Opioid receptors are not involved in the increase of the nociceptive threshold induced by aerobic exercise

Giovane Galdino, *PhD,* ***Thiago Romero,*** *PhD,* ***Ian Andrade,*** *MD,* ***Igor Duarte,*** *PhD,* ***Andrea Perez,*** *PhD.*

ABSTRACT

الأهداف: تشخيص مشاركة المستقبلات الأفيونيه في مضادات الألم الناتجة عن طريق تطبيق تمارين مختلفة على الفئران.

الطريقة: أجريت دراسة تجريبية في الجامعة الفيدرالية في ميناس، مقاطعة بيلو هوريزونتي، البرازيل خلال الفترة من نوفمبر 2011م حتى مايو 2012م اشتملت على 60 فأر أنثى من نوع ويستر قسمت إلى 10 مجموعات كل مجموعة تحتوي على 6 فئران. خضعت إلى بروتوكول تمارين أيروبيك مختلفة منها حاد، قلبي، ضغط وتدريبات. تم قياس بداية الألم عن طريق انسحاب المخالب وقد أعطيت المواد الافيونية المضادة للنالوكسون تحت الجلد قبل ممارسة التمارين لفحص مشاركة النظام الأفيوني الداخلي.

النتائج: جميع التمارين زادت من عتبة الألم لمدة 15 دقيقية. كما أنه لم يغير النالكسون قبل المعالجة مضادات الألم التي سببتها التمارين. كما أن استخدام المضادات الأفيونية قبل العلاج لم يؤثر على مضادات الألم الناتجة من تمارين الايروبيك.

خاتمة: لم يشارك النظام الأفيوني الداخلي في تأثير مضادات الألم التي سببتها تمارين الايروبيك.

Objective: To investigate the involvement of opioid receptors in antinociception induced by different aerobic exercise protocols in rats.

Methods: This experimental study, conducted in the Federal University of Minas Gerais, Belo Horizonte, Brazil from November 2011 to May 2012, included 60 female Wistar rats, divided into 10 groups of 6 animals per group. The rats were subjected to different aerobic exercise protocols: acute, cardiac stress, eccentric, and training. The nociceptive threshold was measured by the paw-withdrawal test. To investigate the involvement of the endogenous opioids system, the non-selective opioid receptor antagonist naloxone (5 mg/kg) was administered subcutaneously before the beginning of the exercise.

Results: All exercise protocols increased the nociceptive threshold for 15 minutes. The naloxone pre-treatment did not alter the antinociception induced by aerobic exercise protocols.

Conclusion: The endogenous opioids system did not participate in the antinociceptive effect produced by the aerobic exercise protocols.

Neurosciences 2014; Vol. 19 (1): 33-37

From the Department of Pharmacology, Federal University of Minas Gerais, Belo Horizonte, Brazil.

Received 3rd July 2013. Accepted 10th October 2013.

Address correspondence and reprint request to: Dr. Giovane Galdino, Department of Pharmacology, Federal University of Minas Gerais, Avenida Presidente Antônio Carlos, 6627 Pampulha, Belo Horizonte, MG, 31270-901, Brazil. Tel/Fax. +55 (31) 34092721. E-mail: giovanegsouza@yahoo.com.br

Physical exercise has been demonstrated as an important non-pharmacological therapy for patients with chronic and acute pain. Exercise is effective treatment for different pain disorders, such as chronic low back pain, osteoarthritis, and fibromyalgia.[1] Several registers concerning nociceptive threshold elevation following physical activity have been demonstrated in recent decades, and the main substance responsible for this effect has been the endogenous opioids.[2] These peptides produce a variety of physiological and pharmacological effects, such as antinociception, after binding to opioids receptors.[3] Opioid receptors are distributed widely throughout the central and peripheral nervous systems. Three opioid receptor subtypes (mu,

Disclosure. This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

delta, and kappa) have been cloned and each shows structural features characteristic of G-protein coupled receptors.[3] Additionally, previous studies have shown a role for endogenous opioids in controlling pain in that stimulation of specific brain sites produces a decrease in pain perception, an effect that is blocked by naloxone (an opioid receptor antagonist).[3]

The relation between exercise-induced analgesia and the endogenous opioids was found in different models of aerobic exercise, such as the bicycle ergometer and treadmill in humans, and swimming in both humans and rodents.[2] Recently, our group demonstrated that naloxone blocked the antinociceptive effect induced by resistance exercise in rats, suggesting the involvement of the endogenous opioids in this effect.[4] However, there is a lack of research comparing, in parallel, different aerobic exercise protocols with the opioid system. Therefore, the present study investigated the involvement of opioid receptors in exercise-induced antinociception by different aerobic exercise protocols.

Methods. This is experimental study was conducted in the Federal University of Minas Gerais, Belo Horizonte, Brazil from November 2011 to May 2012. The study was conducted in concordance with the International Association for the Study of Pain guidelines on use of laboratory animals, and the Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais approved all the experiments and protocols.

Animals. All experiments were performed on 60, 230-250 g female Wistar rats (from CEBIO-UFMG, MG, Brazil). The rats were housed in a temperature-controlled room (23±1°C) on an automatic 12h light/dark cycle (06:00-18:00 h). All tests were conducted during the light phase (08:00-16:00 h). Food and water were freely available until the onset of the experiments.

Drugs. Naloxone (Nx) (Sigma, MO, USA; 5 mg/kg) was dissolved in saline. Rats in the control group were injected with saline. Naloxone and saline were injected subcutaneously (1 ml/kg) into the dorsal nuchal area, 10 minutes before the onset of exercise. The control groups received the same volume of physiological saline solution in the same area as the experimental groups.

Exercise. The exercise was performed in a rodent treadmill following the exercise protocols previously described.[5] The animals were randomly divided into the following groups: control (Co, N = 6): rats that did not run; acute exercise (Ac, N = 6): rats that ran until fatigue at 20 m/min and 0% grade; cardiac stress (Cs, N = 6): animals that ran to exercise protocol that started at 10 m/min, 0% grade followed by gradual increase of treadmill speed and of the grades every 4 minutes up to 30 m/min, 15% grade; eccentric exercise (Ec, N = 6): rats that ran an intermittent downhill protocol (-16° incline) at 16 m/min for a total of 90 minutes, 5 bouts separated by 2 minutes rest (total 18 bouts); training protocol (Tr, N = 6): rats that received saline and were habituated to treadmill running over a 4 week period during which the intensity of the exercise was gradually increased to 27 m/min, 0% grade for 45 minutes, 3 days per week for another 8 weeks. To evaluate the involvement of opioids receptors, the Nx was administered 10 minutes before the onset of each exercise protocol: (Ec+Nx, N = 6; Ac+Nx, N = 6; Cs+Nx, N = 6; Tr+Nx, N = 6). One group non-exercised was also pre-treated with naloxone (Co + Nx, N = 6).

Nociceptive test. Mechanical nociceptive threshold was assessed by measuring the response to a paw pressure test.[6] In a quiet room, rats were placed in acrylic cages (12 x 20 x 17 cm) that had wire grid floors one hour 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 3 consecutive trials. In the trained group, the nociceptive threshold was also measured every 15 days during the 12-week training period.

Statistical analysis. Data were expressed as mean ± standard error of the mean. All measurements were analyzed with 2-way ANOVA and post-Hoc Bonferroni multiple comparisons test by using GraphPad Prism 5 (GraphPad Prism®, La Jolla, CA, USA). For all data sets, $p<0.05$ was accepted to be statistically significant.

Results. All exercise protocols (Ec, Ac, Cs, Tr) significantly increased ($p<0.001$) the nociceptive threshold after the first minute at the end of exercise when compared with control group (Co, non-exercised animals) (**Figure 1**). This effect lasted for 15 minutes ($p<0.01$, Tr x Co; $p<0.01$ Cs x Co; $p<0.01$, Ac x Co; $p<0.05$, Ec x Co), returning to baseline levels after 30 minutes. To investigate the involvement of opioid receptors in the antinociception induced by exercise protocols, we used the opioid receptor antagonist naloxone. The pre-treatment with naloxone (5 mg/kg, subcutaneously did not alter ($p>0.05$) the antinociceptive effect produced by aerobic exercise protocols (**Figure 2**).

The rats ran approximately 39.25 (±4.3) minutes in the Ac protocol, 15.75 (±3.2) minutes in the Cs protocol, 90 minutes in the Ec protocol, and 45 minutes in the Tr protocol. Naloxone was tested alone and did not alter the nociceptive threshold of exercised and non-exercise rats, or change the performance of the rats on the treadmill.

Discussion. The results of the present study demonstrated that the different exercise protocols increased the nociceptive threshold in the paw-withdrawal test. Although each protocol evaluated produced antinociception through different mechanisms, the duration and intensity of the effect were similar in all.

The eccentric exercise protocol (Ec) is similar to downhill running, where the contracting quadriceps muscle controls the rate of knee flexion against the force of gravity, and in this process the muscle undergoes an eccentric contraction with each step.[7] Eccentric contractions involve forced lengthening of active muscle, causing release of muscle enzymes to plasma, ultrastructural muscle damage, and performance deterioration.[8] In addition, muscle injury produced by eccentric exercise also results in an increased muscle concentration of bradykinin, serotonin, potassium, and histamine that might activate or sensitize nociceptive afferent fibers.[8] Thus, the Ec protocol might cause an increase in peripheral sensory nerve terminal activation and axonal transport that activates the inhibitory pain descendent control, thereby producing antinociception. Even in Ec protocols producing an increase in nociceptive threshold and a possible tissue injury, naloxone did not reverse the antinociceptive effect, suggesting that opioid receptors are not involved. Although, other authors reported an increase in opioid receptors in the inflamed tissue, such as the muscle and skin of rats.[9] However, other receptors described as being involved in antinociception, such as cannabinoid receptors, have also been found in inflamed muscle and can participate in this effect.[10]

In the present study, we showed that exercise until fatigue (Ac) also produced antinociception. This effect may be found in athletes after prolonged exercise, such as a marathon running, cycling, and swimming. An early study described a case of a woman, who continued to run with one complete fracture of the tibia, without experiencing pain.[2] Another study also found similar results after acute running or cycling until fatigue, in which the participants were submitted to mechanical or electrical nociceptive stimulus applied to the finger or dental pulp.[2] Unlike our results, most of these studies found that naloxone reversed analgesia after acute aerobic exercise. Furthermore, other substances

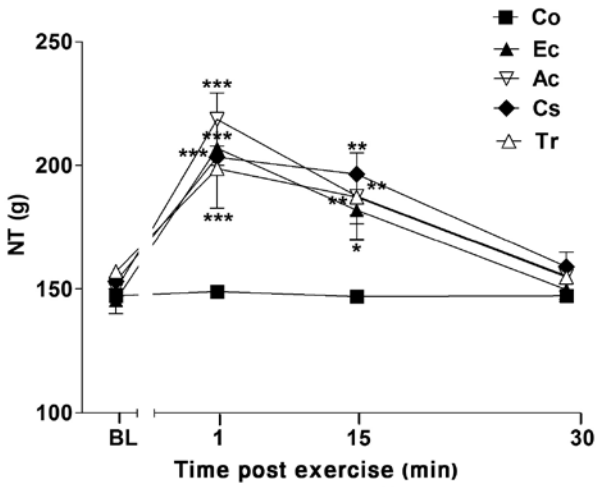

Figure 1 - Effect of different aerobic exercise protocols in the nociceptive threshold (NT) of the following groups of rats (N = 6 per group): eccentric exercise (Ec), acute exercise (Ac), cardiac stress protocol (Cs), and training protocol (Tr). Each point represents nociceptive threshold mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001, represents the significance level compared to the control group (Co), by 2-way ANOVA variance followed by Bonferroni test. BL - baseline latency

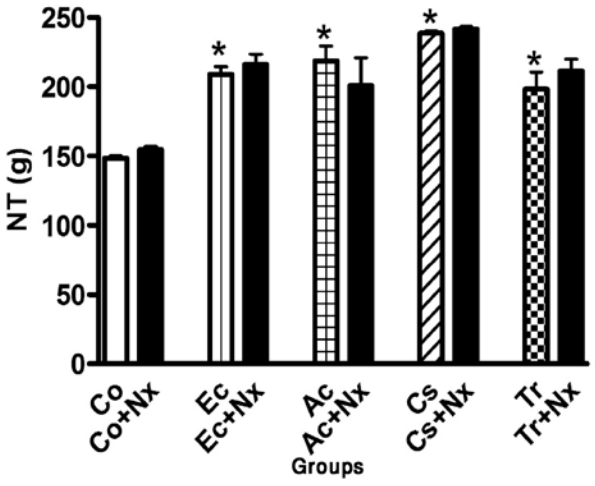

Figure 2 - Effect of naloxone (Nx) in the antinociception produced by different aerobic exercise protocols. Naloxone (5 mg/kg) subcutaneously administered did not alter the antinociceptive effect produced immediately after each aerobic exercise protocol (eccentric [Ec], acute [Ac], cardiac stress [Cs], training [Tr]) and the nociceptive threshold of the control group (Co) in the paw-withdrawal test. Each bar represents mean ± S.E.M. *p<0.05 represents increase of nociceptive threshold (NT) produced by different exercise protocols compared to the Co, by 2-way ANOVA variance followed by Bonferroni test (N = 6).

may be involved in the antinociceptive effect produced by the Ac protocol, such as the nitric oxide/cGMP/KATP pathway. The involvement of this pathway was demonstrated in a recent study conducted by our group.[11]

In our experiments, the Cs protocol also produced antinociception. Similar to running up hill, the Cs protocol challenges the muscles, heart, and lungs, burns more calories and provides additional toning, and is clinically similar to a stress test used in humans, which can evaluate cardiac problems, such as coronary artery disease. Thus, the Cs protocol may induce a greater overload on the heart with an acute consequent increase of blood pressure.[12] Corroborating this, many studies have demonstrated that arterial hypertension can reduce pain sensibility, and some suggested mechanisms underlying this effect have included the increased activation of baroreceptors, and consequently a coordinated response between the descendent control of pain and the cardiovascular regulatory center.[13] Although studies have shown the involvement of endogenous opioids in the hypoalgesia induced by hypertension and exercise,[13] in our study the participation of this system was not observed. However, other mechanisms have also been described as involved in this effect (hypertension induced analgesia), such as the renin-angiotensin system, which may be involved in the antinociception induced by the Cs protocol.[14]

Similar to results obtained in the acute exercise protocols (Ec, Ac, and Cs), the training protocol (Tr) also increased the nociceptive threshold. Previous studies have demonstrated that regular athletes presented with similar thresholds to non-athletes after exercise.[15] These authors suggest that during competition there is a greater incidence of injuries, mainly in the muscle and joints, that may activate the descendent pain modulatory pathway as a protective mechanism for the athletes and to prevent the exercise from stopping. The analgesia induced by exercise during training may be due to a form of short-term adaptation to pain, that can also occur as a result of systematic exposure to periods of intense, but limited pain. The characteristics of this adaptation indicate a possible role of the hypothalamic-pituitary adrenocortical axis.[2] Additionally, a study demonstrated that both naloxone pre-treatment and bilateral adrenalectomy attenuated the antinociception produced by swimming in mice, suggesting the participation of the adrenal glands in this effect.[16] However, the naloxone pre-injection did not alter the antinociception induced by the Tr protocol. This result is consistent with other studies that used different doses of naloxone (1, 5, 10, 20 mg/kg), in both rodents and in trained humans, and also did not find reversion of the antinociceptive effect.[2] Although, our group demonstrated that the antinociception induced by acute resistance exercise in rats was reversed by naloxone.[4] However, the resistance exercise used in our previous study had a lower duration and intensity, which can activate endogenous opioid that has not been seen in the Tr protocol. In addition, previous work suggests that the differences in exercise protocols can explain opposite results.[16]

One factor that could interfere with the effect of naloxone would be the dose used. The dose of naloxone used in the present study was the same as that which reversed the antinociception induced by morphine and *Danae racemosa* in different nociception models in rats.[17] These results support the hypothesis that other endogenous systems may be involved in exercise-induced analgesia. In addition, studies demonstrated that some endogenous substances, such as serotonin, N-Methyl-D-aspartic acid (NMDA), adrenergic system, the nitric oxide/cGMP/KATP pathway and the endocannabinoid system also are involved with antinociception induced by aerobic exercise.[2,6,11,18]

The majority of studies that evaluated the involvement of opioids in exercise-induced analgesia were performed in males. Furthermore, this analgesic effect may be different between genders. One study[19] demonstrated that males display greater analgesic effects after morphine systemic administration than in female humans. This difference may be due to greater immunoreactive density of the endogenous opioid peptides and their proteolytic enzymes, and greater opioid receptor sensitivity in males.[19] Another factor may be the influence of gonadal steroid hormones. For example, gonadectomy in adult female rats has been shown to increase opioid antinociception.[19] Furthermore, a study found that females reported a decrease in pain after isometric contractions, when compared with males.[20] Thus, exercise-induced antinociception found in females and not in males may be due to activation of other analgesic pathways.

Based on the evidence provided by the present study, we can conclude that all exercise protocols were effective in producing antinociception, and this effect occurs without the participation of opioid receptors. Future studies are needed to investigate other mechanisms involved.

References

1. Nijs J, Kosek E, Van Oosterwijck J, Meeus M. Dysfunctional endogenous analgesia during exercise in patients with chronic pain: to exercise or not to exercise? ***Pain Physician*** 2012; 15 (3 Suppl): ES205-ES213.

2. Koltyn KF. Analgesia following exercise: a review. ***Sports Med*** 2000; 29: 85-98.
3. Millan MJ. Descending control of pain. ***Prog Neurobiol*** 2002; 66: 355-474. Review.
4. Galdino GS, Duarte ID, Perez AC. Participation of endogenous opioids in the antinociception induced by resistance exercise in rats. ***Braz J Med Biol Res*** 2010; 43: 906-909.
5. Perez AC, Franca V, Daldegan VM Jr, Duarte ID. Effect of Solanum lypocarpum St. Hill on various haematological parameters in diabetic rats. ***J Ethnopharmacol*** 2006; 106: 442-444.
6. de Souza GG, Duarte ID, de Castro Perez A. Differential involvement of central and peripheral α2 adrenoreceptors in the antinociception induced by aerobic and resistance exercise. ***Anesth Analg*** 2013; 116: 703-711.
7. Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. ***J Physiol*** 2001; 537: 333-345.
8. Mense M. The Pathogenesis of Muscle Pain. ***Curr Pain Headache Rep*** 2003; 7: 419-425.
9. Zollner C, Shaqura MA, Bopaiah CP, Mousa S, Stein C, Schafer M. Painful inflammation-induced increase in mu-opioid receptor binding and G-protein coupling in primary afferent neurons. ***Mol Pharmacol*** 2003; 64: 202-210.
10. Guindon J, Hohmann AG. The endocannabinoid system and pain. ***CNS Neurol Disord Drug Targets*** 2009; 8: 403-421.
11. Galdino GS, Cortes SF, Duarte ID, Perez AC. Involvement of the nitric oxide/(C)GMP/K(ATP) pathway in antinociception induced by exercise in rats. ***Life Sci*** 2010; 86: 505-509.
12. Pescatello LS. Exercise and hypertension: recent advances in exercise prescription. ***Curr Hypertens Rep*** 2005; 7: 281-286.
13. Guasti L, Zanotta D, Mainardi LT, Petrozzino MR, Grimoldi P, Garganico D, Diolisi A, et al. Hypertension related hypoalgesia, autonomic function and spontaneous baroreflex sensitivity. ***Auton Neurosci*** 2002; 99: 127-133.
14. Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, et al. Blockade of angiotensin system increases adiponectin concentrations in patients with essential hypertension. ***Hypertension*** 2003; 42: 76-81.
15. Sternberg WF, Bokat C, Kass L, Alboyadjian A, Gracely RH. Sex-dependent components of the analgesia produced by athletic competition. ***J Pain*** 2001; 2: 65-74.
16. Mazzardo-Martins L, Martins DF, Marcon R, Dos Santos UD, Speckhann B, Gadotti VM, et al. High-intensity extended swimming exercise reduces pain-related behavior in mice: involvement of endogenous opioids and the serotonergic system. ***J Pain*** 2010; 11: 1384-1393.
17. Maleki-Dizaji N, Fathiazad F, Garjani A. Antinociceptive properties of extracts and two flavonoids isolated from leaves of Danae recemosa. ***Arch Pharm Res*** 2007; 30: 1536-1542.
18. Galdino G, Romero TR, Silva JP, Aguiar DC, de Paula AM, Cruz JS, Parrella C, Piscitelli F, Duarte ID, Di Marzo V, Perez AC. The endocannabinoid system mediates aerobic exercise-induced antinociception in rats. ***Neuropharmacol*** 2014; 77: 313-324.
19. Niesters M, Dahan A, Kest B, Zacny J, Stijnen T, Aarts L, et al. Do sex differences exist in opioid analgesia? A systematic review and meta-analysis of human experimental and clinical studies. ***Pain*** 2010; 151: 61-68.
20. Koltyn KF, Trine MR, Stegner AJ, Tobar DA. Effect of isometric exercise on pain perception and blood pressure in men and women. ***Med Sci Sports Exerc*** 2001; 33: 282-290.