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Relatório Final

(Anexo 9)

Tryptophan-induced central fatigue in exercising rats is related to serotonin content in preoptic area

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Abstract

To assess the effects of increased hypothalamic tryptophan (TRP) availability on 5-HT content in preoptic area on thermoregulation and work production during exercise on treadmill, 20.3 \textmu M of L-TRP (n = 7) or 0.15 M NaCl (n = 6) was injected into the lateral cerebral ventricle of male Wistar rats immediately before the animals started running (18 m min\textsuperscript{-1} 5\% inclination). Exercise time to fatigue (min), and workload (kgm) were analysed. Core temperature was measured by telemetry. At fatigue, brains were quickly removed and preoptic area (POA), hypothalamus (HP), frontal cortex (FC), hippocampi (HC) were rapidly dissected and frozen immediately in dry ice. Serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were measured by HPLC. TRP-exercised rats showed the highest content of 5-HT in the POA and the lowest in the hippocampi compared to the rested and SAL-exercised rats. An inverse relationship between TF and a direct correlation with body temperature changes and POA-SHT levels were observed. A correlation between HC-SHT content and TF was also found. However, there was no correlation between HC-SHT content and changes in Tb at fatigue. Finally, our results bring further evidences that increased 5-HT content in POA is involved with an increase in heat production during exercise. In addition, the direct correlation of 5-HT level in hippocampi and TF of TRP-exercised rats suggests that this brain area is also related to motor activity control during exercise. In conclusion, our data indicated that tryptophan-induced central fatigue in exercising rats is related to serotonin content in preoptic area.

Keywords: Time to fatigue; Tryptophan; Serotonin; Preoptic area; Heat storage

Fatigue during prolonged exercise may be influenced by the activity of the brain serotonergic system and this is referred as the "central fatigue hypothesis" [25]. It is suggested that increased central tryptophan (TRP) availability increases 5-HT activity during prolonged exercise, which may cause fatigue by increasing lethargy and loss of central drive/motivation [25]. Previously we showed that increased hypothalamic TRP availability during exercise precipitates fatigue that was related to a heat imbalance due to increased heat production and decreased dissipation [31,32]. These results indicate an important role of TRP in thermoregulation during exercise and are in agreement with the observation that high body temperature reduces the CNS drive for exercise performance [26,34] and that hyperthermia would precipitate feelings of fatigue at a sublethal threshold and establishes a safe guard against heat stroke, protecting the brain from thermal damage [2,34].

The PO/AH is the major brain region involved in thermoregulation [5,10,23,29] that integrates thermal inputs with energy-linked metabolic processes [5,10,23,29]. However, there is no evidence that serotonergic content on the PO area is involved on thermoregulation and work production during exercise in rats despite of well established that central serotonergic mechanisms are involved in rats thermoregulatory responses [16,23,36].

Therefore, this study aims to investigate the effects of increased hypothalamic TRP availability on 5-HT content in areas of CNS involved in thermoregulation such as preoptic area and hypothalamus and in areas that 5-HT turnover has been described to change in exercising rats [4].

Male Wistar rats (250–290 g) were individually housed under 14/10 h light–dark cycles and had free access to water and rat
chow. Under anesthesia with 1.0 mL kg$^{-1}$ (i.p.) of 2.5% tribromoethanol a VitalView Mini-Mitter TR3000 XM-FM (Sun River, Oregon, USA) temperature sensor was implanted in the peritoneal cavity through a small incision in the linea alba, after calibration to a precision of 0.01 $^\circ$C. After telemeter implantation, the animals were fixed in a stereotaxic apparatus (David Kopf Instruments, M-900, Tujung, CA, USA) and a brain guide cannula (22 G) was implanted according to a previously described technique [21,22]. All animals were allowed to recover for 1 week before being used further. 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 consecutive days before the experiments. The purpose of this preliminary exercise was to show the animals in which direction to run. The rats that ran naturally without the use of electric shock were selected for the experiment. All experiments were carried out according to guidelines established by the Ethical Committee for care and use of laboratory animals in the Federal University of Minas Gerais.

Exercise was performed on a motor-driven treadmill from 0 AM to 14 PM at room temperature of 23 ± 2 $^\circ$C. The intensity of exercise was 18 m min$^{-1}$ and 5% inclination, corresponded to an oxygen uptake of ~66% of VO$_{2}$max [1,19]. Exercise time to fatigue (min) and workload (kgs) were taken as indexes of maximal capacity for exercise. Fatigue was defined as the point when animals were unable to keep pace with the treadmill [18,20,27]. Core temperature (Tb) was measured by telemetry every 15 s as described previously [31].

On the day of the experiment, the animals were allowed to rest on the treadmill chamber for 1 h in the experimental room before the running test. A 30 G needle protruding 0.3 mm from the tip of the guide cannula was introduced into the right lateral ventricle and connected to a Hamilton syringe. Each rat received 2.0 $\mu$L of 0.15 M NaCl ($n = 6$) or 2.0 $\mu$L of 20.3 $\mu$M of L-TRP solution ($n = 7$) (Sigma, St. Louis, MO) injected into the right lateral cerebral ventricle [31,32]. Immediately after the injections the syringe and needle were removed and the animals were submitted to a regime of running until fatigue.

Rats were divided into 3 groups; one served as a nontreated control that remained on the treadmill resting for the time equivalent to time to fatigue of TRP or SAL exercising rats. The other groups were rats receiving TRP or SAL and submitted to running until fatigue. As soon as the fatigue point was reached or an equal time for nontreated controls, the animals were killed by decapitation. The brain was quickly removed and washed with ice-cold saline. The frontal cortex (FC), hippocampus (HC), hypothalamus (HP) and hypothalamic preoptic area (POA) were rapidly dissected out on an ice-cold plate [6,7] and frozen immediately in dry ice and stored thereafter at ~80 $^\circ$C, until serotonin and 5-hydroxyindoleacetic acid were measured by high-pressure liquid chromatography (HPLC). The HPLC system was equipped with a reverse-phase column (Shim Pack CLC-ODS; 25 cm, 5 $\mu$m, Shimadzu). The potential was set at 850 mV versus an Ag/AgCl reference electrode. A mobile phase containing 31.4 g citric acid, 584 mg NaCl, 800 mL millioQ water, 140 mg octylsodium sulfate, 48 mL acetylnitrile and 28 mL tetrahydrofurane (pH 3.0) was filtered and pumped through the system at a flow rate of 1.0 mL min$^{-1}$. The brain tissues were weighed and homogenized in perchloric acid (0.1 M) and centrifuged at 15300 × g for 20 min at 60 $^\circ$C, the supernatant were then filtered through a Millipore membrane (0.22 $\mu$m pore size; 13 mm, Millex, SP, Brazil). Twenty microlitres were injected into the HPLC-EC system for analysis (Shimadzu, Kyoto, Japan). Quantification of 5-HT and 5-HIAA was made by comparing the peak area to a standard curve.

The following equations were used to determine change in workload (kgs) and heat storage (HS, cal). HS = $\Delta T_{wc}$ where $\Delta T_{wc}$ = changes in body temperature ($T_{final} - T_{initial}$); $m$ is body weight in grams, and $c$ is specific heat of body tissues (0.826 cal g$^{-1}$ $^\circ$C$^{-1}$) [13]. Workload ($W$) was calculated as $W$ = body weight (kg), $T_{f}$; treadmill speed (m min$^{-1}$), sine $\theta$ (treadmill inclination) [1,28], where $T_{f}$ is time to fatigue.

All data are expressed as mean ± S.E.M. A two-way analysis of variance (ANOVA) was used for determining differences between time and treatment and also interactions between them to evaluate the differences in changes of body temperature. Significant interactions observed by ANOVA were further evaluated by 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. Pearson's correlation coefficient were used to evaluate the relationship between APO-5HT content at fatigue and time to fatigue or changes in body temperature. The relationship between HC-5-HT concentration and TF, as well as, $W$ and HS were also assessed using Pearson's correlation. The significance level was set at $p \leq 0.05$.

Table 1 shows 5-HT and 5-HIAA content in the studied brain areas. At fatigue, 5-HT content was significantly increased in the hypothalamus, frontal cortex and hippocampi of exercised rats compared to the rested group. Exercise by itself promoted a higher increase of 5-HT in hypothalamus than other brain areas (3.28 ± 0.72 mg kg$^{-1}$ exercised-SAL versus 1.28 ± 0.21 ng mg$^{-1}$ rested; $p \leq 0.05$). However, in fatigued TRP-exercised rats the highest content of 5-HT was in the POA compared to the rested and exercised rats, and the lowest, in the hippocampi. In the other hand, 5-HIAA content was unchanged by exercise in POA, HC and HP. In TRP-exercised rats 5-HIAA levels were lower in POA compared to rested or exercised rats.

As expected, icv TRP reduced running time (17.7 ± 2.3 min TRP versus; 35.8 ± 4.9 min SAL $p \leq 0.001$) and induced a decrease in workload (6.7 ± 0.6 kgm TRP versus; 14.3 ± 1.9 kgm SAL $p \leq 0.001$) compared to saline-treated groups. Exercise promoted a rapid increase in core temperature in both groups and the changes in body temperature at fatigue were significantly higher in TRP-treated rats: 1.23 ± 0.1 $^\circ$C TRP versus, 0.57 ± 0.2 $^\circ$C SAL ($p \leq 0.02$). To compare the total thermal effects of exercise in both experimental groups, HS were calculated. At fatigue, HS of TRP-treated rats were higher than in SAL-treated rats (282.2 ± 27.3 cal TRP versus; 134.5 ± 49.0 cal SAL $p \leq 0.02$). We also observed a positive correlation between changes in body temperature and POA 5-HT concentration ($r = 0.66; p = 0.05$) (Fig. 1A); as well as, an inverse relationship between TF and POA-5HT levels ($r = 0.56; p = 0.05$) (Fig. 1B). As illustrated in Fig. 1D, we also observed a correlation between HC-5-HT content and TF ($r = 0.60; p = 0.05$). However, there was
Table 1

Concentrations of 5-HT and 5-HIAA (ng mg⁻¹) in preoptic area (POA), hippocampus (HC), frontal cortex (FC) and hypothalamus (HP) of rested, exercised saline-treated (Exercised-SAL) and exercise tryptophan-treated (Exercised-TRP) rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rested (n=8)</th>
<th>Exercised-SAL (n=6)</th>
<th>Exercised-TRP (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-HT (ng mg⁻¹)</td>
<td>5-HIAA (ng mg⁻¹)</td>
<td>5-HT (ng mg⁻¹)</td>
</tr>
<tr>
<td>POA</td>
<td>1.27 ± 0.22</td>
<td>1.50 ± 0.28</td>
<td>1.37 ± 0.29</td>
</tr>
<tr>
<td>HC</td>
<td>0.80 ± 0.09</td>
<td>0.87 ± 0.12</td>
<td>1.33 ± 0.24</td>
</tr>
<tr>
<td>FC</td>
<td>0.41 ± 0.08</td>
<td>0.29 ± 0.06</td>
<td>1.09 ± 0.07</td>
</tr>
<tr>
<td>HP</td>
<td>1.28 ± 0.21</td>
<td>1.80 ± 0.48</td>
<td>3.28 ± 0.72</td>
</tr>
</tbody>
</table>

Running time to fatigue: TRP (17.7 ± 2.3 min); SAL (35.8 ± 4.9 min).

* p ≤ 0.05 compared to rested group.

No correlation between HC 5-HT content and changes in Tb at fatigue (Fig. 1C).

The present study demonstrated that the expected tryptophan-induced central fatigue due to hyperthermia and increased heat storage in exercising rats is related to serotonin content in pre-otic area. As already shown, TRP-exercised rats showed higher increase in body temperature and heat storage fatiguing earlier than saline-treated group. Icv injection of TRP increased 5-HT content in POA which was positively correlated with changes in body temperature (Fig. 1A) and inversely correlated to time to fatigue (Fig. 1B). In addition, our data showed that running time to fatigue was also directly correlated with 5-HT concentration in hippocampi (Fig. 1D). However, different from what occurred in POA, 5-HT content in hippocampi was not correlated with hyperthermia or heat storage induced by TRP in exercising rats. Thus, our results showing that increased brain TRP availability elevated 5-HT content in POA and diminished it in HC corroborate earlier findings that 5-HT turnover in areas of CNS involved both in thermoregulation and motor activity control change in a different manner in exercising rats [3,4]. Previously, we showed that, at fatigue, metabolic rate was increased in TRP-exercised rats [32]. Taking all together, our data suggest that icv TRP is

![Fig. 1](image-url) Correlation between 5-HT concentration in preoptic area (POA-5-HT) and changes in body temperature (Tb) (A) and between POA-5-HT concentration and time to fatigue (TF) (B); correlation between 5-HT concentration in hippocampi (HC-5-HT) and changes in Tb (C); correlation between HC-5-HT concentration and TF (D) in rats treated with 2 μL of L-TRP (20.3 μM, filled circle) or 2 μL of 0.15 M NaCl (open circles).
acting through an increase in heat production, but we cannot exclude that heat loss mechanisms could also be disrupted in these animals. The present experiment brings further evidences that increased brain TRP availability promotes hyperthermia reducing time to fatigue and performed workload. Furthermore, our data show that exercise by itself did not increase 5-HT levels in the POA of Sal-exercised rats compared to the rested animals. This suggests that the increased 5-HT concentration in the POA of TRP-exercised rats might be due to elevated TRP levels. However, it should pointed out that we did not measured 5-HT and 5-HIAA contents in brain areas after SAL or TRP treatment in rested animals. There are several evidences suggesting that the effect of TRP to stimulate 5-HT synthesis is dependent on the level of serotoninergic neuronal activity [8,9,30]. So, the thermogenic action of this precursor could result from increased production and release of 5-HT within the brain.

It is known that serotonin increases core temperature when injected into the preoptic area [23]. The POAH is the major brain region involved in thermoregulation [5,10,23,29] that integrates thermal inputs with energy-linked metabolic processes [5,10,23,29]. Besides preoptic area, hypothalamus also may have influence in body temperature control. Lin et al. [23] demonstrated that elevating 5-HT levels in the hypothalamus by infusing 5-hydroxytryptophan resulted in hyperthermic effects which were brought by increased metabolic heat production and a decreased heat loss.

Recently, Hasegawa et al. [14] showed that infusion of tetrodotoxin (TTX) into POAH in exercising rats promoted hyperthermia as result of both an impairment of heat loss and an elevation of heat production. Our data show that exercise increased body temperature and icv TRP induced an additional increase in body temperature. In previous experiment, we have shown that this effect of TRP is due to an enhanced heat production, as shown by increased metabolic rate [32]. This increased heat production was not followed by enhanced heat dissipation mechanism since heat storage was increased during exercise. It was shown that the dissipation of heat from the body is thought to be more important than the control of heat production in the regulation of body temperature during exercise [11,37]. In our experiment, increased heat storage reduced running time and performed workload probably to protect the brain against hyperthermia. Elevated internal body temperature and increased heat storage have been proposed as limiting factors to physical performance in both human [12,26] and animal studies [34]. Rodrigues et al. [28] demonstrated that the heat storage rate was the main predictive factor of fatigue during running in thermo-neutral or hot environments. On the other hand, the data reported by Soares et al. [31] support the hypothesis that both heat storage rate and internal body temperature seem to be important determinants of fatigue during exercise. However, the mechanism involved in these hyperthermia-induced effects and how thermal stress affects brain neurotransmission during exercise are not yet fully understood.

In our experiments, analysis of 5-HT and 5-HIAA changes in the brain following icv TRP in rats running to fatigue revealed regional differences showing that 5-HT system does not respond in an uniform manner to exercise. These results are in agreement with Chauoloff et al. [3,4] who demonstrated that TRP loading and/or exercise caused a region-specific alteration in the conversion of TRP into serotonin synthesis pathway. It is possible that exercise differentially alters the rate of TRP uptake in different brain regions resulting in different levels of 5-HT after exercise.

The role of serotonin in the regulation of motor control is complex. There are many evidences that 5-HT hippocampus is involved in locomotion [24,33,35]. Our data show that at fatigue, hippocampi 5-HT content were directly correlated with TF. This result is in agreement with reports that serotonergic neurons in the median raphe nuclei projecting to the hippocampus are related to motor activity [15,17,33]. Our results indicated that HC 5-HT content might participate in fatigue during exercise through other mechanism rather than thermoregulation. However, the neurochemical mechanisms that underlie the effect of exercise in the hippocampus 5-HT synthesis and metabolism during TRP loading still remained unknown.

The evidences that changes in the brain TRP promote regional differences in the 5-HT synthesis and turnover in the CNS and evidence of subgroups of 5-HT neurons acting in different brain circuit, make difficult the identification which 5-HT neurons are responsive to TRP during exercise [8]. Furthermore, increasing evidence suggests different role of 5-HT receptor subtypes in thermoregulation in rodents. So, it may be important study the interaction between 5-HT receptors involved in the control of endurance performance and thermoregulatory mechanisms to establish which ones are involved on the onset of fatigue during exercise. Furthermore, we can not also exclude that 5-HT in various brain regions might be differentially influenced by other neurotransmitters, such as dopamine, that could be affected by exercise and might alter 5-HT neurotransmission when released from nerve terminals.

Finally, our results bring further evidences that increased 5-HT content in POA is involved with an increase in heat production during exercise. In addition, the direct correlation of 5-HT level in hippocampi and TF of TRP-exercised rats suggests that this brain area is also related to motor activity control during exercise. In conclusion, our data indicated that tryptophan-induced central fatigue in exercising rats is related to serotonin content in preoptic area.

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References


