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Research report

Intrinsic exercise capacity is related to differential monoaminergic activity in the rat forebrain

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ABSTRACT

Monoamines levels in central nervous system have been associated with exercise performance and fatigue. The present study investigated whether intrinsic exercise capacity is associated with differential activity of monoamines in the caudate–putamen (CPu) and accumbens (ACC) nucleus. Male Wistar rats were subjected to a progressive testing protocol. Based on the maximal time of exercise in the progressive testing protocol (TEPmax), the animals were divided into low-performance (LP), high-performance (HP), and standard-performance (SP) groups. After classification, eight animals in each group were chosen randomly and evaluated in two experimental situations: rest ($n = 8$) or moderate exercise (ME) at 60% of maximal velocity ($n = 8$). The CPu and ACC were dissected for analyses of monoamine levels. At rest, HP rats exhibited higher 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine (DA) ratio and lower serotonin (5-HT) concentration compared other groups, and lower 5-hydroxyindoleacetic (5-HIAA) compared with the LP rats. The ME resulted in increased DOPAC/DA ratio in the CPu of all experimental groups. In both the CPu and ACC, ME increased 5-HIAA levels in SP and HP rats and 5-HIAA/5-HT ratio only in HP rats. Thus, our findings demonstrate that rats with natural intrinsic differences in performance to exercise exhibit alterations in dopaminergic and serotonergic systems at rest and after ME exercise until fatigue.

1. Introduction

The phenotype of physical performance is determined by both environmental (e.g., physical training, nutrition) and genetic factors that influence multiple physiologic systems (Koch and Britton, 2008). It is well known that apart from regular physical exercise (Leitzmann et al., 2007; Sabia et al., 2012), the intrinsic ability to perform physical activity is important to survival (Koch and Britton, 2008). Low aerobic exercise capacity increases the risk of developing a large number of diseases (Lessard et al., 2009; Ritchie et al., 2013). In the last decades, the relationship between physical performance and health status has been investigated in divergent lines of rats using a large-scale selective breeding program based on differences in running capacity. In each generation of selection, the lines contrast in numerous physiological variables (Britton and Koch, 2001; Koch et al., 2012). On the other hand, a previous study from our group showed that rats without divergent artificial selection also have intrinsic variation in their capacity to exercise (Primola-Gomes et al., 2009). In this model for exercise performance, the higher capacity to run is related to higher levels of intracellular calcium transients in cardiomyocytes (Primola-Gomes et al., 2009), but the mechanisms determining this natural variability to perform exercise remain to be elucidated.

The central nervous system (CNS) is known to play a role in fatigue during prolonged exercise. The biological mechanism involves the interrelationship between serotonin (5-HT) and dopamine (DA) systems [for reviews, see (Davis and Bailey, 1997; Foley and Fleshner, 2008; Leite et al., 2010; Rodrigues et al., 2009)]. The dopaminergic neurons from the substantia nigra pars compacta and the ventral tegmental area project to the dorsal [caudate–putamen (CPu)] and ventral [accumbens nucleus

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(ACC)] striatum forming the nigrostriatal and mesolimbic pathways, respectively (Knab and Lightfoot, 2010). These forebrain areas are involved with motor control and motivation for performing exercise (and other motivated behaviors), which are critical aspects related to running performance (Balthazar et al., 2009; Kravitz and Kreitzer, 2012; Watson et al., 2005). In contrast, the serotonergic system is formed by neurons originating in the raphe nuclei, which project to numerous structures in the CNS, including CPu and ACC (Berger et al., 2009). The effects of 5-HT are generally associated with lethargy, sleepiness, and loss of motivation (Nakamaru-Ogiso et al., 2012; Nugent et al., 2013), which can impair exercise performance (Cordeiro et al., 2012; Soares et al., 2007). Moreover, DA and 5-HT have been reported to establish a relationship of reciprocal inhibition with impact on the occurrence of fatigue. The activity of both DA and 5-HT terminals increases during physical exercise in different brain areas, such as the striatum, hypothalamus and midbrain, but at fatigue the increase in dopaminergic activity is reported to be attenuated while serotonergic activity remains elevated (Bailey et al., 1993; Balthazar et al., 2009; Davis and Bailey, 1997). Thus, considering that dopaminergic activity is associated with increased mechanical efficiency and motivation, and decreased perception of effort, whereas serotonergic activity is related to lethargy, tiredness and loss of drive, the increase in the activity of 5-HT relative to DA in the CNS is thought to result in interruption of exercise (Balthazar et al., 2009; Coimbra et al., 2012; Leite et al., 2010; Soares et al., 2007). Indeed, the administration of a 5-HT antagonist has been shown to increase the time of exercise and prevent the attenuation in dopaminergic activity at fatigue in the hypothalamus and midbrain (Bailey et al., 1993).

Recent works have shown that differences in the monoaminergic systems may contribute to variation in intrinsic running capacity between lines of genetically selected rats (Foley et al., 2006; Roberts et al., 2012). For example, compared with low-capacity runner (LCR) rats, high-capacity runner (HCR) rats show higher levels of DA receptor D2 (DR-D2) mRNA in the CPu and ACC and 5-HT receptor 1B (5-HT1B) mRNA in the dorsal and median raphe nuclei, suggesting a role for DA and 5-HT in the differences of exercise performance in genetically selected animals (Foley et al., 2006). However, whether differences in the activity of DA and 5-HT systems are related to intrinsic physical exercise capacity in non-genetically selected rats remains to be elucidated. Thus, the aim of this study was to investigate whether the activity of monoamine terminals in the CPu and ACC is associated with differences in intrinsic exercise capacity in rats. In order to control for any interference of the availability of energetic substrates in the exercise performance, we also evaluated the metabolic responses of the rats to the exercise.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (body weight, 240–260 g) were obtained from the Animal Care Center at the Federal University of Minas Gerais. The rats were housed in collective cages (four animals per cage) under a light/dark cycle (14/10 h) and room temperature ($24 \pm 2\,^{\circ}C$) with free access to water and rat chow. All experimental procedures were approved by the Ethical Committee for the Care and Use of Laboratory Animals of the Federal University of Minas Gerais (protocol 061/11).

2.2. Progressive exercise testing protocol

The rats were gradually encouraged to exercise on a motor-driven treadmill (Columbus Instruments, Columbus, OH, USA) by running 5 min at progressive speeds (10, 10, 11, 13, and 15 m/min) over five days. The treadmill inclination was kept at 5° and light electrical stimulus was set at 0.28 mA during all exercise situations. During the second week, the running capacity, based on the time of exercise, in each rat was evaluated three times in a progressive exercise testing protocol. The interval of at 48 h was given between tests for recovery of the animals. Each test began at 10 m/min and the velocity was increased by 1 m/min every 3 min until fatigue. Fatigue was defined as the time at which the animal was unable to keep pace with the treadmill speed for at least 10 s, even when being stimulated by mild electrical shock (Primola-Gomes et al., 2009).

2.3. Animal selection

The selection strategy used in the present study was based on the criteria of Primola-Gomes et al. (2009), which considered the maximal time of exercise in the progressive test protocol (TEPmax) of each rat. According to the TEPmax, a frequency histogram was constructed (Fig. 1) and three experimental groups were assigned: low performance (LP), standard performance (SP), and high performance (HP). To be included in the LP group, the animal needed to have a TEPmax lower than one standard deviation (SD) of the population average. Animals with a TEPmax higher than one SD were classified as HP. Finally, to be included in the SP group, the animal needed to have a TEPmax that fell within one SD of the average. These parameters were based on previous work (Primola-Gomes et al., 2009).

2.4. Experimental design

Six days after the progressive exercise testing protocol, 16 animals were chosen randomly in each exercise performance group (LP, SP and HP rats) and were evaluated in two experimental situations: rest ($n = 8$) or moderate exercise (ME, $n = 8$). The sample number was based on the previous study from our group that also investigated parameters related to physical performance (Wanner et al., 2010). In the rest state, rats were euthanized immediately after removal from their home cage, without previous stimulus. In the ME situation, rats were subjected to ME (60% of maximum velocity) and were euthanized immediately following fatigue. This intensity of exercise was chosen to ensure that all animals were able to run for at least 20 min, required for induction of monoaminergic responses in the brain (Hattori et al., 1994).

2.5. Euthanasia

All procedures were performed during the morning period. Rats were rapidly decapitated, the brains were quickly removed, and trunk blood was collected. Using a brain matrix (Insight, Ribeirao Preto, SP, Brazil), the brain was sliced in 2 mm coronal sections between +2.7 mm and +0.7 mm from bregma, according to the rat brain Atlas (Paxinos and Watson, 2005). The dissections of the ACC and CPu were performed between +1.7 and +0.7 mm from bregma using a punch needle. The visual identification of the anterior commissure was used as anatomical landmark for the ACC dissection. The CPu dissection was made beneath the corpus callosum and visualization of tissue striations was used for identification of this brain region (McCandlish et al., 2000). All dissections were performed in both cerebral hemispheres and by the same researcher. The high concentrations of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) in the CPu and ACC were similar to previous reports in the striatum (Del-Bel et al., 2014) and further confirmed the accuracy of the dissections. The brain areas were immediately stored at −80 °C until assay to measure DA, DOPAC, 5-HT, and 5-hydroxyindoleacetic (5-HIAA)