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Evidence that tryptophan reduces mechanical efficiency and running performance in rats

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Abstract

It has been reported that exercise increases brain tryptophan (TRP), which is related to exhaustive fatigue. To study this further, the effect of increased TRP availability on the central nervous system (CNS) with regard to mechanical efficiency, oxygen consumption (VO_2) and run-time to exhaustion was studied in normal untrained rats. Each rat was anesthetized with thiopental (30 mg/kg ip b. wt.) and fitted with a chronic guiding cannula attached to the right lateral cerebral ventricle 1 week prior to the experiments. Immediately before exercise, the rats were randomly injected through these cannulae with 2.0 μl of 0.15 M NaCl ($n=6$) or 20.3 μM L-TRP solution ($n=6$). Exercise consisted of running on a treadmill at 18 m min^{-1} and 5% inclination until exhaustion. TRP-treated rats presented a decrease in their mechanical efficiency (21.25 ± 0.84%, TRP group vs. 24.31 ± 0.98%, saline-treated group; $P \leq .05$), and increased VO_2 at exhaustion (40.3 ± 1.6 ml kg^{-1} min^{-1}, TRP group vs. 36.0 ± 0.8 ml kg^{-1} min^{-1}, saline group; $P \leq .05$), indicating that the metabolic cost of exercise was higher in the former group. In addition, a highly significant reduction was also observed in run-time to exhaustion of TRP animals compared to those of the saline-treated group (15.2 ± 1.52 min, TRP group vs. 50.6 ± 5.4 min, saline group; $P \leq .0001$). It can be deduced from the data that intracerebroventricular TRP injection in rats increases O_2 consumption and reduces mechanical efficiency during exercise, diminishing running performance.

Keywords: Tryptophan; Fatigue; Exercise; Metabolic rate; Mechanical efficiency

1. Introduction

Several experimental approaches have been used to test the hypothesis that central fatigue is related to increased brain tryptophan (TRP) uptake. These include nutritional manipulation to modify the plasma concentration of the branched-chain amino acid/free tryptophan balance (BCAA/f-TRP) and pharmacological manipulation of brain monoamine turnover (Bailey et al., 1992, 1993a,b; Newsholme and Blomstrand, 1995, 1996; Farris et al., 1998; Yamamoto and Newsholme, 2000). However, nutritional manipulation to increase plasma-free TRP and thereby increase its availability to the brain has produced contradictory results with respect to changes in exercise performance (Alves et al., 1995; Segura and Ventura, 1988; Stensrud et al., 1992; van Hall et al., 1995). Pre-ingestion of TRP has been reported to increase time to exhaustion (Segura and Ventura, 1988) or have no effect on treadmill performance (Stensrud et al., 1992) or on bicycle exercise (Alves et al., 1995; van Hall et al., 1995). On the other hand, inhibition of the L-system transporter for the uptake of TRP in nagase analbuminemic rats (Yamamoto and Newsholme, 2000) resulted in a prolonged run-time, suggesting that the serotonergic system might be involved in central fatigue (Yamamoto and Newsholme, 2000). There is also some evidence that elevated levels of plasma-free TRP in humans are associated with fatigue (Davis et al., 1992; Wilson and Maughan, 1992). The objective of the present study was therefore to verify the role of increased TRP availability to the central nervous system (CNS) on metabolic rate in normal untrained rats, during submaximal exercise until exhaustion. Metabolic rate

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is an important parameter in physical work that influences both mechanical efficiency and run-time to exhaustion (Sonne and Galbo, 1980; Brooks and White, 1978).

2. Materials and methods

2.1. Animals

Male Wistar rats (300 ± 20 g) were housed in individual cages under controlled light (05:00–19:00 h) and temperature (23 ± 2 °C) conditions, with food and water provided ad libitum. After being anesthetized with thiopental (30 mg/kg ip b. wt.), each rat was fixed to a sterotaxic apparatus (David Kopf Instruments, M-900, Tujunga, CA, USA) and a guide cannula (22 G) implanted into the right lateral cerebral ventricle using a previously described technique (Lima et al., 1998). All animals were allowed to recover for at least 1 week before being utilized in the experiments. The animals were familiarized to exercise in the motor-driven treadmill by running them daily for 5 min for 5 days before experiments.

2.2. Exercise

Exercise was performed on a motor-driven treadmill between 10:00 and 14:00 h at 23 ± 2 °C. The intensity of exercise (18 m min^{-1} and 5% inclination) corresponded to an oxygen uptake of 66% of VO_{2max} (Brooks and White, 1978; Lima, 2000; Hussain et al., 2001). Exhaustion was defined as the point when the animals were unable to keep pace with the treadmill despite constant physical prodding for 1 min (Bailey et al., 1993a,b). Time to exhaustion (min) was taken as an index of maximal capacity for exercise.

2.3. Experimental protocol

On the day of the experiment, the animals were allowed to rest on the metabolic rodent treadmill (Columbus Instruments, OH, USA, Modular Treadmill, serie 96002-2) for 1 h in the experimental room before running the test. A needle (30 G) protruding 0.3 mm from the tip of the guide cannula by connecting to a Hamilton syringe was introduced into the right lateral cerebral ventricle. Immediately before the exercise, 2.0 μl of 0.15 M NaCl ($n = 6$) or 2.0 μl of 20.3 μM L-TRP solution ($n = 6$) (Sigma, St. Louis, MO) were injected into the right lateral cerebral ventricle of each animal. Immediately after the injections, the animals were subjected to a regime of running until exhaustion. Oxygen consumption (VO_2) and CO_2 production (VCO_2) were measured by an open-flow indirect calorimeter (Columbus Instruments) that was calibrated before each use with a certified mixture of gases (20.5% O_2 and 0.5% CO_2). VO_2 (ml kg^{-1} min^{-1}), VCO_2 (ml kg^{-1} min^{-1}), respiratory exchange ratio (R) and energy expenditure (kcal min^{-1}) were continuously recorded on-line using a computerized system (Oxymax Apparatus, Columbus Instruments).

Mechanical efficiency (ME) was calculated by the formula: ME=(W/energetic cost) × 100; W=[(exercise intensity) × (time to exhaustion) × (body weight)] (Brooks et al., 1984).

All procedures were in compliance with guidelines laid down by the Ethical Committee for Care and Use of Laboratory Animals of the Federal University of Minas Gerais.

2.4. Statistical analysis

The data are reported as mean ± S.E.M. Differences between groups and the effect of time were determined by analysis of variance (ANOVA) followed by the Newman–Keuls test. The data were also compared by paired or unpaired Student's t-test when applicable. The significance level was considered to be $P \leq .05$.

3. Results

As illustrated in Fig. 1, intracerebroventricular injection of TRP in untrained normal rats (TRP group, $n = 6$ rats) induced a marked decrease in maximal capacity for exercise compared to saline-treated rats (SAL group, $n = 6$): 15.2 ± 1.52 min, TRP vs. 50.6 ± 5.4 min, SAL; $P \leq .0001$.

As illustrated in Fig. 2A, TRP rats showed an increased energy expenditure per meter compared to SAL rats, observed at the time of exhaustion (3.34 ± 0.17 cal m^{-1}, TRP vs. 2.77 ± 0.20 cal m^{-1}, SAL; $P \leq .05$). Oxygen consumption by TRP-treated rats at exhaustion (Fig. 2B)

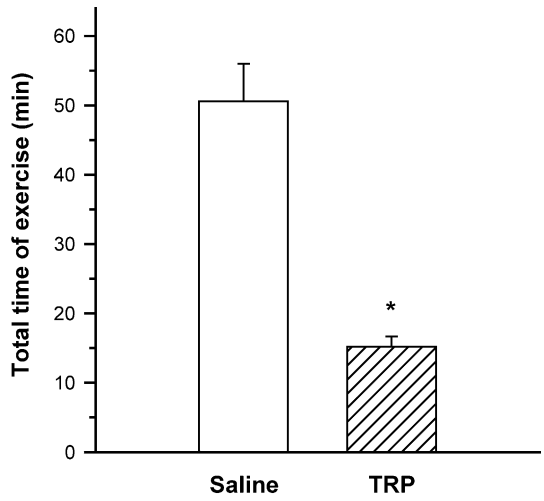

Fig. 1. Effect of intracerebroventricular injection of L-tryptophan (20.3 μM) ($n = 6$) or 0.15 M NaCl ($n = 6$) on total time of exercise (min). Data are expressed as means ± S.E.M. * Significantly different from the control group ($P \leq .0001$).

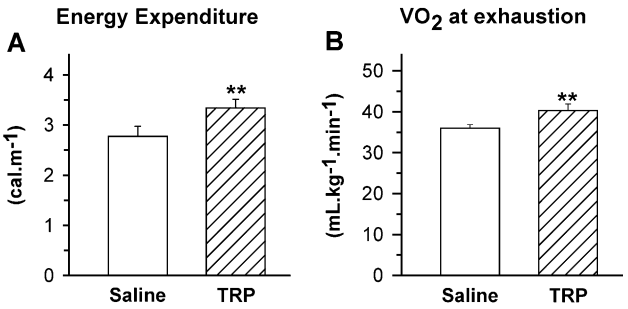

Fig. 2. (A) Effect of intracerebroventricular injection of L-tryptophan (20.3 μM) ($n=6$) or 0.15 M NaCl ($n=6$) on energy expenditure per meter of running distance (cal m^{-1}) and (B) on oxygen uptake at exhaustion (ml kg^{-1} min^{-1}). Data are expressed as means ± S.E.M. ** Significantly different from the control group ($P \leq .05$).

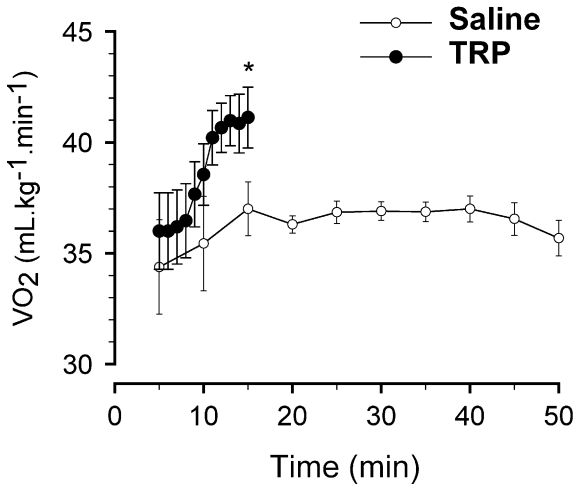

Fig. 4. Effect of intracerebroventricular injection of L-tryptophan (20.3 μM) ($n=6$) or 0.15 M NaCl ($n=6$) on oxygen consumption (ml kg^{-1} min^{-1}) during exercise. Values are expressed as means ± S.E.M. * Significantly different from the control group at exhaustion ($P \leq .05$).

was also higher than in saline-treated rats: 40.3 ± 1.6 ml kg^{-1} min^{-1}, TRP vs. 36.0 ± 0.8 ml kg^{-1} min^{-1}, SAL; $P \leq .05$. The calculated mechanical efficiencies at exhaustion point were 24.31 ± 0.98% in saline-treated rats and 21.25 ± 0.84% in TRP-treated rats, showing a higher metabolic cost in the TRP group than in SAL group ($P \leq .05$) (Fig. 3). These differences can be better evaluated by analysis of the O_2 consumption time curve (Fig. 4). As illustrated in Fig. 4, TRP rats showed an increased O_2 consumption compared to that of the controls (36.5 ± 0.2 ml kg^{-1} min^{-1}). This was already evident 11 min after the animals started running and persisted until the fatigue point (Fig. 4, $P \leq .01$). However, treatment with intracerebroventricular TRP did not induce significant changes in respiratory exchange ratio compared to saline-treated rats (0.96 ± 0.07, TRP vs. 0.88 ± 0.05, SAL; P=.402).

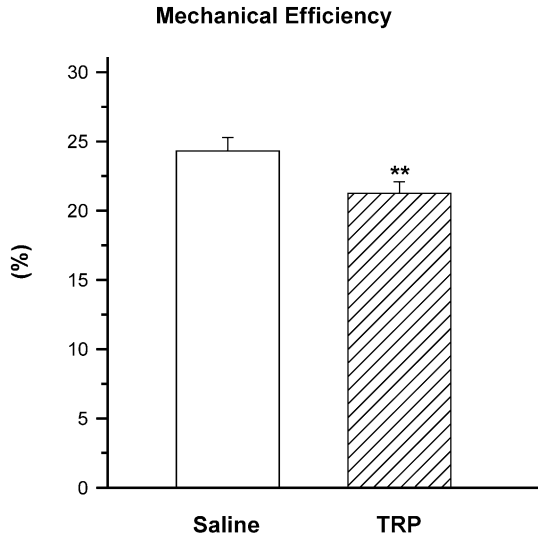

Fig. 3. Effect of intracerebroventricular injection of L-tryptophan (20.3 μM) ($n=6$) or 0.15 M NaCl ($n=6$) on mechanical efficiency (%). Values are expressed as means ± S.E.M. ** Significantly different from the control group ($P \leq .05$).

4. Discussion

The results of the present study show that intracerebroventricular injection of TRP induces a decrease in mechanical efficiency associated with a drastic reduction (~70%) in running time to exhaustion in rats submitted to submaximal exercise. The increased energy expenditure per meter of running distance (Fig. 2A) observed during exercise was followed by a higher metabolic rate at exhaustion compared to control rats (Fig. 2B). Our data indicate that an increased availability of TRP in the CNS affects metabolic rate during exercise, reducing time to fatigue (Fig. 1). These results are consistent with the central fatigue hypothesis and confirm the indications that run-time to exhaustion can be related to increased TRP extracellular concentration in the CNS (Newsholme and Blomstrand, 1995, 1996; Farris et al., 1998; Yamamoto and Newsholme, 2000). However, this is the first study to describe an effect on mechanical efficiency, metabolic cost of exercise and metabolic rate at exhaustion induced by intracerebroventricular injection of TRP.

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). However, altered 5-HT activity can also affect thermoregulation, pain tolerance and activity of the hypothalamic–pituitary–adrenocortical and sympathoadrenal systems (Korte et al., 1991;

Alper, 1990; Bagdy et al., 1989; Goodwin et al., 1985; Prieto-Gomez et al., 1989). Therefore, manipulation of any of these systems could potentially alter performance.

Several studies have focused on the influence of exercise on TRP uptake by brain and 5-HT synthesis and turnover (Chaouloff, 1997; Chaouloff et al., 1987; Davis et al., 1992; Meeusen et al., 1996). Analysis of whole brain (Chaouloff, 1997; Chaouloff et al., 1985, 1986a), brain regions (Blomstrand et al., 1989; Chaouloff et al., 1989; Lookingland et al., 1986) or cerebrospinal fluid samples (Chaouloff et al., 1986b) from exercising rats showed an increased brain TRP content. Although both Chaouloff et al. (1985, 1986a,b, 1987) and Meeusen et al. (1996) demonstrated that prolonged exercise results in increased availability of TRP to brain and increased brain 5-HT and 5-hydroxyindole-3-acetic acid (5-HIAA) concentrations in the rat; a causal link between increased 5-HT activity and reduced physical and mental performance has not been established. It is important to point out that these experiments were not designed to study fatigue but rather the effects of TRP injection and/or mild exercise on CNS extracellular 5-HT concentration. The total workload to which these animals were submitted was mild compared to experiments designed to study fatigue (Bailey et al., 1992, 1993a,b; Lima et al., 2001). Data from Bailey et al. (1992, 1993a) showed that run-time to exhaustion in the rat can be significantly affected by intraperitoneal administration of 5-HT agonist and antagonist drugs. Specifically, administration of a 5-HT agonist reduces run-time to exhaustion, while administration of a 5-HT antagonist increases run-time to exhaustion. On the other hand, support for the involvement of TRP and 5-HT in fatigue can be found in studies where the brain concentration of 5-hydroxytryptophan (5-HTP), the immediate precursor of serotonin, was altered by means of pharmacological agents. When 5-HTP levels were elevated in this way, performance was impaired in rats, while a decrease resulted in an improvement. In a related study, Yamamoto et al. (1997) showed that intrasynaptosomal concentration of TRP and 5-HT in trained Nagase analbuminemic rats increased after exercise and suggested that it could be responsible for the shortening of running time to exhaustion. These results agree with those of the present study, suggesting that the increased TRP level available in the CNS since the onset of exercise could drastically reduce run-time to exhaustion. In addition, our data showed increased energy expenditure in TRP-treated rats during exercise on a treadmill with low mechanical efficiency and higher O_2 uptake already observed when only 75% of the time to exhaustion was attained (Figs. 3 and 4).

TRP increase induced by exercise has been described as occurring simultaneously with a significant rise in 5-hydroxyindoleacetic acid concentration, indicating that exercise increases 5-HT synthesis and turnover. Evidence that TRP alters 5-HT within the brain was provided by Denizeau and Sourkes (1977), who demonstrated a positive relationship between endogenous serotonin content and activity of TRP transport mechanisms in hypothalamic brain slice or synaptosome preparations. They observed a decreased synaptosomal TRP accumulation in rats with reduced nerve terminals due to electrolytic lesions of raphe nuclei or intracerebroventricular injection of 5,7-dihydroxytryptamine. In addition, Schaechter and Wurtman (1990) demonstrated that both spontaneous and electrically evoked 5-HT release from rat hypothalamic slices is dependent on precursor availability, which causes parallel changes in brain 5-HT levels and 5-HT release. The possibility that, in the present study, the intracerebroventricular injection of TRP could have decreased the running time to exhaustion by activating the kynurenine pathway is unlikely. TRP-injected intracerebroventricularly (Luthman et al., 1996), or by means of dialysis (Speciale et al., 1989), was unable to act as a precursor of quinolinic acid in the rat brain. The authors suggested that there is an incomplete kyrunenine metabolism in the rat brain, and that this may constitute an important protective mechanism against build-up of neuroactive concentrations of quinolinic acid. This becomes important, considering that TRP is required in normal brain functioning as a precursor of serotonin.

Besides their observed effect on metabolic rate during exercise (Fig. 4), changes in extracellular TRP induced by intracerebroventricular injection of TRP may also affect thermoregulatory function, which in turn might limit exercise performance. Increased body temperature is a consequence of an increase in metabolic rate and the failure of heat loss to keep pace with heat production. Therefore, the decrease in run-time to exhaustion (Fig. 1) observed in our study might also have resulted from thermogenic action of central TRP not compensated by heat loss, as shown by the increased metabolic cost of exercising in TRP-treated rats. TRP has been described as inducing thermogenic effects on preoptic area/anterior hypothalamus (PO/AH) (Serra et al., 1992; Lin et al., 1998). The PO/AH is the major brain region involved in thermoregulation (Hammel, 1968; Coimbra and Migliorini, 1988; Santos et al., 1990, 1991; Lin et al., 1998; Ferreira et al., 1999). Therefore, changes in extracellular TRP and serotonin in rat PO/AH due the intracerebroventricular injection of TRP could have affected thermoregulatory function and metabolic adjustments during exercise. Increase in O_2 consumption in conscious resting rats in response to central or peripheral injections of TRP has been observed (Serra et al., 1992; Lin et al., 1998). Thus, the effect of TRP on thermoregulation might contribute to decreased mechanical efficiency, reducing the run-time to exhaustion in TRP-treated rats.

In conclusion, our data showed that intracerebroventricular TRP injection reduced mechanical efficiency, increased O_2 consumption at exhaustion and induced a marked reduction in the run-time to exhaustion. These results indicate that increased availability of TRP into the CNS during exercise affects metabolic rate and reduces running performance in rats.

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