value ± S.E.M. of heat storage rate (cal min^{-1}) after intracerebroventricular injection of TRP (20.3 μM) or 0.15 M NaCl in exercising rats. $^{\delta}P \leq .002$ from control group. (B) Correlation between heat storage rate (cal min^{-1}) as function of time to fatigue (min) after intracerebroventricular injection of TRP (20.3 μM) or 0.15 M NaCl in exercising rats.

decreases mechanical efficiency in running rats (Soares et al., 2003). Taking into account the present data and those from previous study (Soares et al., 2003), there are strong indications that increased TRP availability in the CNS accelerates metabolic rate, producing a faster increase in internal temperature and HSR with a marked decrease in mechanical efficiency during exercise, thus reducing time to fatigue. This hypothesis is reinforced by the results of the present study, not only the marked elevation in the rate of core temperature increase but also the 110% higher HS observed at the fatigue point despite the reduced running time in TRP-treated rats. Thus, the more rapid increase in core temperature and HSRs together with decreased mechanical efficiency may have precipitated feelings of fatigue and eventually impaired work performance, protecting the animal against thermal stress.

Although the exact locations and precise pathways involved in TRP mediation of thermoregulation remain to be elucidated, there are strong indications that one of these sites is the preoptic/anterior hypothalamus (PO/AH). The PO/AH is the major brain region involved in thermoregulation (Ferreira et al., 1999; Lin et al., 1998; Santos et al., 1990, 1991; Coimbra and Migliorini, 1986) that integrates thermal inputs with energy-linked metabolic processes (Ferreira et al., 1999; Lin et al., 1998; Santos et al., 1990, 1991; Coimbra and Migliorini, 1986).

Taking into account that TRP is rapidly converted to 5-hydroxytryptophan (5-HTP), a precursor of 5-HT in CNS (Lookingland et al., 1986; Denizeau and Sourkes, 1977) that increases core temperature when injected into the preoptic area (POA), the principal effect of TRP other than its direct thermogenic action could be the activation of serotonergic receptors in the POA (Lin et al., 1998). Serotonin is considered to be an activator of thermogenesis (Imeri et al., 1999; Lin et al., 1998; Rothwell and Stock, 1987; Myers, 1981; Feldberg and Myers, 1964). Furthermore, it has been shown that in humans, increasing core temperature increases central 5-HT activity, either passively through exposure to high ambient temperature at rest, or actively through exercise (Bridge et al., 1999). It was observed that an incremental change of 5-hydroxyindole-3-acetic acid (5-HIAA) in the PO/AH of freely moving rats was accompanied by hyperthermia, suggesting a link between core temperature regulation and serotonergic neuronal mechanisms (Yasumatsu et al., 1998). In addition, elevated levels of hypothalamic 5-HT in unanesthetized untrained rats were associated with hyperthermia (Lin et al., 1998), which was brought about by an increase in metabolic heat production and decrease in heat loss. On the other hand, Serra et al. (1992) demonstrated that intracerebroventricular TRP promoted a thermogenic effect, similar to that observed following central injections of 5-HT. These authors suggested that this effect was not mediated through 5-HT release, because it was not completely blocked by the 5-HT antagonist, methysergide. The thermogenic action of TRP and metabolic adjustments during exercise may therefore result from a direct effect of TRP and/or increased production and release of serotonin due to increased TRP availability. However, our experiments did not allow us to postulate whether TRP acts directly, by increased serotonin release, or by both mechanisms.

Because cerebral uptake of TRP is the rate-limiting step in the synthesis of 5-HT (Fernström, 1991) and provides an indication of changes in the serotonin level within the brain, several studies have focused on the influence of exercise on TRP uptake by brain, as well as 5-HT synthesis and turnover (Chaouloff, 1997; Chaouloff et al., 1987; Davis et al., 1992). Analysis of whole brain (Chaouloff, 1997; Chaouloff et al., 1986a, 1989), brain regions (Gomez-Merino et al., 2001; Blomstrand et al., 1989; Lookingland et al., 1986) or cerebrospinal fluid samples (Chaouloff et al., 1986b) from exercising rats showed an increased brain TRP content. Special attention has been given to the synthesis and metabolism of serotonin since Newsholme et al. (1992) hypothesized that fatigue during prolonged exercise may be influenced by activity of the brain serotonergic system. This is commonly referred to as the central fatigue hypothesis. Its major premise is that increased 5-HT activity during prolonged exercise may cause fatigue by increasing lethargy and loss of central drive/motivation (Newsholme et al., 1992). A relationship between increased serotonergic activity and the early onset of fatigue has been demonstrated in both rats (Bailey et al., 1993) and humans (Davis et al., 2000). Although both Chaouloff et al., 1986a,b, 1987) and Meeusen et al. (1996) demonstrated that prolonged exercise results in increased availability of TRP to brain and increased 5-HT and 5-HIAA concentrations in the rat, a causal link has not been established between increased 5-HT activity and reduced physical and mental performance. However, to the best of our knowledge, this is the first study to describe an effect of TRP both in disrupting the thermal balance during exercise and accelerating the increase in exercise-induced hyperthermia. It may be at least one of the mechanisms by which increased TRP availability during exercise would reduce time to fatigue, as predicted by the central fatigue hypothesis.

It is important to point out that to maintain a thermal balance, heat produced by exercising muscles should be offset by increased heat loss, and otherwise, physical activity could result in excessive hyperthermia. Thus, the faster hyperthemia observed in TRP-treated rats (Fig. 2A) should be a consequence not only of increased metabolic rate but also due to a failure of heat loss mechanisms to keep pace with exercise-induced heat production. This maladjustment between heat production and heat loss induced by central TRP would accelerate exercise-induced hyperthermia, decreasing mechanical efficiency and running performance as seen previously (Soares et al., 2003).

It has been shown that hyperthermia reduces physical performance in many mammalian species, including rodents (Walters et al., 2000; González-Alonso et al., 1999; Fuller et al., 1998; Nielsen et al., 1993; Brück and Olschewski, 1987), reduces CNS drive for exercise performance (Mckinley et al., 1996; Nielsen et al., 1993) and precipitates feelings of fatigue at a sublethal threshold by establishing a safety level against heat stroke, thus protecting the brain, among other tissues, from thermal damage (Jessen, 1987; Caputa et al., 1986). On the other hand, termination of work is also associated with increased body temperature both in humans (Galloway and Maugham, 1997) and in animals (Walters et al., 2000; González-Alonso et al., 1999; Fuller et al., 1998). Our data show that despite the faster increase in core temperatures of the TRP-treated animals, they fatigued at the same internal temperature as the control group (38.32 TRP vs. 38.03 SAL). More importantly, internal temperatures of both groups were below the values previously described as being critical (40.4–41.5 °C, Fuller et al., 1998) and they fatigued without showing signs of heat stroke. These include poor limb coordination after exercise and exhaustion, characterized by the point at which the rat is unable to right itself when placed on its back (Fuller et al., 1998). These data suggest that TRP does not change hypothalamic setpoint for body temperature regulation but act by disrupting the adjustment between heat production and the heat dissipation mechanism. This is reflected by the marked increase in TI as well as by HS values that are 110% higher in TRP-treated animals but do not affect core temperature at fatigue. Although there is controversy as to whether there is an absolute critical value for internal temperature, critical HSR or both that determines the point of fatigue, the results of the present study support the hypothesis that both HSR and critical body internal temperature seem to be important determinants of fatigue. This assumption is supported by the fact that fatigue occurred at the same core temperature value in both TRP- and SAL-treated rats and HSRs in each group were directly related to a reduction in time to fatigue.

It has been proposed that there is, in rats, a possible interaction between brain serotonin and dopamine during exercise, and that together, these neurotransmitters could play a regulatory role in the onset of fatigue (Bailey et al., 1993). Recently, Bridge et al. (2003) suggested that high ratio of dopaminergic to serotonergic activities is better associated with exercise performance because it allows a better tolerance of high core temperature during exercise. Considered together, the literature investigating a possible "central" component in the onset of fatigue during exercise is inconclusive and further research is needed in this area.

In conclusion, the present study provides further evidence that increased hypothalamic TRP availability is related to fatigue. It also provides evidence that central TRP affects thermoregulation and exercise performance by elevating the rate of core temperature increase, HSR and HS until fatigue. Furthermore, our data suggest that TRP does not change hypothalamic setpoint for body temperature regulation but acts disrupting the adjustment between the heat production and dissipation mechanisms. Thus, we can conclude that increased hypothalamic TRP availability reduces run time to fatigue by changing the thermoregulatory ability of running rats.

Acknowledgements

The authors are indebted to CAPES, CNPq, FAPEMIG and PRONEX for financial support.

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