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Inhibition of tryptophan hydroxylase abolishes fatigue induced by central tryptophan in exercising rats

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Fatigue during prolonged exercise is related to brain monoamines concentrations, but the mechanisms underlying this relationship have not been fully elucidated. We investigated the effects of increased central tryptophan (TRP) availability on physical performance and thermoregulation in running rats that were pretreated with parachlorophenylalanine (p-CPA), an inhibitor of the conversion of TRP to serotonin. On the 3 days before the experiment, adult male Wistar rats were treated with intraperitoneal (ip) injections of saline or p-CPA. On the day of the experiment, animals received intracerebroventricular (icv) injections of either saline or TRP (20.3 μM) and underwent a submaximal exercise test until fatigue. Icv TRP-treated rats that received ip saline presented higher heat storage rate and a 69% reduction in time to fatigue compared with the control animals. Pretreatment with ip p-CPA blocked the effects of TRP on thermoregulation and performance. Moreover, ip p-CPA administration accelerated cutaneous heat dissipation when compared with saline-pretreated rats. We conclude that an elevated availability of central TRP interferes with fatigue mechanisms of exercising rats. This response is modulated by serotonergic pathways, because TRP effects were blocked in the presence of p-CPA. Our data also support that a depletion of brain serotonin facilitates heat loss mechanisms during exercise.

Physical exercise-induced changes in the concentrations of different neurotransmitters, including the monoamines [dopamine, noradrenaline, and serotonin (5-HT)], have been associated with central fatigue. The first evidence suggesting that the serotonergic system plays a role in the modulation of exercise performance was provided by Chaouloff et al. (1985), who showed that concentrations of brain 5-HT increased in rats after moderate-intensity running exercise. Later, Newsholme et al. (1987) suggested that fatigue during prolonged exercise might be influenced by the activity of the brain serotonergic system. This idea was referred as the "central fatigue hypothesis," which has become a topic of great interest. According to this hypothesis, an increase in central 5-HT concentration may cause fatigue by increasing lethargy, tiredness, and the loss of central drive/motivation (Davis & Bailey, 1997).

Various studies have used pharmacological and nutritional manipulations to increase or decrease central 5-HT, thereby assessing the effects of the neurotransmitter on exercise performance [for reviews, see Newsholme & Blomstrand (2006) and Meeusen and Roelands (2010)]. Because prolonged running exercise increases brain levels of tryptophan (TRP; Chaouloff et al., 1985), the amino acid precursor of 5-HT synthesis (Davis et al., 1992; Fadda, 2000), one manipulation that has been studied entails changing the central availability of TRP; a high availability of TRP leads to increases in cerebral concentrations of 5-HT (Chaouloff, 1997; Castell et al., 1999; Davis et al., 2000; Soares et al., 2007). However, nutritional manipulation of central TRP levels yielded conflicting results with respect to changes in performance (Stensrud et al., 1992; Alves et al., 1995; Mittleman et al., 1998; Cheuvront et al., 2004; Javierre et al., 2010).

In contrast, direct administration of TRP into the central nervous system through intracerebroventricular (icv) injections consistently decreased the time to fatigue in running rats by approximately 60% (Soares et al., 2003, 2004, 2007). The reduced running performance of icv TRP-treated rats was related to an increased preoptic concentration of 5-HT (Soares et al., 2007) and decreased mechanical efficiency (Soares et al., 2003), which led to higher rates of increase in internal temperature (Soares et al., 2004). Whether this higher exercise

hyperthermia caused by central TRP was also a consequence of impaired cutaneous heat loss mechanisms has yet to be determined.

Another experimental approach to study the serotonergic system is the administration of parachlorophenylalanine (*p*-CPA), an agent that potently and selectively depletes cerebral 5-HT through the inhibition of TRP hydroxilase enzyme activity (Koe & Weissman, 1966). According to this report, intraperitoneal (ip) injections of *p*-CPA (100 mg/kg/day) on three consecutive days reduced the central concentration of 5-HT to approximately 11% of basal values. However, experiments using *p*-CPA yielded conflicting results with respect to the regulation of body temperature. Giacchino et al. (1983) observed that resting rats that were pretreated with *p*-CPA had diminished heat dissipation when exposed to a hot environment. Sharma et al. (1995) did not observe changes in rectal temperature in rats pretreated on three consecutive days with *p*-CPA that were then forced to swim for 30 min. These contradictory results indicate that more studies are required to better understand the effects of brain 5-HT depletion on the activation of heat dissipation responses. Moreover, considering that the systemic administration of *p*-CPA induced long-lasting locomotor hyperactivity in a variety of experimental paradigms (Fibiger & Campbell, 1971), we also hypothesized that the depletion of brain serotonin would extend the running time until the interruption of the effort.

An alternative pathway of TRP thermogenic activity, not mediated by a serotonergic pathway, was suggested by Serra et al. (1992). They observed that rats receiving an icv injection of TRP had increased resting oxygen consumption rates that were not abolished by treatment with methylsergide, a serotonergic antagonist. Considering this finding, Soares et al. (2004) hypothesized that TRP could exert thermogenic activity during exercise from a direct effect of this amino acid.

Because different experimental manipulations of brain 5-HT levels did not consistently confirm or refute the validity of the central fatigue hypothesis, additional studies using alternative tools are important for elucidating the role of 5-HT in fatigue. To date, no studies addressed if the effects of the increased central TRP availability on the regulation of body temperature and fatigue in exercising rats are dependent on 5-HT synthesis. Therefore, this study investigated the effect of icv administration of TRP in untrained rats that were pretreated with *p*-CPA on thermoregulatory responses and running performance in thermoneutral ambient conditions. Moreover, there is no evidence if blocking the physiological, exercise-induced conversion of central TRP to 5-HT could increase exercise performance, and this hypothesis was addressed in the experiments performed with rats that were pretreated with ip *p*-CPA and then received icv injections of saline (SAL).

Materials and methods

Animals

Male adult Wistar rats (250–300 g) were housed in individual cages under controlled lights (05:00 a.m.–7:00 p.m.) and room temperature (23 ± 2 °C) conditions, with food and water provided *ad libitum*. Under anesthesia with ip ketamine (115 mg/kg body mass) and xylazine (5.75 mg/kg body mass), each rat was fixed in a stereotaxic apparatus (David Kopf Instruments, M-900, Tujunga, California, USA) and was implanted with a brain guide cannula (22 G) into the right lateral cerebral ventricle using the technique that was previously described by Lima et al. (1998). A SAL-filled manometer attached to the cannula showed a drop in pressure when the tip of the cannula reached the ventricular space. Immediately after the brain cannulation, rats were implanted with a temperature sensor (TR3000-XM-FM, Mini-Mitter, Sunriver, Oregon, USA) in the peritoneal cavity through a small incision in the linea alba of the abdominal wall.

Over a period of 5–7 days, the animals were allowed to recover to their presurgical body mass, and then they were familiarized to exercise on a motor-driven treadmill (Columbus Instruments, Columbus, Ohio, USA) for 5 days before the experiment. Each daily session consisted of running for 5 min at a constant speed of 18 m/min and a grade of 5%. 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 number 82/2006), and were conducted in accordance with the Committee's Guiding Principles Manual.

Treatment

All rats received ip injections of DL-*p*-chlorophenylalanine (Sigma, St. Louis, Missouri, USA) (*p*-CPA; 100 mg/kg/day) or SAL solution (0.5 mL/100 g/day) on the last 3 days of the familiarization protocol (Koe & Weissman, 1966; Sharma et al., 1995).

Exercise

One day after the last ip injection, the rats were exercised at a moderate intensity (18 m/min and 5% grade), which corresponds to an oxygen uptake of approximately 67% VO_{2max} (Sonne & Galbo, 1980), until they were fatigued. Total time to fatigue (TTF) was recorded from the beginning of exercise until the moment when the animals were unable to keep pace with the treadmill (Soares et al., 2003).

Experimental protocol

On the day of the experiments, the rats were placed in an environmental chamber (Russels Technical Products WMD 1150-5, Holland, Michigan, USA). Ambient temperature was held constant at 23 °C and relative humidity at 50%. A thermocouple (series 409-B, YSI Inc., Yellow Springs, Ohio, USA) was fixed on the rat's tail to measure the skin temperature. Then, a 30-G needle connected to a Hamilton syringe (Hamilton Company, Reno, Nevada, USA) and protruding 0.3 mm from the tip of guide cannula was introduced into the lateral cerebral ventricle. The rats that had been previously treated with either ip *p*-PCA or SAL were randomly assigned to receive 2.0 µL of SAL (0.15 M) or L-TRP (20.3 µM) solution into the right lateral cerebral ventricle. Immediately after the icv injection, the animals underwent a bout of treadmill running until they were fatigued.

The dose selected for icv injections of TRP was based on previous results showing that 2.0 µL of a 20.3 µM TRP solution, administered immediately before the exercise, produced twofold higher pre-optic concentration of 5-HT measured at fatigue when compared with SAL-treated rats (Soares et al., 2007).

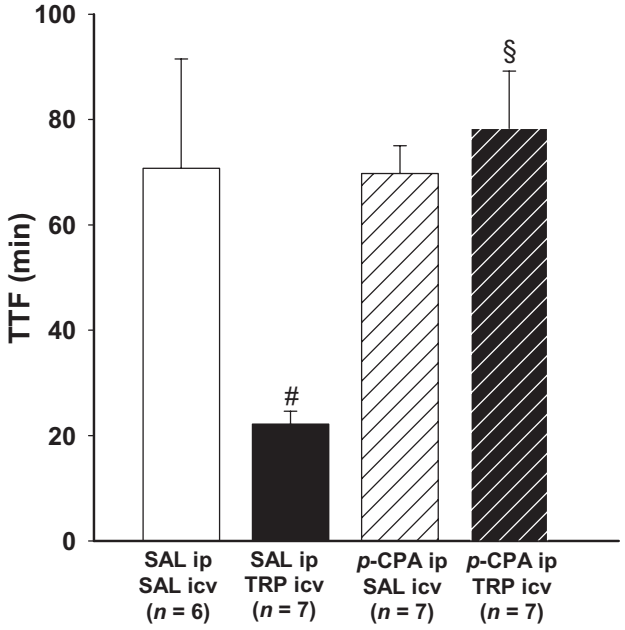

Fig. 1. Effect of intracerebroventricular (icv) injection of tryptophan (TRP) (20.3 µM) or saline (SAL) (0.15 M NaCl) on time to fatigue (TTF; min) in rats pretreated with intraperitoneal (ip) SAL or parachlorophenylalanine (p-CPA) and that underwent submaximal exercise until they were fatigued. Values are expressed as mean ± standard error of the mean. The number of animals is indicated in parentheses. #Significantly different from ip SAL–icv SAL (P = 0.03); §Significantly different from ip SAL–icv TRP (P = 0.0003).

Temperature recording

The internal temperature (T_{INT}) was continuously recorded during exercise using a telemetric apparatus and the data were acquired by software (Vital View, Mini-Mitter). The tail skin temperature (T_{TAIL}) and ambient temperature inside the treadmill were measured with thermocouples.

Heat storage rate (HSR) was calculated by the formula: (ΔT_{INT}/TTF).m.c; where ΔT_{INT} = change in internal temperature; TTF = total time to fatigue; m = body mass (g); c = specific heat of the tissues of animals (0.826 cal/g/°C).

Statistical analysis

The data are reported as mean ± standard error of the mean. Differences in the thermoregulatory responses were determined by a two-way analysis of variance followed by the post-hoc Student–Newman–Keuls test. All data collected as single-point measurements (TTF and HSR) were compared by unpaired Student's *t*-tests. The significance level was set at $P < 0.05$.

Results

As illustrated in Fig. 1, in SAL-pretreated animals, icv injection of TRP reduced physical performance by 69% in relation to the group that received icv SAL (22.2 ± 2.5 min ip SAL–icv TRP vs 70.7 ± 20.8 min ip SAL–icv SAL; $P = 0.03$). Whereas, in *p*-CPA-pretreated rats, no modification was observed in the TTF after the icv injection of TRP when compared with the control group (78.3 ± 10.9 min ip *p*-CPA–icv TRP vs 69.7 ± 5.3 min ip *p*-CPA–icv SAL), indicating that pretreatment with *p*-CPA abolished the effect of central TRP on decreasing TTF (78.3 ± 10.9 min ip *p*-CPA–icv TRP vs 22.2 ± 2.5 min ip SAL–icv TRP; $P = 0.0003$). However, an ergogenic effect of *p*-CPA was not observed in animals receiving central administration of SAL (69.7 ± 5.3 min ip *p*-CPA–icv SAL vs 70.7 ± 20.8 min ip SAL–icv SAL).

Figure 2(a,b) represents the T_{INT} and T_{TAIL} of the animals pretreated with ip SAL that received an icv injection of SAL or TRP. Physical exercise induced an increase in T_{INT} in both groups. In the icv SAL group, T_{INT} increased from the seventh min after the beginning of exercise (38.05 ± 0.06 °C at 7 min vs 37.45 ± 0.10 °C at 0 min) and remained elevated until the point of fatigue (38.51 ± 0.14 °C; Fig. 2a). In the icv TRP group, T_{INT} increased from the sixth min after the beginning of exercise (37.99 ± 0.16 °C at 6 min vs 37.44 ± 0.13 °C at 0 min) and remained high until the point of fatigue (38.43 ± 0.15 °C; Fig. 2a). As illustrated in Fig. 2(b), T_{TAIL} transiently decreased when the animals were subject to treadmill running and then increased from the 14th min of exercise in the SAL group (28.98 ± 0.73 °C at 14 min vs 26.28 ± 0.31 °C at 0 min) and from the 12th min in the TRP group (29.11 ± 1.01 °C at 12 min vs 26.54 ± 0.26 °C at 0 min), indicating that heat loss mechanisms had been activated. No differences were observed between treatments in either T_{INT} or T_{TAIL} throughout the exercise period.

Figure 2(c,d) represents the T_{INT} and T_{TAIL} of the animals pretreated with ip *p*-CPA that also received icv injection of SAL or TRP. No changes were observed in T_{INT} during the exercise until fatigue in either group (38.39 ± 0.42 °C at the point of fatigue vs 37.79 ± 0.22 °C at 0 min in the SAL group; 38.38 ± 0.40 °C at the point of fatigue vs 37.99 ± 0.29 °C at 0 min in the TRP group; Fig. 2c). T_{TAIL} increased after the beginning of exercise in both the SAL (29.07 ± 0.93 °C at 8 min vs 26.88 ± 0.22 °C at 0 min) and TRP groups (29.72 ± 0.28 °C at 6 min vs 26.90 ± 0.17 °C at 0 min), indicating that cutaneous heat loss was enhanced (Fig. 2d). Thereafter, the T_{TAIL} remained elevated and stable until the point of fatigue.

To address the effects of ip pretreatment with *p*-CPA, which might have blocked the conversion of TRP to 5-HT, on thermoregulatory responses during exercise, the data presented in Fig. 2 were replotted as changes in temperatures against ip *p*-CPA and ip SAL for both icv treatments groups. Because no differences in T_{INT} between pretreatments were observed at the beginning of exercise (37.79 ± 0.22 °C ip *p*-CPA vs 37.45 ± 0.10 °C ip SAL), the data were analyzed as changes from pre-exercise levels. As shown in Fig. 3(a,c), the overall increase of T_{INT} was attenuated by ~0.6 °C in animals that received the pretreatment with ip *p*-CPA

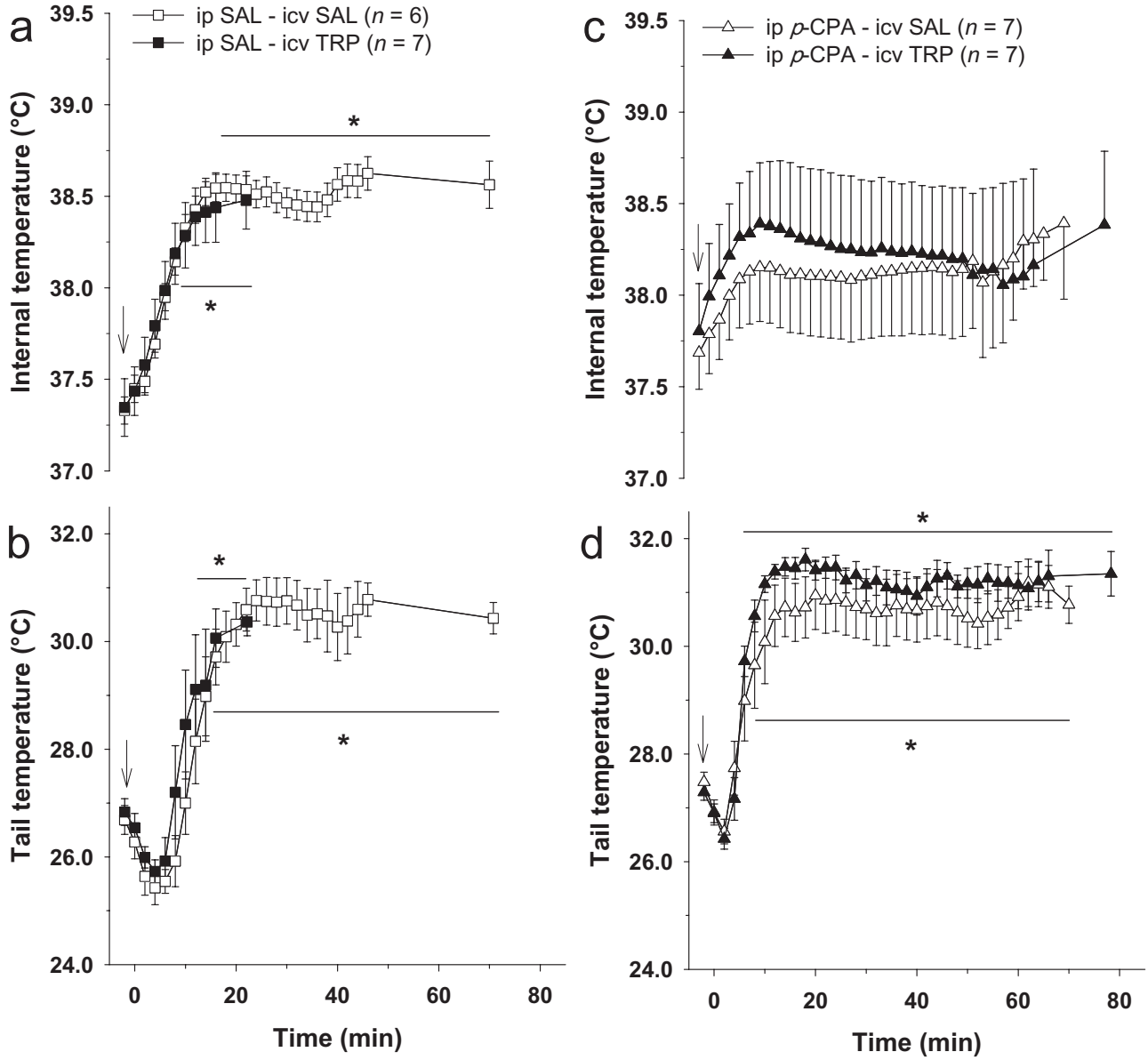

Fig. 2. Effect of intracerebroventricular (icv) injection of tryptophan (TRP) (20.3 µM) or saline (SAL) (0.15 M NaCl) on internal and tail temperatures (°C) in rats pretreated with intraperitoneal (ip) SAL (2a and 2b) or parachlorophenylalanine (p-CPA) (2c and 2d) and that underwent submaximal exercise until they were fatigued. Values are expressed as mean ± standard error of the mean. The arrows represent the moment of central injection. Legends: ip SAL–icv SAL (white squares); ip SAL–icv TRP (black squares); ip p-CPA–icv SAL (white triangles); ip p-CPA–icv TRP (black triangles). *Significantly different from the beginning of exercise ($P < 0.05$).

(0.50 ± 0.16 °C ip *p*-CPA vs 1.08 ± 0.10 °C ip SAL, grouped data, $P < 0.01$). Regarding the heat loss mechanisms, exercise induced earlier increases in T_{TAIL} in rats pretreated with *p*-CPA as compared with control animals, irrespective of whether the animals received icv SAL or TRP (Fig 3b,d).

To compare the total thermal effects of exercise in the four experimental groups, HSR was calculated (Fig. 4). HSR was 260% higher in the ip SAL–icv TRP group as compared with the control group (12.6 ± 2.3 cal/min ip SAL–icv TRP vs 4.8 ± 0.9 cal/min ip SAL–icv SAL; $P = 0.01$). In contrast, pretreatment with *p*-CPA blocked the hyperthermic effect of TRP and reduced HSR by 90% compared with the respective control group (1.3 ± 0.6 cal/min ip *p*-CPA–icv TRP vs 12.6 ± 2.3 cal/min ip SAL–icv TRP; $P = 0.0004$).

Discussion

Our data demonstrated that, as expected, treatment with icv TRP induced a higher HSR and reduced TTF. One novel finding is the observation that these responses

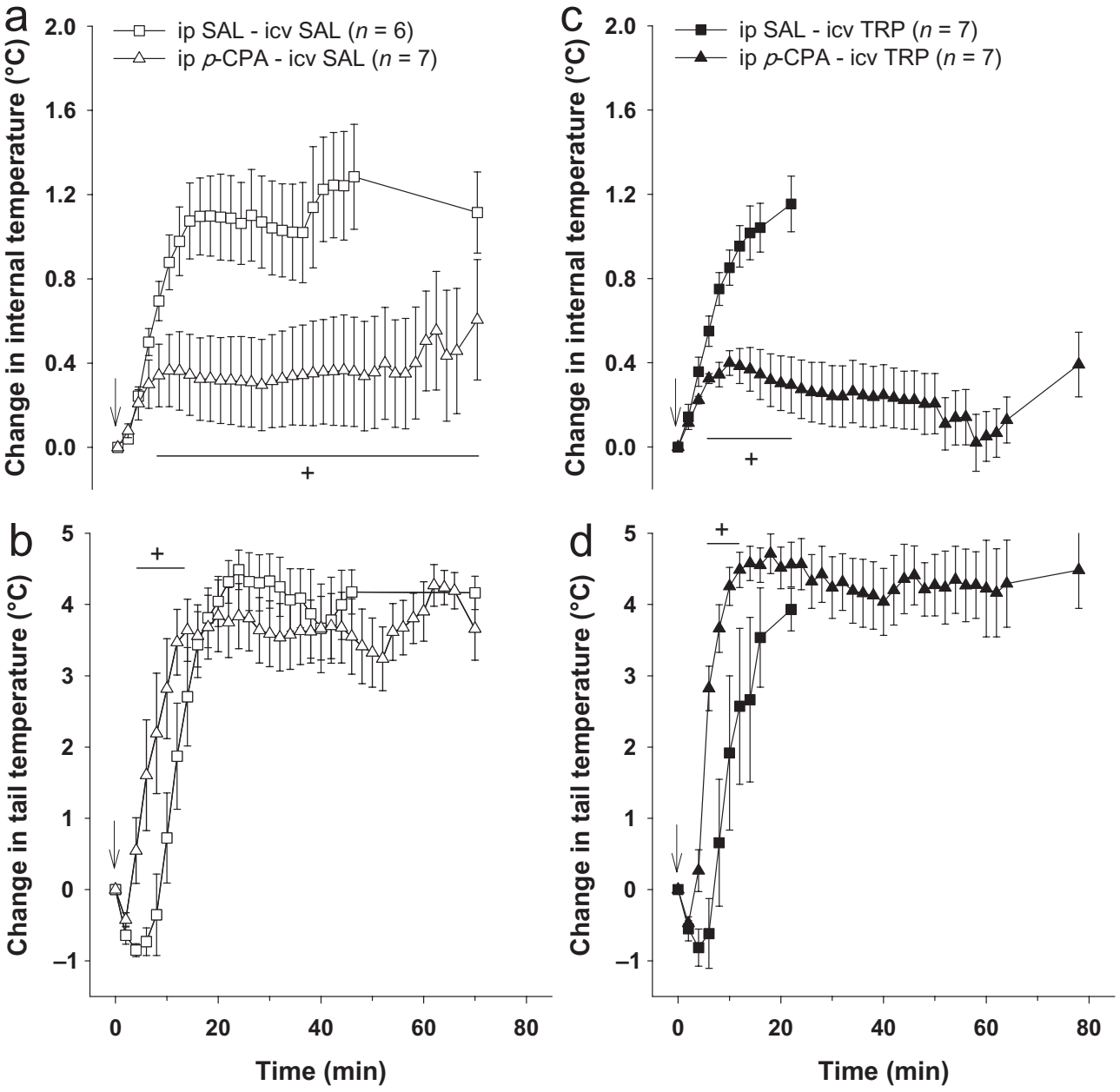

Fig. 3. Effect of pretreatment with intraperitoneal (ip) saline (SAL) or parachlorophenylalanine (p-CPA) in the change of internal and tail temperatures (°C) in rats that underwent submaximal exercise until they were fatigued after an intracerebroventricular (icv) injection of SAL (0.15 M NaCl; 3a and 3b) or tryptophan (TRP) (20.3 μM; 3c and 3d). Values are expressed as mean ± standard error of the mean. The arrows represent the moment of central injection. Legends: ip SAL–icv SAL (white squares); ip p-CPA–icv SAL (white triangles); ip SAL–icv TRP (black squares); ip p-CPA–icv TRP (black triangles). +Significantly different from control group ($P < 0.05$).

were completely abolished by pretreatment with ip *p*-CPA. Considering that *p*-CPA treatment had the expected effect of blocking brain 5-HT synthesis from TRP (Koe & Weissman, 1966; Howard et al., 2008), the present results indicate that brain 5-HT is involved in fatigue induced by central injection of TRP. These findings also exclude a direct role for the amino acid in the modulation of physical performance.

Fatigue is thought to be a multifaceted process involving different factors that act in an integrated manner to interrupt exercise in order to maintain body homeostasis (Noakes, 2000). Both elevated T_{INT} (Gonzalez-Alonso et al., 1999; Walters et al., 2000) and high HSR (Rodrigues et al., 2003; Soares et al., 2004, 2007) are important determinants of fatigue during prolonged exercise. Evidence implicates 5-HT in the physiological

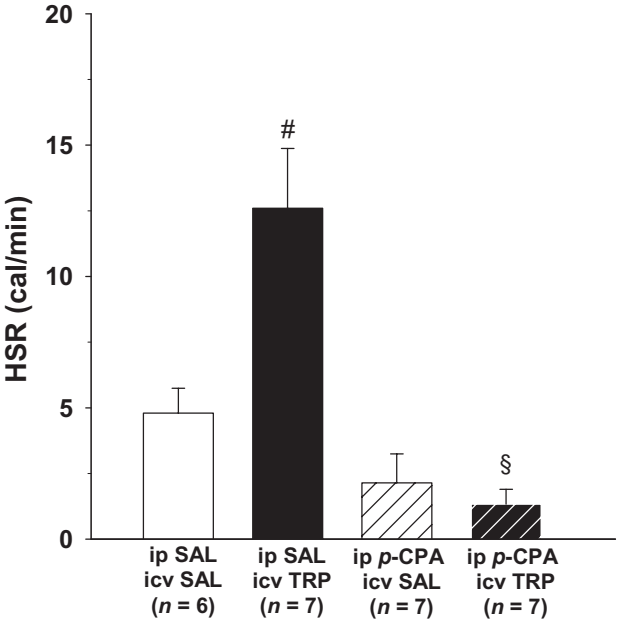

Fig. 4. Effect of intracerebroventricular (icv) injection of tryptophan (TRP) (20.3 μM) or saline (SAL) (0.15 M NaCl) on heat storage rate (HSR; cal/min) in untrained rats pretreated with intraperitoneal (ip) SAL or parachlorophenylalanine (p-CPA) and that underwent submaximal exercise until they were fatigued. Values are expressed as mean ± standard error of the mean. [#]Significantly different from ip SAL–icv SAL (P = 0.01); [§]Significantly different from ip SAL–icv TRP (P = 0.0004).

mechanisms by which thermoregulation modulates performance as supported by the observation that, in TRP-exercised rats, fatigue was accelerated because of increased HSR and high 5-HT concentrations in the preoptic area (Soares et al., 2007).

To further understand the role of the central serotonergic system in thermoregulation and fatigue during exercise, it is important to separate the possible effects mediated by 5-HT and TRP. In resting rats, Serra et al. (1992) demonstrated that icv injection of TRP promoted a thermogenic effect similar to that observed following central injections of 5-HT. The authors suggested that this effect was not mediated through 5-HT release, because it was not blocked by methysergide, an antagonist of 5-HT. Therefore, in the present experiments, we used *p*-CPA, an agent that potently and selectively depletes cerebral 5-HT in rats (Koe & Weissman, 1966; Howard et al., 2008). By using this pharmacological tool, we blocked the detrimental effects of increased brain TRP availability on physical performance. These observations suggest that *p*-CPA treatment was successful in limiting the synthesis of central 5-HT, therefore preventing serotonin-mediated fatigue. This result is demonstrated by the ip *p*-CPA–icv TRP rats that showed similar physical performance to the control groups (ip *p*-CPA–icv SAL or ip SAL–icv SAL). Moreover, the enhanced exercise hyperthermia observed in TRP-exercising rats previously reported by our group (Soares et al., 2003, 2004) was not observed in the ip *p*-CPA–icv TRP running rats. This finding suggests that the thermoregulatory effects and fatigue induced by icv TRP depend on 5-HT synthesis. In addition, the lack of an ergogenic effect evoked by pretreatment with *p*-CPA rules out the possibility that *p*-CPA prevents TRP-mediated fatigue through actions in a pathway independent of TRP hydroxylase.

As observed in the present study, a large number of rodent studies provide evidence supporting the role for increased brain 5-HT concentrations in the fatigue process (Bailey et al., 1992; Calders et al., 1997; Soares et al., 2007); however, it is unclear why these ergolytic effects of 5-HT are not extrapolated to human studies (Roelands et al., 2009). Some explanations for this disparity can be raised: existence of intraspecies differences in the regulation of serotonergic transmission; the pharmacological tool used (administration of an antagonist, agonist, precursor of the synthesis or inhibitor of the neuronal reuptake); and route of administration of the drug (ingested in capsules, administrated systemically or directly into the brain). It is important to emphasize that the icv injection of TRP was used, in the present study, as an investigative tool meant to identify a 5-HT-related function, rather than an attempt to mimic a change in 5-HT brain concentrations that occurs during exercise. Therefore, we cannot compare the amount of TRP that was directly injected into the cerebral ventricles (injected during 1 min) with the amount of physiologically TRP delivered to the brain during exercise. This methodological approach is one of the possible explanations to the contradictory outcomes yielded from studies with icv injections of TRP in exercising rodents and studies with nutritional and pharmacological manipulations intended to increase brain 5-HT levels in humans (Stensrud et al., 1992; Alves et al., 1995; Roelands et al., 2009; Javierre et al., 2010).

A novel finding reported in the present study is that depleting brain 5-HT and blocking the physiological exercise-induced conversion of TRP to 5-HT does not delay the time to fatigue. This result is in agreement with an emerging idea that 5-HT is not the only neurotransmitter involved in fatigue. The central fatigue hypothesis has been revised and now it is proposed that an increase in the central ratio of serotonin to dopamine is associated with feelings of tiredness and lethargy, whereas a decreased ratio improves performance through the maintenance of motivation and arousal (Meeusen et al., 2006). In support to this hypothesis, the manipulations of the dopaminergic neurotransmission either by inhibiting dopamine reuptake (Roelands et al., 2008) or by blocking of the dopamine D1 and D2 receptors (Balthazar et al., 2010) modified physical performance. Recent findings also support that the noradrenergic neurotransmission impairs performance in both normal and high ambient temperatures [for review, see Meeusen & Roelands (2010)].

Another question addressed in the present study was whether increasing the availability of central TRP impairs cutaneous heat loss mechanisms during exercise. Animals treated with ip SAL and icv TRP did not exhibit any changes in tail temperature compared with the control animals, and this result possibly indicates that icv TRP does not interfere with cutaneous heat loss mechanisms during exercise. However, we suggest that the absence of thermoregulatory changes may be due to the drastically impaired performance of these rats. Actually, in rats treated with icv TRP, exercise was interrupted while T_{INT} and T_{TAIL} were still increasing, therefore, we cannot rule out that T_{INT} would have increased more and that T_{TAIL} would have stabilized at lower levels in the rats treated with icv TRP than in the control animals.

Evidence that subgroups of 5-HT neurons act in different brain circuits makes it difficult to identify which neurons are responsive to icv TRP during exercise (Fernstrom & Fernstrom, 2006). However, previous reports indicate that TRP loading and/or exercise promotes a region-specific conversion of TRP into 5-HT (Chaouloff et al., 1987, 1989; Soares et al., 2007). The pre-optic area, which is involved in the modulation of autonomic thermoeffectors (Romanovsky, 2007), is an interesting candidate; 5-HT increases T_{INT} when it is injected into the pre-optic area of resting rats (Lin et al., 1998). Although prolonged exercise by itself does not increase 5-HT content in this brain area (Hasegawa et al., 2008; Takatsu et al., 2010), Soares et al. (2007) reported that the increase in the pre-optic levels of the neurotransmitter was among the factors related to icv TRP-induced fatigue resulting from enhanced exercise hyperthermia. These results are consistent with the observation that early fatigue and increased body heating rate induced by blockade of central angiotensin II AT1 receptors in exercising rats were related to higher 5-HT content in the pre-optic area and hypothalamus (Leite et al., 2010). Interestingly, attenuated exercise hyperthermia caused by central cholinergic stimulation was closely related to lower serotonin levels in the pre-optic area (Rodrigues et al., 2009). Therefore, it is reasonable to assume that, in ip *p*-CPA–icv TRP-exercising rats, decreased 5-HT in the pre-optic area might have restored the running time to fatigue.

Also of interest are the data related to the effects of ip injections of *p*-CPA on thermoregulatory responses. Animals treated with *p*-CPA had more rapid increases in tail temperature, with consequent reductions in exercise hyperthermia and HSR. This earlier increase in T_{TAIL}, probably caused by an early withdrawal of the sympathetic activity over the skin vessels, is suggestive of a tonic control by the central serotonergic system on cutaneous heat dissipation. The latter hypothesis is corroborated by previous reports showing the participation of the serotonergic neurons that project from the medulla to the spinal cord preganglionic neurons in the control of skin blood flow. Direct stimulation of 5-HT2A receptors evokes marked hyperthermia as a consequence of sympathetically mediated vasoconstriction, selective for the cutaneous bed (Blessing & Seaman, 2003). An alternative hypothesis that may explain the *p*-CPA mediated earlier increase in T_{TAIL} is related to the hypertensive effects caused by the central depletion of 5-HT [reported in resting rats by Kellett et al. (2005)]; previous studies showed that higher exercise-induced increase in arterial pressure promotes an early cutaneous heat loss (Pires et al., 2007) through a mechanism mediated by arterial baroreceptors activation (Pires et al., 2010).

The present experiments conducted in rodents allow us to conclude that the blockade of TRP hydroxylase with *p*-CPA prevented a reduction in performance caused by increased availability of central TRP, indicating that early fatigue might have been a consequence of an increased synthesis of central 5-HT rather than an effect of the amino acid by itself. Furthermore, pretreatment with ip *p*-CPA facilitates cutaneous heat loss mechanisms during exercise, without altering exercise performance.

Perspectives

An interesting finding reported in the present study is that depleting brain 5-HT and blocking the physiological exercise-induced conversion of TRP to 5-HT does not improve physical performance. This result is in agreement with an emerging idea that 5-HT is not the only neurotransmitter involved in fatigue; previous reports showed that the neurotransmissions mediated by acetylcholine (Guimaraes et al., 2011), dopamine, and noradrenaline (Hasegawa et al., 2008; Meeusen & Roelands, 2010; Takatsu et al., 2010), among others, are determinant for physical performance. Therefore, future measurements of the brain levels of noradrenaline, dopamine, and acetylcholine in running rats treated with *p*-CPA are important to understand why depleted levels of brain 5-HT does not increase time to fatigue. Another intriguing finding was that *p*-CPA accelerated the activation of cutaneous heat loss, attenuating exercise hyperthermia. Additional experiments using different pharmacological tools to determine if, during exercise, the physiological synthesis of brain 5-HT inhibits cutaneous heat loss are warranted. Considering that the animal species have developed different strategies to cope with different environmental challenges, future experiments are important to investigate if these results regarding 5-HT obtained in rodent's experiments can be extrapolated to human thermoregulation.

Key words: heat loss, p-CPA, serotonin, skin temperature, thermoregulation, treadmill.

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