Improved exercise anaerobic power and less central fatigue in CD73 knockout mice

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ACKNOWLEDGMENTS: Supported by Prémio Maratona da Saúde, CAPES-FCT (039/2014), CNPq (302234/2016-0), LaCaixa Foundation (LCF/PR/HP17/52190001), FCT (POCI-01-0145-FEDER-03127 and UIDB/04539/2020), and ERDF through Centro 2020 (project CENTRO-01-0145-FEDER-000008:BrainHealth 2020 and CENTRO-01-0246-FEDER-000010). A.S.A.Jr is a CNPq fellow. We thank Flávio Reis and Frederico Pereira (University of Coimbra) for making available the treadmill and gas analyzer.

AVAILABILITY OF DATA AND MATERIAL: The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICTS OF INTEREST: RAC is a scientific consultant for the Institute for Scientific Information on Coffee. All other authors declare no conflict of interests.

ABSTRACT

Ecto-5'-nucleotidase CD73 is the main source of extracellular adenosine involved in the activation of adenosine A_{2A} receptors responsible for the ergogenic effects of caffeine. We now investigated the role of CD73 in exercise by comparing female wild-type (WT) and CD73 knockout (KO) mice in a treadmill graded test to evaluate running power, oxygen uptake ($\dot{V}O_2$), and respiratory exchange ratio (RER) – the gold standards characterizing physical performance. Spontaneous locomotion in the open field and submaximal exercise performance in the treadmill were similar between CD73-KO and WT mice; $\dot{V}O_2$max also demonstrated equivalent aerobic power. However, CD73-KO displayed higher anaerobic power indexes, namely a 43.7±4.2% larger critical power (large effect size, $P < 0.05$) and 3.8±0.4 increase of maximum RER (small effect size, $P < 0.05$). Thus, KO of CD73 was ergogenic, i.e., it increased physical performance, suggesting that CD73-mediated formation of extracellular adenosine signals fatigue.

Keywords

Adenosine; CD73; fatigue; respiratory exchange ratio; running; $\dot{V}O_2$max

INTRODUCTION

Adenosine is a prototypical inter-cellular modulator, signaling altered activity and metabolic stress within a tissue by activating adenosine receptors [1]. Accordingly, exercise temporarily increases the circulating levels of adenosine in humans [2] and rats [3], and the non-selective adenosine receptor antagonist, caffeine, is well established to cause an ergogenic effect [4]. Adenosine is also a neuromodulator in brain circuits [5] that causes drowsiness and tiredness at rest [5,6] and is a candidate molecule to signal central exercise fatigue [7-9]. The observations that the ergogenic effects of caffeine in rodents are mimicked by selective antagonists of adenosine A_{2A} receptors ($A_{2A}R$) and abrogated in forebrain $A_{2A}R$ knockout mice [8] and eliminated upon intracerebroventricular administration of the A_2R agonist, NECA [9], strongly implies central $A_{2A}R$ as critical regulators of the impact of adenosine on exercise performance.

The activation of $A_{2A}R$ is achieved by a particular pool of extracellular adenosine formed by ecto-5'-nucleotidase, or CD73, responsible for the final formation of ATP-derived extracellular adenosine in the brain [10,11]. There is a physical association of CD73 and $A_{2A}R$ in central synapses [12] and the pharmacological or genetic inhibition of CD73 phenocopies the inhibition of $A_{2A}R$ in physiological and pathological conditions of brain functioning [13-15], as well as in immune [16] or adaptive vascular control [17]. However, the role of CD73 in exercise remains to be defined, which prompted the present study to characterize the impact of deleting CD73 on mice's exercise performance.

METHODS

Animals

We used 17 female mice (20.8±0.2 g, 8-10 weeks old) from our inbred colony with CD73-KO mice and wild-type littermates. The global-CD73 KO mice have a C57BL/6 genetic background and were bred as previously described [12,15]. Mice were housed in collective cages in HEPA-filtered ventilated racks (n=3-5) under a controlled environment (12 h light-dark cycle, lights on at 7 AM, and room temperature 22±1°C) with *ad libitum* access to food and water. WT and CD73-KO mice were housed

together, with no genotype separation, following European Union guidelines (2010/63), and the Ethical Committee approved the study of the Center for Neuroscience and Cell Biology (University of Coimbra).

Mice were habituated to handling and the treadmill (9 m/min) in the three days before starting the experiments, which were performed between 9 AM and 5 PM, within the light phase of the mouse dark/light cycle, in a sound-attenuated and temperature/humidity controlled room (20.3±0.6 °C and 62.8±0.4% humidity) under low-intensity light (≈ 10 lux). The open field apparatus and the treadmill were cleaned with 10% ethanol between individual experiments. The allocation for the experimental groups was random. For each test, the experimental unit was an individual animal.

Open field

Mice explored an unaccustomed open field (38×38 cm) for 15 min. Locomotion was analyzed using an ANY-Maze video tracking system (Stoelting Co.), as previously described [12].

Graded exercise test – ergospirometry

Mice were accustomed to a single-lane treadmill (Panlab LE8710, Harvard apparatus) at 9 m/min (10 min, slope 5°, and 0.2 mA) with a 24 h interval between each habituation session. The incremental running protocol started at 9 m/min, with an increment of 3 m/min every 2 min at 5° inclination [18]. The exercise lasted until running exhaustion, defined by the animal's inability to leave the electrical grid for five seconds [18,19].

Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were estimated in a metabolic chamber (Gas Analyzer ML206, 23×5×5 cm, AD Instruments, Harvard) coupled to the treadmill, as previously described [18]. The animals remained in the chamber for 15 min before exercise testing. Atmospheric air (≈21% O_2, ≈0.03% CO_2) was renewed at a rate of 120 mL/min, using the same sampling rate for the LASER

oxygen sensor (Oxigraf X2004, resolution 0.01%) and infrared carbon dioxide sensor (Servomex Model 15050, resolution 0.1%).

We estimated the running and critical power output in the treadmill based on a standard conversion of the vertical power, body weight, and running speed [18,20]. Running power is the sum (Σ) of all stages of the exercise test, and critical power is the running power performed above $\dot{V}O_2$max. $\dot{V}O_2$max is the maximum capacity to capture (respiratory), transport (cardiovascular), and consume (muscles) oxygen [8]. The respiratory exchange ratio (RER) is the ratio between the amount of carbon dioxide production ($\dot{V}CO_2$) and the consumed oxygen ($\dot{V}O_2$) [8].

Statistics

Data are presented as mean±SEM in graphs built using the GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Statistical analyzes were performed according to an intention-to-treat principle using StatSoft, Inc. (2007). STATISTICA (data analysis software system), version 13.0. www.statsoft.com. A Student's t-test was used to evaluate body mass, open field, running power, $\dot{V}O_2$max, and RER. The evolution of running power and submaximal $\dot{V}O_2$ were evaluated by ANOVA for repeated measures followed by the Bonferroni *post hoc* test. The differences were considered significant when $P<0.05$. Effect sizes (Cohen's *d*) were calculated for between-group changes in mean differences for open field, running power, and RER, where a Cohen's $d=0.2$ represents a 'small' effect size, 0.5 represents a 'medium' effect size, and 0.8 a 'large' effect size [21]. Cohen's η^2 was used for $\dot{V}O_2$ kinetics, defined as small (0.02), medium (0.13) large (0.26) [21].

RESULTS

WT and CD73-KO mice's body mass did not differ ($t=0.5$, $df=15$, Fig.1A), an essential feature since the running power depends on this variable. Locomotion in the open field was not different between genotypes; neither the total distance indicated by the average speed ($t_{15}=0.75$, $P=0.4$, Fig.1B) nor the maximum speed ($t_{15}=0.16$, $P=0.87$, Fig.1B).

Running power increased with belt speed acceleration ($F_{7,56}$=30k, P<0.05, η^2=0.99, Fig.1C), with no differences between genotypes up to 33/min ($F_{7,56}$=1.5, P=0.16, Fig.1C); then, only CD73-KO mice continued to run until the maximum belt speed of 42 m/min. Thus, the running power was greater in CD73-KO than WT mice (t_{15}=4.2, P<0.05, *d*=0.74, Fig.1D). $\dot{V}O_2$ increased ($F_{7,56}$=67, P<0.05, η^2=0.94, Fig.1E) in line with the intensity of running power, with no difference in submaximal ($F_{7,56}$=0.7, P=0.6, Fig.1E) and maximal $\dot{V}O_2$ (t_{15}=0.25, P=0.8, Fig.1F) between WT and CD73-KO mice.

CD73-KO mice reached a greater critical power (t_{15}=4.3, P<0.05, *d*=1.2, Fig.1D – highlighted in red) and RER (t_{15}=2.4, P<0.05, *d*=0.37, Fig.1G) at the maximum stage of the exercise graded test. Both WT (t_6=6.9, P<0.05, Fig.1G) and CD73-KO mice (t_6=9.5, P<0.05, Fig.1G) did not reach the maximum RER value of 1.0.

DISCUSSION

This study shows that the genetic deletion of CD73 results in an ergogenic profile in mice. Although CD73-KO mice displayed submaximal values of running power and $\dot{V}O_2$ and $\dot{V}O_2$max values similar to their WT littermate, CD73-KO mice reached surprisingly high exercise stages with improved anaerobic power, as demonstrated by the greater critical power (large effect) and maximum RER (small effect).

We propose that the ergogenic effect of CD73 inhibition results from a central effect. This stems from the lack of evident peripheral changes in CD73-KO mice typified by the lack of altered blood pressure [22], cardiac output and ejection fraction [22], $\dot{V}O_2$ on running wheels during the light and dark phase of circadian rhythm [23], oxygen saturation erythrocytic and pH [23], blood glucose and 2,3-biphosphoglycerate levels [23]. This is in accordance with the presently observed lack of differences in the submaximal and maximum $\dot{V}O_2$ in a graded exercise test between CD73-KO mice and their WT littermates and the lack of alteration of spontaneous locomotion either in CD73-KO mice [12,15,24] or upon administration of the CD73 inhibitor α,β-methylene-ADP (AOPCP) [14]. Kulesskay et al. [25] observed a hyperlocomotion in CD73-KO mice but attributed this difference to their isolation in cages with running wheels, contrasting with the collective housing in the present study.

The size effect of the ergogenic effect in CD73-KO mice was large for critical power and small for RER, indicating that the animals ran faster and stronger. Since the absence of submaximal $\dot{V}O_2$ differences excludes metabolic effects, the genetic deletion of CD73 likely delayed limiting fatigue. Thus, our results indicate that CD73-mediated adenosine formation might be the likely source of the extracellular involved in developing exercise-induced fatigue, as well as tiredness and drowsiness [5,6]. Notably, an exercise session transiently increases adenosine, which causes sleep and tiredness [7], whereas caffeine causes arousal [5] and is ergogenic [4]. Moreover, the ergogenicity of CD73-KO mice phenocopies the ergogenic profile of forebrain A_{2A}R-KO mice [8]. Indeed, CD73 and A_{2A}R are functionally coupled in different brain regions [12-15], namely, in basal ganglia [12,14], A_{2A}R antagonism with SCH58261 is ergogenic [8], and the hyperlocomotion stimulated by SCH-58261 is reduced in CD73-KO mice [12].

In conclusion, we now show the critical participation of CD73 in developing fatigue by continuing exercise at maximum intensities. This fully mimics the impact of forebrain A_{2A}R in controlling fatigue [8], in line with the prominent contribution of central effects of adenosine to control fatigue [26-28].

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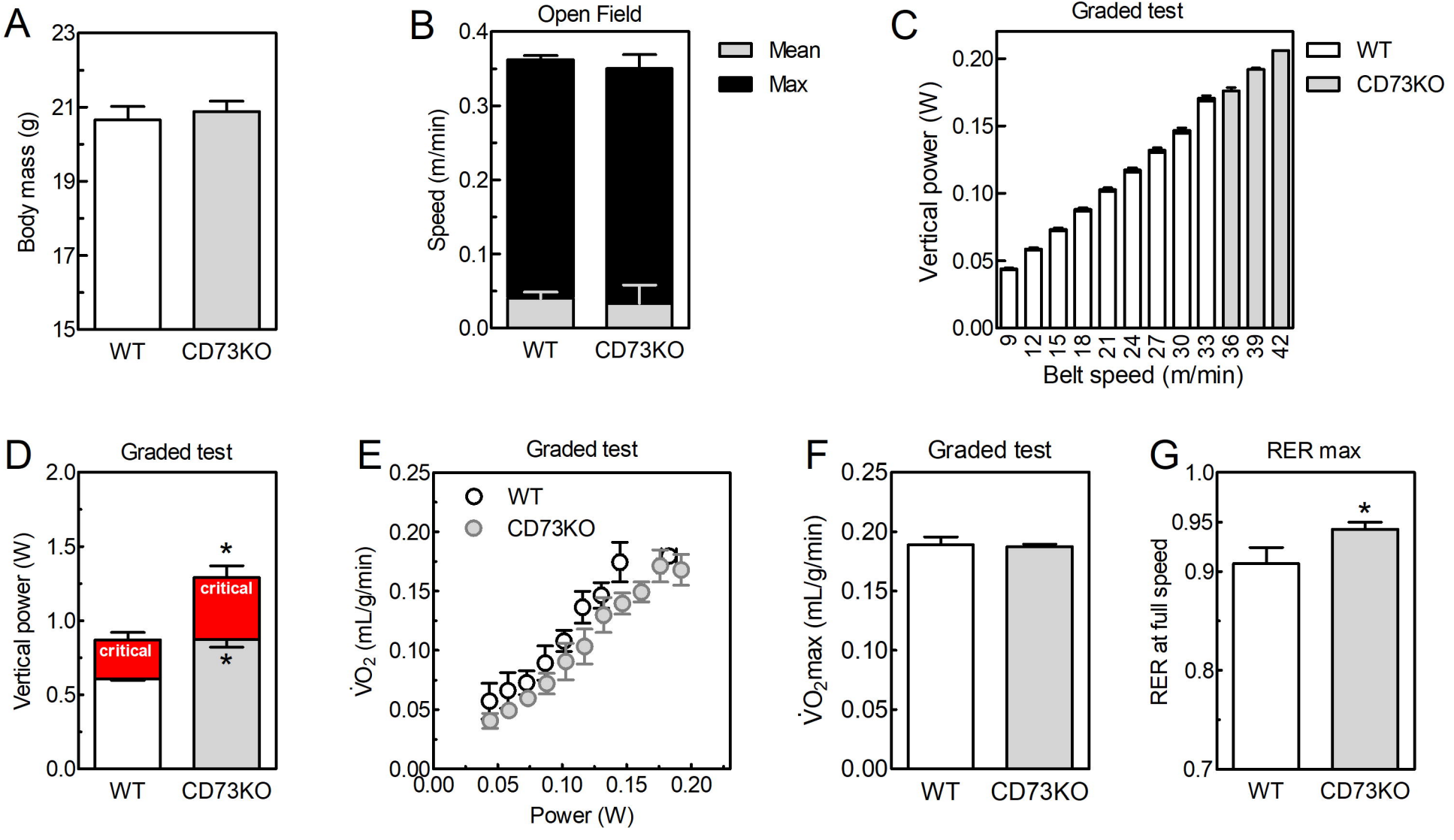
https://doi.org/10.1038/srep26614

Captions

Fig.1 – Body mass (A) and locomotion (B) did not differ between wild-type (WT) and CD73 knockout (KO) mice. CD73-KO mice showed greater running power (C and D) and critical power (indicated in red in D), a greater anaerobic power due to the equal $\dot{V}O_2$max (E and F), and higher carbohydrate consumption indicated by the maximum RER - Respiratory Exchange Ratio (G). Data are described as mean ± SEM. N=7-8 animals/group for three independent experiments. *P<0.05 *vs.* WT (Student's t-test t). $\dot{V}O_2$ – oxygen consumption.


A
Body mass (g)
23
21
19
17
15
WT
CD73KO
B
Open Field
Speed (m/min)
0.4
0.3
0.2
0.1
0.0
Mean
Max
WT
CD73KO
C
Graded test
Vertical power (W)
0.20
0.15
0.10
0.05
0.00
WT
CD73KO
9
12
15
18
21
24
27
30
33
36
39
42
Belt speed (m/min)
D
Graded test
Vertical power (W)
2.0
1.5
1.0
0.5
0.0
critical
critical
*
*
WT
CD73KO
E
Graded test
$\dot{V}O_2$ (mL/g/min)
0.25
0.20
0.15
0.10
0.05
0.00
WT
CD73KO
0.00
0.05
0.10
0.15
0.20
Power (W)
F
Graded test
$\dot{V}O_2$max (mL/g/min)
0.25
0.20
0.15
0.10
0.05
0.00
WT
CD73KO
G
RER max
RER at full speed
1.0
0.95
0.9
0.8
0.7
*
WT
CD73KO