Deletion of CD73 increases exercise power in mice

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ABSTRACT

Ecto-5'-nucleotidase or 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 running power and $\dot{V}O_2$ in the treadmill were similar between CD73-KO and WT mice; $\dot{V}O_2$max also demonstrated equivalent aerobic power, but CD73-KO mice displayed 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.

Keywords

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

INTRODUCTION

Adenosine is an inter-cellular modulator that signals altered cellular activity and metabolic stress within a tissue by activating adenosine receptors [1]. Acute exercise increases adenosine levels in human blood [2,3] and the rat brain [4]. Accordingly, adenosine contributes to exercise-induced vasodilation [5] and also causes drowsiness and tiredness at rest, being a candidate molecule to signal exercise fatigue [6-8]. The non-selective adenosine receptor antagonist, caffeine, is well established to cause an ergogenic effect [8,9], which is mimicked by selective antagonists of adenosine A_{2A} receptors ($A_{2A}R$), abrogated in forebrain $A_{2A}R$ knockout mice [8], and eliminated upon administration of the A_2R agonist NECA [6,10]. These observations strongly imply central $A_{2A}R$ as critical regulators of the impact of adenosine on exercise performance, whereas there is no consistent evidence for the involvement of A_1, A_{2B}, or A_3 receptors in the control of 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 fact, the pharmacological or genetic inhibition of CD73 has effects identical to the inhibition of $A_{2A}R$ in the control of brain functioning [11-13], of the immune system [14] or adaptive vascular control in the periphery [15]. However, the role of CD73 in exercise remains to be defined, which prompted the present study to characterize the impact of deleting CD73 on the exercise performance of mice.

METHODS

Animals

Since we previously characterized the discrete impact of the estrous cycle on exercise performance of mice [16], 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 (WT) littermates, cross-bred as previously described [13,17]. Mice were housed in collective home cages (n=3-5) under a controlled environment (12 h light-dark cycle, lights on at 7 AM, and room temperature of 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 approval by the Ethical Committee of the Center for Neuroscience and Cell Biology (University of Coimbra, ORBEA 138-2016/1507201).

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 dark/light cycle, in a sound-attenuated and temperature (20.3±0.6 °C) and humidity (62.8±0.4%) controlled room, under low-intensity light (≈ 10 lux). The open field apparatus and the treadmill were cleaned with 10% ethanol between individual experiments. 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 [17].

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 [8,16]. The exercise lasted until running exhaustion, defined by the animal's inability to leave the electrical grid for five seconds [8,18].

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 [8]. 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 [8,19]. 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 [20]. The respiratory exchange ratio (RER) is the ratio between the amount of carbon dioxide production ($\dot{V}CO_2$) and the consumed oxygen ($\dot{V}O_2$) [21].

Statistics

Data are presented as mean±SD in graphs built using the GraphPad Prism version 5.00 (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 a Bonferroni *post hoc* test. The differences were considered significant at $P<0.05$. 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 [22]. Cohen's η^2 was used for $\dot{V}O_2$ kinetics, defined as small (0.02), medium (0.13) large (0.26) [22].

RESULTS

The body mass of WT and CD73-KO mice did not differ ($t_{15}=0.5$, Fig.1A), an essential feature since the running power depends on this variable. Locomotion in the open field, either the total distance indicated by the average speed ($t_{15}=0.75$, $P=0.4$, Fig.1B) or the maximum speed ($t_{15}=0.16$, $P=0.87$, Fig.1B), was not different between genotypes.

Running power increased with belt speed acceleration ($F_{7,56}=30k$, $P<0.05$, $\eta^2=0.99$, Fig.1C), with no differences between genotypes up to 33 m/min ($F_{7,56}=1.5$, $P=0.16$, Fig.1C); then, only CD73-KO mice continued to run until a maximum belt speed of 42 m/min. Thus, the running power was larger in CD73-KO than WT mice ($t_{15}=4.2$, $P<0.05$, $d=0.74$, Fig.1D). $\dot{V}O_2$ increased ($F_{7,56}=67$, $P<0.05$, $\eta^2=0.94$, 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}=5.8, P<0.05, *d*=1.2, Fig.1D) 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 high exercise stages with improved anaerobic power as demonstrated by the greater critical power (large effect) and maximum RER (small effect). This anaerobic power is developed during all-out, short-term exercise above $\dot{V}O_2$max that involves anaerobic muscle metabolism as hinted by the higher maximum RER values characteristic of greater anaerobic consumption of glycolysis.

CD73 controls the formation of adenosine in the periphery and the brain, impacting functions such as motor control, inflammatory responses, adaptive blood pressure, or fatigue. Most of these responses are well-established to be modulated by $A_{2A}R$, in line with the predominant effect of $A_{2A}R$ in the ergogenic effect of the non-selective adenosine receptor antagonist, caffeine [8]. $A_{2A}R$ is present in blood vessels and their activation triggers reactive vasodilation [23]. Adenosine derived from muscle contraction is responsible for 20-40% of exercise-induced vasodilation [5]. However, adenosine treatment during exercise does not change myocardial and muscle $\dot{V}O_2$ [24,25] and adenosine receptor blockade with 8-phenyltheophylline does not modify tachycardia, mean aortic pressure, coronary blood flow, and myocardial $\dot{V}O_2$ of running dogs [25]. Overall, this suggests a limited impact of vascular $A_{2A}R$ on exercise performance, although this remains to be directly tested.

We did not directly assess the alteration of the extracellular levels of adenosine in the blood and different tissues of CD73-KO mice, which is a significant limitation of the present study and a challenge for future studies. However, the combination of current and previous observations also prompts the suggestion that the altered levels of adenosine in the blood of CD73-KO mice [26] might not be the prime contributor to the ergogenic profile CD73-KO mice. CD73-KO mice display few peripheral changes, typified by the lack of altered blood pressure [26], cardiac output and ejection fraction [27], $\dot{V}O_2$ on running wheels during the light and dark phase of circadian rhythm [27], oxygen saturation erythrocytic and pH [27], blood glucose and 2,3-biphosphoglycerate levels [27]. 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 WT and CD73-KO mice. The ergogenic effect resulting from knocking out CD73 cannot also be attributed to motor differences in CD73-KO mice since they do not display alterations of spontaneous locomotion [13,17,28]. Kulesskay et al. [29] 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 most parsimonious explanation is the possibility that the ergogenic effect of knocking out CD73 might result from a central effect of dampening exercise-induced fatigue. Exercise transiently increases adenosine that can cause sleep and tiredness [4,10,30], whereas caffeine causes arousal and is ergogenic [6,8]. Moreover, the ergogenicity observed in CD73-KO mice phenocopies the previously observed ergogenic profile of forebrain $A_{2A}R$-KO mice [8]. Indeed, CD73 and $A_{2A}R$ are functionally coupled in different brain regions [11-13,17], namely, in basal ganglia [12,17], and the hyperlocomotion caused by $A_{2A}R$ antagonists is dampened in CD73-KO mice [17].

In conclusion, we now show the critical participation of CD73 in defining the maximum intensity of exercise performance. Although peripheral effects of adenosine cannot be excluded, this effect of knocking out CD73 mimics the impact of knocking out forebrain $A_{2A}R$ in controlling fatigue [8], in line with the prominent contribution of central effects of adenosine to control fatigue [6,7].

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Conflict of Interest

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

Ethical approval :

Animal experiments were approved by the Ethical Committee of the Center for Neuroscience and Cell Biology (University of Coimbra, ORBEA 138-2016/1507201) and followed the European Union guidelines (2010/63).

Informed consent: not applicable

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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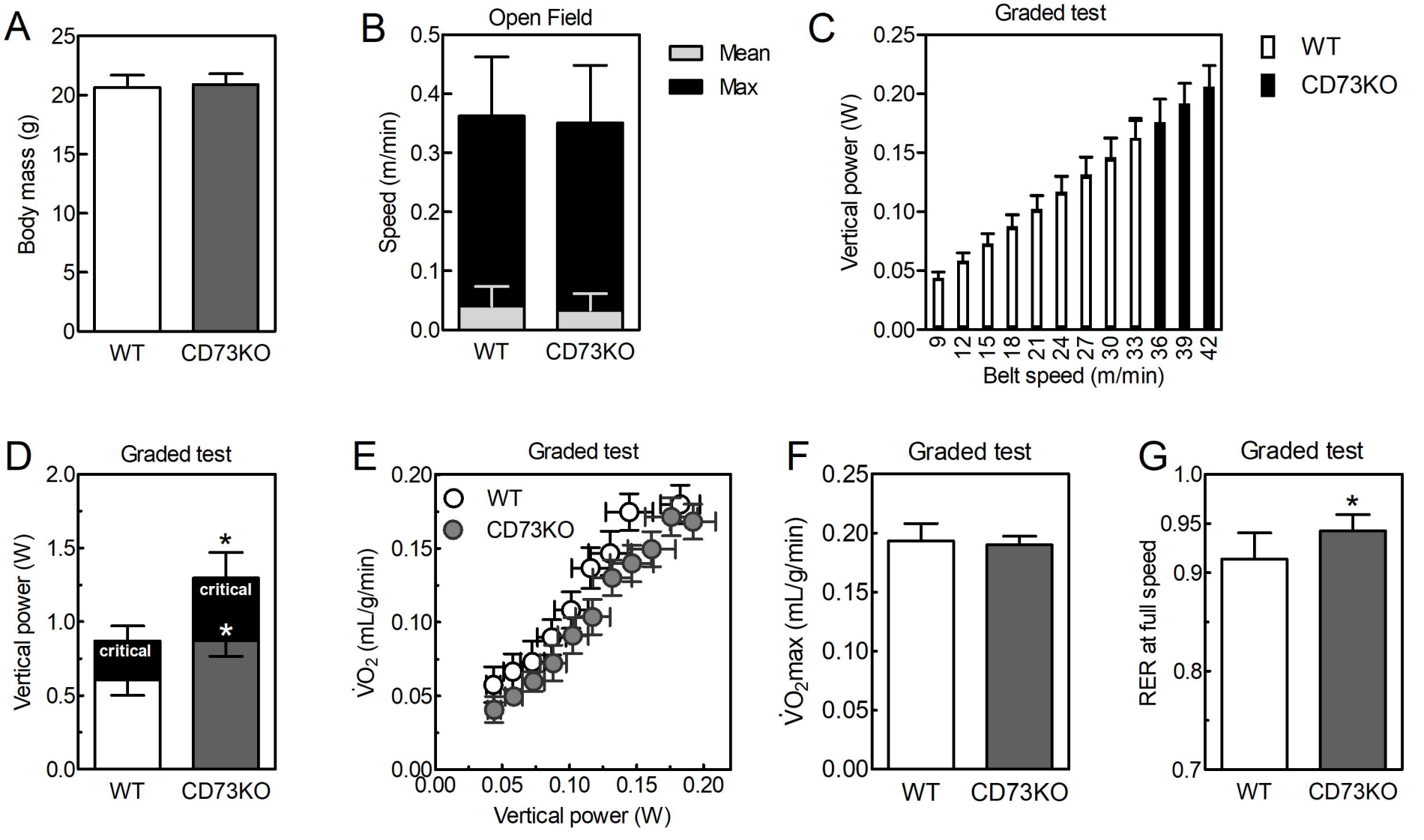
Captions

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 critical power due to the equal $\dot{V}O_2max$ (E and F), and higher carbohydrate consumption indicated by the maximum RER - Respiratory Exchange Ratio (G). Data are described as mean ± SD. 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)
25
20
15
10
5
0
WT
CD73KO
B
Open Field
Speed (m/min)
0.5
0.4
0.3
0.2
0.1
0.0
Mean
Max
WT
CD73KO
C
Graded test
Vertical power (W)
0.25
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
WT
CD73KO
V̇O2 (mL/g/min)
0.20
0.15
0.10
0.05
0.00
0.00
0.05
0.10
0.15
0.20
Vertical power (W)
F
Graded test
V̇O2max (mL/g/min)
0.25
0.20
0.15
0.10
0.05
0.00
WT
CD73KO
G
Graded test
RER at full speed
1.0
0.95
0.9
0.8
0.7
*
WT
CD73KO