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The endocannabinoid system mediates aerobic exercise-induced antinociception in rats

Giovane Galdino [a,*], Thiago R.L. Romero [a], José Felipe P. Silva [b], Daniele C. Aguiar [a], Ana Maria de Paula [c], Jader S. Cruz [d], Cosimo Parrella [e], Fabiana Piscitelli [e], Igor D. Duarte [a], Vincenzo Di Marzo [e], Andrea C. Perez [a]

[a] Department of Pharmacology, Institute of Biological Sciences, Federal University of Minas Gerais, Ave. Antônio Carlos 6627, 31270-100 Belo Horizonte, Brazil
[b] Department of Physiology, Institute of Biological Sciences, Federal University of Minas Gerais, Ave. Antônio Carlos 6627, 31270-100 Belo Horizonte, Brazil
[c] Biophotonics Lab, Department of Physics, Federal University of Minas Gerais, Ave. Antônio Carlos 6627, 31270-901 Belo Horizonte, Brazil
[d] Department of Biochemistry, Institute of Biological Sciences, Federal University of Minas Gerais, Ave. Antônio Carlos 6627, 31270-100 Belo Horizonte, Brazil
[e] Endocannabinoid Research Group, Institute of Biomolecular Chemistry, National Research Council, via Campi Flegrei 34, Compresorio Olivetti, 80078 Pozzuoli, Napoli, Italy

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ABSTRACT

Exercise-induced antinociception is widely described in the literature, but the mechanisms involved in this phenomenon are poorly understood. Systemic (s.c.) and central (i.t., i.c.v.) pretreatment with CB_1 and CB_2 cannabinoid receptor antagonists (AM251 and AM630) blocked the antinociception induced by an aerobic exercise (AE) protocol in both mechanical and thermal nociceptive tests. Western blot analysis revealed an increase and activation of CB_1 receptors in the rat brain, and immunofluorescence analysis demonstrated an increase of activation and expression of CB1 receptors in neurons of the periaqueductal gray matter (PAG) after exercise. Additionally, pretreatment (s.c., i.t. and i.c.v.) with endocannabinoid metabolizing enzyme inhibitors (MAFP and JZL184) and an anandamide reuptake inhibitor (VDM11) prolonged and intensified this antinociceptive effect. These results indicate that exercise could activate the endocannabinoid system, producing antinociception. Supporting this hypothesis, liquid-chromatography/mass-spectrometry measurements demonstrated that plasma levels of endocannabinoids (anandamide and 2-arachidonoylglycerol) and of anandamide-related mediators (palmitoylethanolamide and oleoylethanolamide) were increased after AE. Therefore, these results suggest that the endocannabinoid system mediates aerobic exercise-induced antinociception at peripheral and central levels.

1. Introduction

Several studies have demonstrated the involvement of the endocannabinoid system in pain relief (Pertwee, 2001). This system comprises two G protein-coupled membrane receptors (CB_1 and CB_2) negatively coupled to adenylate cyclase and positively coupled to mitogen-activated protein kinase (Mu et al., 1999; Pertwee, 2001). CB_1 receptors have been found in the central nervous system (CNS), including structures that participate in the descending control of pain, such as the periaqueductal gray matter (PAG), the rostroventromedial medulla (RVM) and the dorsal horn of the spinal cord (Herkenham et al., 1991; Tsou et al., 1998). These receptors are also found in the peripheral nervous system in the dorsal root ganglia (DRG) (Fan et al., 2011). In contrast, CB_2 receptors are mostly restricted to immune cells (Pertwee and Ross, 2002). However, recent studies have described these receptors in the central nervous system (Onaivi et al., 2012). CB_1 receptors, when activated by their endogenous ligands (endocannabinoids), promote hyperpolarization and a reduction in the rate of firing of excitable cells, suppressing neurotransmitter releasing, as well as a reduction in the nociceptive impulse (Di Marzo et al., 2004). The endocannabinoid arachidonoylethanolamide (anandamide, or AEA) is hydrolyzed by the enzyme fatty acid amide hydrolase (FAAH), whereas the other endogenous cannabinoid receptor ligand, 2-arachidonoylglycerol (2-AG), is degraded by the enzyme monoacylglycerol lipase (MGL) (Di Marzo et al., 2004). FAAH also

* Corresponding author. Ave. Jovino Fernandes Sales, 2600, 37130-000 Alfenas, Brazil. Tel.: +55 35 3299 1000.
E-mail address: giovanegsouza@yahoo.com.br (G. Galdino).

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partly regulates the levels of *N*-acylethanolamine, i.e., palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), which have AEA-like molecular structures and metabolic pathways, but do not potently activate cannabinoid receptors (Matias et al., 2007). Both PEA and OEA can, however, interact with several targets, including the peroxisome proliferator-activated receptor-α (PPAR-α) and the transient receptor potential vanilloid channel-1 (TRPV1) (Fu et al., 2003; Piomelli et al., 2005). However, some studies have shown that some effects of PEA are inhibited by cannabinoid receptor antagonists, thus confirming the hypothesis that this compound can also act as an "entourage" compound for endocannabinoids by enhancing their activity at their receptors or by inhibiting their degradation (Calignano et al., 1998; Onaivi et al., 2008; Petrosino et al., 2010; Re et al., 2007).

In addition, the antinociceptive activity of AEA, 2-AG, PEA, and OEA has been confirmed in experiments using central or peripheral administration into mice and rats, in different acute pain models (Costa et al., 2008; Pertwee, 2001; Suardiaz et al., 2007). Finally, FAAH and MGL inhibitors reduce hyperalgesia in mice and rats and increase stress-induced analgesia in rats (Connell et al., 2006; Costa et al., 2008; Guindon et al., 2011; Haller et al., 2006; Naidu et al., 2009). These effects are reversed by cannabinoid receptor antagonists.

The endocannabinoid system is activated by exercise. Sparling et al. (2003) found an increase in anandamide plasma levels in humans after running and cycling. Voluntary exercise produced increased endocannabinoid signaling within the hippocampus of rats and cannabinoid receptor sensitization in the striatum of mice (De Chiara et al., 2010; Hill et al., 2010). More recently, it was also shown that intense exercise increases circulating endocannabinoid levels in humans (Feuerecker et al., 2011; Heyman et al., 2011).

Exercise-induced analgesia has been reported in the literature since the early 1980s. Aerobic exercises are more frequently studied in rats and mice subjected to treadmills or swimming and in humans subjected to running and cycling (Koltyn, 2002). However, after 30 years of research, few mechanisms have been shown to underlie this effect, including, for example, those involving endogenous opioid, serotonin and nitric oxide/cGMP/K^+_{ATP} pathways (Galdino et al., 2010; Koltyn, 2000). Thus, to elucidate the mechanisms involved in the exercise-induced antinociception, we investigated the possible role of the endocannabinoid system in this phenomenon.

2. Material and methods

2.1. Animals

The study was conducted in concordance with the International Association for the Study of Pain guidelines on the use of laboratory animals (Zimmermann, 1983), and all the experiments were approved by the Ethics Committee for Animal Experimentation of the Federal University of Minas Gerais (UFMG). The experiments were performed using male Wistar rats that weighed 180–200 g obtained from UFMG Brazil. The animals were divided in groups and housed in individual cages that were kept at a constant temperature 23 °C with a 12 h light–dark cycle, and they had free access to food and water. Animals were kept under these conditions until they were taken to the testing room, at least 1 h before the experiments.

2.2. Drugs

The following drugs were used in this study: *N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251), a CB_1 cannabinoid receptor antagonist; [6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone (AM630), a CB_2 cannabinoid receptor antagonist; (5*Z*,8*Z*,11*Z*,14*Z*)-5,8,11,14-eicosatetraenyl-methyl-ester phosphonofluoridic acid (MAFP), an irreversible FAAH inhibitor; 4-[Bis(1,3-benzodioxol-5-yl)hydroxymethyl]-1-piperidinecarboxylic acid 4-nitrophenyl ester (JZL184), a potent and selective MGL inhibitor; (5*Z*,8*Z*,11*Z*,14*Z*) *N* (4Hydroxy-2methylphenyl) 5,8,11,14 eicosatetraenamide (VDM11), a selective inhibitor of the anandamide membrane transporter, all purchased from Tocris Bioscience (Ellisville, MO). AM251, AM630 and JZL184 were dissolved in physiological saline and dimethyl sulphoxide (20%, Sigma, MO, USA) vehicle. MAFP was dissolved in physiological saline and ethanol (2.0%, Merck, NJ, USA) vehicle, and VDM11 was dissolved in Tocrisolve (Tocris, MN, USA) vehicle. All the drugs were administered in a volume of 1 ml/kg (s.c.), 10 μl (i.t.) and 5 μl (i.c.v.).

2.3. Injections

2.3.1. Subcutaneous injection

The subcutaneous injections were into the dorsal nuchal area of rats in a volume of 1 ml/kg, 10 min before exercise in the exercised groups.

2.3.2. Intrathecal injection

The intrathecal injections (i.t.) were performed in a volume of 10 μl in the subarachnoid space between L5 and L6 using a 30 G × 1/2-inch needle and a 50 μl Hamilton syringe as described by Mestre et al. (1994). Intrathecal injection is stressful for rats and, according to IASP recommendation, requires anesthesia. Thus, before injection, rats were slightly anesthetized with volatile isoflurane (3.5%) and recovered 5 min after the removal from the anesthesia chamber. Correct i.t. positioning of the needle tip was confirmed by a characteristic tail-flick response in the animal. Lidocaine 4% (10 μl), was administered to a group of test animals, using temporary paralysis of the hind limbs as an end point to confirm the effectiveness of the injection technique. Intrathecal injections were administered immediately before to exercise.

2.3.3. Intracerebroventricular injection

Initially before intracerebroventricular injections, each rat was anaesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) injected i.p., and then placed in a stereotaxic apparatus (Stoelting, IL, USA). The scalp was incised, and the skull was leveled off around the bregma. A 22 gauge, 12 mm stainless-steel guide cannula was inserted in the right lateral ventricle of the brain. The type of the cannula aimed at the following coordinates: 1.5 mm posterior to the bregma, 2.5 mm lateral to the midline, and 3.3 mm below the top of the skull (Paxinos and Watson, 1986). The cannula was then fixed to the skull using three screw and dental acrylic. 12.5 mm stylet was inserted in the cannula to keep in patent prior to injection. Animals were allowed 5-day recovery period before experiments were initiated. For i.c.v. drugs injections, a gauge, 12.5 mm injection needle was attached to a 30 cm polyethylene tube fitted to a 10 μl Hamilton syringe. Then, the rats were restrained by hand, the stylet was withdrawn, and the injection needle inserted into the guide cannula. The volume of the solutions to be injected into lateral ventricle was 5 μl, and the injection was made over a period of 120 s. Intracerebroventricular injections were administered immediately before the exercise.

2.4. Exercise

Acute aerobic exercise (AE) was performed using a rodent treadmill. Animals ran with a progressive speed at 20 m/min and 0% inclination, an average time of 49.06 (±3) min, until fatigue (Ji et al., 1992). Fatigue was defined as the point at which the animals were unable to keep pace with treadmill (Soares et al., 2003). The back of the treadmill has an electrical stimulator (3 v) in order to encourage the animals to run. To familiarize the rats to exercise, reducing the effects of stress, they ran daily on the treadmill at 5 m/min for 5 min/day during 3 consecutive days prior to the experiments.

The groups were as follows ($n = 6$ per group): Control (Co): animals that did not perform exercise and received saline; Acute aerobic exercise (AE): rats that ran and received saline; AE + AM251: animals pretreated with CB_1 cannabinoid receptor antagonist and exercised; AE + AM630: animals pretreated with CB_2 cannabinoid receptor antagonist and exercised; AE + MAFP: animals pretreated with irreversible FAAH inhibitor and exercised; AE + JZL184: animals pretreated with MGL inhibitor and exercised; AE + VDM11: animals pretreated with inhibitor of the anandamide membrane transporter and exercised. Furthermore, were used different groups composed of animals that received the drugs previously described via s.c., i.t. and i.c.v. administration. A group received the same numbers of electrical stimulus of AE group, and there was no change on the nociceptive threshold.

2.5. Assessment of mechanical nociceptive threshold

Mechanical nociceptive threshold was assessed by measuring the response to a paw pressure test described by Randall and Selitto (1957). In a quiet room, rats were placed in acrylic cages (12 × 20 × 17 cm) that had wire grid floors 1 h before the testing began. An analgesimeter (Ugo Basile, Comerio, Italy) with a cone-shaped paw-presser that had a rounded tip (9 mm base diameter) was used to apply a linearly increasing force to the hind paw. The pressure intensity in grams (g) that caused an escape reaction was defined as the nociceptive threshold. A maximum intensity of 300 g was used to reduce the possibility of damage to the paws. The nociceptive threshold was measured in the right paw and determined as the average of three consecutive trials.

2.6. Assessment of thermal nociceptive threshold

Thermal nociceptive threshold was assessed by measuring the latency to tail withdrawal from a noxious heat source, as described by D'amour and Smith (1941). Rats were placed in acrylic cages (12 × 20 × 17 cm) that had wire grid floors in a