UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

CAROLINA ORTIGOSA CUNHA

**The effect of specific and non-specific opioids' receptors
antagonists on Exercise-induced Hypoalgesia (EIH)**

**Efeito de antagonistas de receptores opióides específico e não-
específicos na Hipoalgesia-induzida por Exercício (HIE)**

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Helen Keller

ABSTRACT

The effect of specific and non-specific opioids' receptors antagonists on Exercise-induced Hypoalgesia

The opioid system is involved in a well-documented biological phenomenon named Exercise-induced Hypoalgesia (EIH) but the exact mechanism(s) are not clear. Studies demonstrate that exercise effects could be reversed by naloxone but there are no studies that evaluate the role of specific opioid receptors in the EIH levels. The present study aimed to evaluate the role of a non-specific (naltrexone) and specific opioids' receptors (mu, kappa and delta) antagonists on the EIH levels induced by aerobic exercise in the Rota-Rod in a hundred and eighty (n=180) healthy male adult Sprague-Dawley rats. Rats were divided into five groups (naltrexone, vehicle, mu, kappa, delta) and each group was subdivided into high ($67\% \leq \text{EIH} \leq 100\%$), medium ($34\% \leq \text{EIH} \leq 66\%$) or low ($\text{EIH} \leq 33\%$) EIH profile level. After 3 days of habituation, EIH baseline measurements (percentage of response to 30 mechanical stimuli with 60g Von Frey monofilaments) at 1, 5, 10 and 20 minutes following exercise were assessed. In the day after the baseline measurements, thirty rats from each group (10 high, 10 medium and 10 low EIH) rats were injected with naltrexone hydrochloride, CTAP (mu receptor antagonist), GNTI (kappa receptor antagonist), Naltrindole hydrochloride (delta receptor antagonist), or vehicle (distilled water solution). Then, after 10 minutes (for naltrexone and vehicle), 20 minutes (for mu), 15 minutes (for kappa) and 10 minutes (for delta), EIH measurements were obtained again, just once. Data were analyzed with repeated-measurements ANOVA, followed by post-hoc Fisher test. Alpha was set at 0.05. Injection with the vehicle (distilled water) did not reduce the EIH ($p=0.904$), differently, naltrexone hydrochloride ($p=0.000$), kappa ($p=0.002$) and delta ($p=0.000$) antagonist's drugs significantly reduced the EIH 1 minute following exercise when compared to baseline. It was concluded that rats with high EIH profile had a significant EIH reduction after injection of a non-specific and some specific opioid receptors antagonist (delta and kappa). The EIH effect, however, was just partially reduced, suggesting that others mechanism are involved in EIH phenomenon. The findings reveal that exercise induces hypoalgesia in healthy rats. More studies are needed to evaluate the phenomenon and the mechanisms of EIH in humans with painful disorders such as chronic musculoskeletal pain and orofacial pain.

KEYWORDS: Exercise. Opioid receptors. Pain Measurement.

RESUMO

Efeito dos antagonistas específicos e não-específicos dos receptores opióides na Hipotalgesia-induzida por exercício

O sistema opióide está envolvido em um fenômeno biológico bem documentado na literatura chamado Hipotalgesia-induzida por Exercício (HIE), mas o exato mecanismo ainda não é claro na literatura. Estudos demonstram que os efeitos do exercício podem ser revertidos por substâncias antagonistas não-específicas dos receptores opióides, mas não existem estudos que avaliem o papel específico dessas substâncias na HIE. O presente estudo objetivou avaliar o papel de antagonistas não-específico e específicos de receptores opióides nos níveis de HIE com exercício aeróbico em cento e oitenta (180) ratos (Sprague-Dawley) machos adultos e saudáveis. Os ratos foram divididos em cinco grupos (naltrexona, veículo, mu, kappa, delta) e cada grupo, subdividido em alto ($67\% \leq \text{HIE} \leq 100\%$), médio ($34\% \leq \text{HIE} \leq 66\%$) ou baixo ($\text{HIE} \leq 33\%$) nível de HIE. Após 3 dias de habituação, medidas de HIE (porcentagem de resposta a 30 estímulos mecânicos realizados com o monofilamento de von Frey de 60g) em 1, 5, 10 e 20 minutos após o exercício foram realizadas. No dia seguinte, trinta ratos de cada grupo (10 de cada perfil de HIE) foram injetados com naltrexona, CTAP (antagonista do receptor mu), GNTI (antagonista do receptor kappa), cloridrato de naltrindole (antagonista do receptor delta), ou veículo (solução de água destilada) e, em seguida, depois de 10 minutos (para a naltrexona e veículo), 20 minutos (para mu), 15 minutos (para kappa) e 10 minutos (para delta), as medições de HIE foram novamente realizadas (apenas uma vez). Dados foram analisados com ANOVA seguido pelo teste post-hoc Fisher. Valor geral de alfa foi de 0,05. Injeção com o veículo (água destilada) não reduziu a HIE ($p = 0,904$). Diferentemente, naltrexona ($p=0,000$), kappa ($p=0,002$) e delta ($p=0,000$) antagonistas reduziram significativamente a HIE no primeiro minuto seguinte ao exercício quando comparados com o valor basal. Concluiu-se que os ratos com alto nível de HIE tiveram uma redução significativa do HIE após a injeção de naltrexona e antagonistas dos receptores delta e kappa, mas o efeito HIE foi parcialmente reduzido, sugerindo que outros mecanismos estão envolvidos na HIE. Os achados revelam que o exercício induz hipotalgesia em ratos saudáveis. Mais estudos são necessários para avaliar o fenômeno e os mecanismos da HIE em seres humanos com distúrbios dolorosos como dor crônica musculoesquelética e dor orofacial.

KEYWORDS: Exercício. Receptores opióides. Medição de dor.

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1 Introduction

1 INTRODUCTION

In recent years, increasing attention has been given to the treatment of acute and chronic pain, and all the health associated conditions, including orofacial pain conditions. Despite the considerable number of studies and consequent improvements in creating more reliable animal models, understanding the pathophysiology of pain and evaluating new drugs, pain continues to be a major health and social problem. For these reasons, it continues to be a growing need to update scientific knowledge and understand the complex phenomenon of pain and painful conditions (Allegri *et al.*, 2012).

Nociceptive signal stimulates higher brainstem centers, but it can be attenuated by inhibitory pathways from the higher centers, that send signals downstream, reducing or eliminating the strength of the pain signal. Centrally released inhibitory neurochemicals travel downward along inhibitory pathways that serve to modulate these signals at dorsal horn connections (Melzack and Wall, 1965). Endogenous substances (neurotransmitters) usually control the nociceptive stimulus information in the CNS. These inhibitory systems can be activated by various mechanisms, other than the noxious stimulus. Many of the pharmacological and non-pharmacological therapies used in orofacial pain treatment activate these inhibitory control mechanisms. Exercise is one of this non-pharmacological therapies considered a trigger for pain modulation activation (Gurevich *et al.*, 1994; O'Connor and Cook, 1999; Koltyn, 2002; Dietrich and McDaniel, 2004; Whiteside *et al.*, 2004; Hoffman *et al.*, 2005; Stagg *et al.*, 2011; Ellingson *et al.*, 2013; Galdino *et al.*, 2014; Khan *et al.*, 2014).

The involvement of pain modulation during aerobic exercise leads the common use of this modality as part of rehabilitation programs for patients with chronic pain, including fibromyalgia, chronic neck pain, osteoarthritis, rheumatoid arthritis, and chronic low back pain. Aerobic exercise programs reduce pain, fatigue and depression, and improve peak oxygen uptake, health-related quality of life and physical fitness in patients with fibromyalgia (Dinler *et al.*, 2009; Hauser *et al.*, 2010). In chronic low back pain, aerobic endurance exercises are also commonly used and have been shown to reduce pain perception (Hoffman *et al.*, 2005). Nowadays, chronic musculoskeletal orofacial pain has the aerobic exercise as part of its management (Overath *et al.*, 2014). It has been demonstrated that the aerobic exercise may increase or optimize level of several neurotransmitters such as serotonin,

dopamine, acetylcholine and norepinephrine. Indeed, it activates the endocannabinoid and endogenous opioid system (Deslandes *et al.*, 2009; Matta Mello Portugal *et al.*, 2013).

Exercise reducing pain has been reported in the literature since the early 1980s (Black *et al.*, 1979; Gurevich *et al.*, 1994; Haier *et al.*, 1981; Janal, 1996; Janal *et al.*, 1984; Koltyn, 2000; O'Connor and Cook 1999) and the effect of physical activity on pain perception is commonly termed as Exercise-induced Hypoalgesia (EIH) (Black *et al.* 1979). Studies have shown increased pain thresholds after aerobic exercise for both mechanical (Hoffman *et al.*, 2005; Hoeger Bement *et al.*, 2008; Hoeger Bement *et al.*, 2009) and thermal stimuli (Staud *et al.*, 2005) and generalized increased of pressure pain thresholds following isometric muscle contraction (Kosek and Lundberg, 2003; Hoeger Bement *et al.*, 2008).

The exact mechanisms of EIH are unknown. However, it is widely believed that activation of the endogenous opioid system and release of peripheral and central beta-endorphins (Bement and Sluka 2005; Stagg *et al.*, 2011) play a major role in this phenomenon. Other suggested mechanisms include activation of neurotransmitters like serotonin and norepinephrine (Dietrich and Mcdaniel, 2004), involvement of the adenosinergic system (Martins *et al.*, 2013) and interactions with the cardiovascular system (Lovick, 1993).

The mechanisms underlying EIH have proven to be complex. Studies demonstrate that exercise effects could be reversed by naloxone, a non-specific opioid antagonist (Haier *et al.*, 1981; Janal *et al.*, 1984; Stagg *et al.*, 2011) but, to the best of our knowledge, there are no studies that evaluated the role of specific opioid receptors antagonist in the EIH levels, developed after an aerobic exercise program.

The endogenous opioid system consists of 3 families of opioid peptides (that derive from proteolytic cleavage of large protein precursors): β -endorphin, enkephalins, and dynorphins, and 3 families of receptors: μ (mu) (MOR), encoded by the OPRM1 gene; δ (delta) (DOR), encoded by the OPRD1 gene; and κ (kappa) (KOR), encoded by the OPRK1 gene. Whereas all 3 types of opioid receptors are defined pharmacologically by their blockade by naloxone, these receptors include different subtypes, as defined by their different affinity to a variety of agonists and antagonists (Dietis *et al.*, 2011). For example, μ opioid receptors include $\mu 1$ receptors, responsible for supraspinal analgesia, and $\mu 2$ receptors, which mediate opioid-triggered respiratory depression and miosis. The β -endorphin acts primarily via μ and

δ receptors, enkephalins via δ receptors, and dynorphins via κ receptors. Opioid peptides and their receptors have a widespread but selective distribution in the central and peripheral nervous systems, particularly in circuits involved in pain modulation, reward, responses to stress, and autonomic control (Benarroch, 2012).

As cited above, studies demonstrate that exercise effects could be reversed by naloxone, a non-specific opioid antagonist (Haier *et al.*, 1981; Janal *et al.*, 1984; Stagg *et al.*, 2011) but there are no studies that evaluate the role of specific opioid receptors antagonist in the EIH levels (higher or low EIH) developed after an aerobic exercise program. The studies presented in this thesis aimed to evaluate the role of non-specific (naltrexone) and specific opioids' receptors (mu, kappa and delta) antagonists on the EIH levels, produced by aerobic exercise in male rats. The two studies were performed using a recently developed model for EIH in rats (Khan *et al.*, 2014). These studies allow an evaluation of any association between the effect of exercise on the rat's ability to modulate pain and activation of a specific opioid receptor. Therefore it is hypothesized that rats with high levels of EIH will have more reduction on post-exercise analgesia after injection of specific opioid receptors antagonist drug.

2 Articles

2 ARTICLES

The articles presented in this Thesis were written according to *The Clinical Journal of Pain* instructions and guidelines for articles submission.

2.1 ARTICLE 1

THE EFFECT OF NON-SPECIFIC OPIOIDS' RECEPTORS ANTAGONIST ON EXERCISE-INDUCED HYPOALGESIA IN MALE RATS

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ABSTRACT

Opioids are known to play a central role in endogenous pain control mechanisms. The opioid system is involved in a widely studied biological phenomenon named Exercise-induced Hypoalgesia (EIH), but the exact mechanism(s) and how it is triggered are not clear. Studies demonstrate that exercise effects could be reversed by naloxone but there are no studies that evaluate if this effect happens in all individuals equally. The present study aimed to evaluate the role of non-specific opioids' receptors antagonist (naltrexone hydrochloride) on the EIH levels induced by aerobic exercise in the Rota-Rod equipment in sixty (n=60) healthy male adults Sprague-Dawley rats. Rats were divided into two groups (injected with naltrexone hydrochloride or distilled water) and subdivided into high ($67\% \leq \text{EIH} \leq 100\%$), medium ($34\% \leq \text{EIH} \leq 66\%$) or low ($\text{EIH} \leq 33\%$) EIH profile level. After 3 days of habituation, EIH baseline measurements (percentage of withdrawal response to 30 mechanical stimuli with 60g Von Frey monofilaments) at 1, 5, 10 and 20 minutes following exercise were assessed. In the day after the baseline measurements, rats were injected with naltrexone or distilled water. Then, after 10 minutes, EIH measurements were obtained once again. Data were analyzed with repeated-measurements ANOVA followed by post-hoc Fisher test. Alpha was set at 0.05 for all analyses. Injection of distilled water did not reduce the EIH ($p=0.904$) differently; naltrexone hydrochloride ($p=0.000$) significantly reduced the EIH assessed 1 minute following exercise when compared to baseline. It was concluded that only the rats with high EIH profile had a significant EIH reduction after injection of naltrexone. The EIH effect, however, was just partially reduced, suggesting that others mechanisms are involved in the EIH phenomenon. Exercise is commonly used as part of the treatment and rehabilitation programs for patients with chronic pain. The findings reveal that exercise induces hypoalgesia in healthy rats. More studies are needed to evaluate the phenomenon and the specific mechanisms of HIE in humans.

KEYWORDS: Exercise-induced Hypoalgesia. Opioid receptor. Opioid receptor antagonist. Pain behavior.

INTRODUCTION

Opioids are well known to have potent central analgesic actions, playing a central role in pain control^{1,2,3}. Opioid receptor (OR) is activated endogenously by the opioid peptides enkephalins, β -endorphin and dynorphins. These peptides and their derivatives exhibit different affinity and selectivity for specific receptors such as the mu-, delta- and kappa-opioid receptors (MOR, DOR and KOR, respectively), located on the central and the peripheral neurons, neuroendocrine, immune, and mucosal cells and on many other organ systems³. In the past two decades, researches in pharmacological and genetic area have clarified the specific role of each opioid receptor in many aspects of opioid-related behaviors, physiology and disorders². In recent years, a great number of studies have shown the role of opioids in the pathophysiology of diseases as well as in biological phenomenon of therapeutic interest as seen in Exercise-induced Hypoalgesia (EIH).

Aerobic exercise is commonly used as part of treatment and rehabilitation programs for patients with chronic pain, including fibromyalgia, chronic neck pain, osteoarthritis, rheumatoid arthritis, and chronic low back pain. Nowadays, chronic musculoskeletal orofacial pain has the aerobic exercise as part of its management⁴. It has been demonstrated that the aerobic exercise may increase or optimize level of several neurotransmitters such as serotonin, dopamine, acetylcholine and norepinephrine, also it can activates the endocannabinoid system and endogenous opioid system^{5,6,7}. Exercise-induced hypoalgesia has been reported in the literature since the early 1980s^{8,9,10,11,12,13,14}. Kosek *et al.*, observed that isometric muscle contractions were associated with increased generalized pressure pain thresholds¹⁵. Normal controls have shown increased pain thresholds after aerobic exercise for both pressure^{16,17,18,19} and temperature²⁰.

The most commonly tested hypothesis for EIH has been that activation of the endogenous opioid system during exercise may be responsible for the analgesic response that occurs following exercise. The most attractive evidence to support analgesia following exercise and the involvement of the endogenous opioid system on it have been provided by animal experimentation¹³. Furthermore, in animal models, exercise diminishes measures of inflammatory pain²¹, chronic muscle pain²² and chronic neuropathic pain^{23,24}. A single episode of exercise increases the production of endogenous opioids, leading to transient antinociception in both animals and humans¹³. The mechanisms underlying EIH have proven to be complex, and the exact mechanism(s) responsible for it is not entirely clear at this time^{13,6,7}. Studies demonstrate that exercise effects could be reversed by naloxone, a non-

specific opioid antagonist^{10,12,25} but the literature is poor about studying in which individual this EIH phenomenon actually happens, in which intensity for each individual and what is the role of the opioid system in the EIH profile levels (high, medium or low EIH profile) developed after an aerobic exercise program, presuming that individuals respond differently regarding the hypoalgesia effect of exercise.

The present study was performed using a recently method developed model for EIH in rats²⁶ to evaluate the role of the non-specific antagonist naltrexone hydrochloride on the EIH profile levels produced by aerobic exercise in male rats. This study allowed an evaluation of any association between the effect of exercise on the rat's ability to modulate pain and activation of the opioid system. Therefore it is hypothesized that rats with high EIH profile level will have more reduction on analgesia post exercise after injection of naltrexone hydrochloride.

MATERIAL AND METHODS

Animals

Healthy adults male Sprague-Dawley rats, weighing 250-300g, were used in the present study (n=60). Animals were sourced from a single breeder and housed in the on-site animal facility under veterinary supervision. They were fed a standard rodent chow, given *ad libitum* access to reverse osmosis water, and maintained on a 12hr day/night cycle. To familiarize the rats to the environment and to the exercise program, they were habituated daily during three consecutive days in the lab environment and for the Rota-RodTM (Stoelting Inc., Wood Dale, IL, USA) (equipment used for the rat's aerobic exercise). Habituation for exercise involved slowly and gentles exposure to the exercise equipment. Once the rat was successful habituated to the Rota-RodTM in motion, subsequent habituation days reinforced a 180 seconds exercise routine. Across the three days, the rats learned to walk for 180 seconds in the Rota-RodTM without failure or verbal protest. The Institutional Animal Care and Use Committee of the Rutgers University – School of Dental Medicine, approved the experimental procedures and protocols used in the present study.

Exercise-induced Hypoalgesia (EIH) evaluation

The EIH test is typically performed applying a noxious stimulus before and following exercise, creating a percentage of response. In the present study, EIH was evaluated using same methodology of Khan *et al.* 2014. The EIH calculation is showed in the Figure 1.

The noxious stimulus performed was 30 successive mechanical stimuli using a 60g force von Frey monofilament, applied in the left rat's mid-plantar hind paw at a rate of approximately 1 stimulus per second. A positive response was recorded when the rat withdrew the paw following the stimulus. When the filament passively elevated the paw (without paw withdrawal by the rat) the stimulus was considered a non-response.

The percentage of response out of 30 were calculated before exercise (baseline), and re-evaluated within 1 minute, 5, 10, and 20 minutes following the cessation of exercise. The higher the EIH score, the higher the analgesic effect of the exercise. An EIH score of 100% means that the rat, after exercising for 3 minutes had a decrease in the percentage of response for the mechanical stimuli of 100% when comparing to the percentage of response before exercise. High EIH means more efficient EIH and low means less efficient EIH.

After the EIH calculation and according to the EIH score obtained, the rats were divided into thirds as high ($67\% \leq \text{EIH} \leq 100\%$) ($n=10$), medium ($34\% \leq \text{EIH} \leq 66\%$) ($n=10$) and low ($\text{EIH} \leq 33\%$) ($n=10$) EIH responders²⁶.

$$\text{EIH} = 100 \times (\text{baseline \% response} - 1 \text{ minute \% response}) / \text{baseline \% response}$$

Figure 1: EIH equation

Experimental conditions

The same researcher performed all the tests at the same time of the day (morning) for all the groups. For exercise, the rats were placed on a rotating surface (Rota-RodTM, Stoelting Inc., Wood Dale, IL, USA) and were required to walk against the motion of the rotating drum with the speed accelerating from 8 to 16 rpm over 100 seconds and maintaining the 16 rpm for the remainder of the 180 seconds. After 3 days of habituation, the baseline measurements for EIH were obtained. In the day after, the injections were administered and measurements for EIH were recorded again.

The non-specific opioid receptor antagonist administered (injected) was Naltrexone

hydrochloride, purchased from Sigma-Aldrich Company (St Louis, MO) and dissolved in distilled water (vehicle) at the time of the experiment. The vehicle was the substance injected in the control group. Naltrexone and vehicle were administered intraperitoneally (i.p.) in a volume of 0.25ml/rat with a 1ml syringe. The solution was prepared few minutes before injection. Drug administration protocol is described in Table 1. The drug dose selection was based on selected paper that used this drug for experiments involving pain and opioid system block²⁵.

Rats were injected only one time and measurements of EIH before and after injection were recorded only once. The study design is showed in the Figure 2.

Figure 2: Study design

Table 1 - Drugs administration protocol

<i>Groups</i>	<i>Dosage</i>	<i>Time waiting to start EIH test after injection</i>
Vehicle (distilled water)	0.25ml/rat	10 min
Naltrexone hydrochloride	1mg/ml/rat	10 min

Data Analysis

The study sample size was calculated with the help of the Office of Research, Rutgers University, School of Dental Medicine. For the purposes of this study, alpha was set at 0.05 for all analyses. Statistical analyses were performed with Stat View 5.0 software (SAS Institute Inc. NC, USA).

The EIH response following exercise, before and after injection, was analyzed with Repeated-measurements Analysis of Variance (RMANOVA). Factorial analysis of variance (ANOVA) was performed to compare groups at the same time point (1, 5, 10 and 20 minutes following exercise). All statistical tests were followed by post-hoc Fisher test. All results are presented as mean $\pm$ standard error of mean ($\pm$ SEM).

RESULTS

EIH

It was compared the EIH score obtained after injection with that obtained at baseline, for all time points, for each group of rats, previously categorized as high, medium and low EIH. The percentage number for EIH represents the level of analgesia presented by the rats following exercise.

Results obtained before injection

The EIH mean scores of the rats after 1, 5, 10 and 20 minutes following exercise, in percentage, are described in Table 2. The minus score in the low EIH group represents more response out of 30 stimuli after exercise than the amount of response at baseline.

EIH	1 minute	5 minutes	10 minutes	20 minutes
High	79%	48%	44%	29%
Medium	53%	36%	46%	41%
Low	-6%	-5%	11%	-4%

Chart 1: EIH mean score (percentage) of the rats within 1, 5, 10 and 20 minutes following exercise.

In the **high EIH group**, the percentage of response to the 30 successive stimuli within 1 minute following exercise decreased significantly ($p < 0.05$) when compared to baseline. After 5, 10 and 20 minutes of exercise, it was still significantly different from baseline ($p < 0.05$) but increasing. In the **medium EIH group**, the percentage of response significantly decreased compared to baseline after 1, 5, 10 and 20 minutes following exercise ($p < 0.05$). The **low EIH group** had no statistical difference when compared to baseline ($p > 0.05$) (Figure 3).

Figure 3: Response stratified by High, Medium and Low EIH profile rats before naltrexone injection. (* $p < 0.05$)

Results obtained after injection

In the **high EIH group**, at the time point of **1 minute following exercise**, the injection of vehicle did not reduce the EIH of the rats ($p = 0.904$). Differently, naltrexone hydrochloride significantly affected the EIH results at 1 minute following exercise, reducing it significantly comparing to the same time point (1 minute) at baseline measurement (before injection) ($p < 0.000$) (Figure 4). There was no significant difference in the EIH before and after injection for 5, 10 and 20 minutes in High group. In the medium and low EIH group, at 1, 5, 10 and 20 minutes following exercise, there was no significant difference in EIH values before and after injection.

Figure 4: Percentage of change from baseline (before and after injection) in the response to mechanical stimuli (EIH score) 1 minute following exercise, in the High EIH rats (n=10 for each group) (* $p < 0.05$)

Figure 5: Percentage of change from baseline (before and after injection) in the response to mechanical stimuli (EIH score) 1 minute following exercise, in the High, Medium and Low EIH rats (n=10 for each group). Statistical significance difference was found in the High EIH rats after injection.

DISCUSSION

It is well known in the EIH research field that injection of naloxone or naltrexone, both non-specific opioid receptor's antagonist, decreases the hypoalgesic effect of exercise^{10,12,13,25}. Similar result was found in the present study, in which naltrexone hydrochloride significantly decreased the EIH. The present study shows interesting results about the role of the opioid system in the hypoalgesic effect following aerobic exercise in male rats, emphasizing that the EIH phenomenon do not happen in an universal way. It seems to have a likelihood, based on the classification according to the amount of EIH was obtained after the aerobic exercise. All rats had a hypoalgesic effect of exercise at baseline, but after naltrexone injection, the significant reduction (50% of reduction comparing to baseline measurements) of EIH was found only in rats with high EIH profile.

As cited above, the reduction in the EIH of the rats with high EIH profile was partial (50%). It suggests that a non-opioid mechanism could be involved in the EIH. Blood pressure, cardiovascular system, conditioned pain modulation (CPM) and endocannabinoid system are being studied as possible additional mechanisms involved in the EIH phenomenon⁶. Systemic and central pre-treatment with CB1 and CB2 cannabinoid receptor antagonists (AM251 and AM630) blocked the antinociception induced by an aerobic exercise protocol in both, mechanical and thermal nociceptive tests at peripheral and central levels²⁷. This recent study concluded that the endocannabinoid system is involved in EIH. So, further studies are needed to clarify the role of both systems, the opioid and endocannabinoid, simultaneously.

Based on the type of exercise, different mechanisms may be involved in the regulation of beta-endorphin release during exercise²⁸. This beta-endorphin changes and alterations in its receptors could explain the variation among rats in the present study, regarding the EIH profile. Some opioids receptors interact with each other²⁹, and this could be also an explanation for the partial reduction in the hypoalgesic effect after naltrexone's injection.

In the present study, during the EIH profile levels' measurements of the rats, before injection, there was a significant linear decline in pain threshold still after 20 minutes following exercise, in which the percentage of response was still significant lower comparing to the baseline ($p=0.0004$). But the most important results for EIH before injection in the present study were obtained within 1 minute following exercise. Our findings suggest that there is a minimum time of exercise required to elicit EIH in rats and that the EIH effect lasts more than 20 minutes. It is in accordance to some studies^{30,31,32}. Cooper and Carmody examined the time course of analgesia induced by swimming. Male mice swum for different

time periods in different water temperatures, and pain thresholds were assessed before and following the swim by recording the time of a flick of a hind limb after the animal was placed on a hot plate. The results showed that pain thresholds were increased significantly 1 minute after the swim, with a linear decline in pain thresholds through 30 minutes³⁰.

Conditioned pain modulation is another potential factor that could influence EIH, especially given that exercise is sometimes perceived to be painful, especially isometric exercise. With CPM, pain from a noxious stimulus (conditioning stimulus, i.e., exercise) results in the inhibition of pain during the application of a second noxious stimulus (test stimulus) applied elsewhere (i.e., “pain inhibits pain”). The difference in pain experienced with the test stimulus applied with and without the conditioning stimulus is a measure of CPM. The noxious conditioning stimulus activates descending inhibitory pathways, resulting in inhibition of extra-segmental spinal and trigeminal wide dynamic range neurons³³, thereby decreasing pain associated with the test stimulus. It has been proposed that pain experienced during exercise may act as a conditioning stimulus, resulting in EIH³⁴. In the present study, the aerobic exercise was used and CPM could have been activated, as well as the endocannabinoid system.

It is unclear whether individuals with chronic pain would experience similar results with exercise as healthy young adults. Results from studies examining EIH in individuals with chronic pain are currently equivocal, with EIH occurring in some chronic pain conditions (eg, osteoarthritis and rheumatoid arthritis) but not consistently in others (eg, fibromyalgia, temporomandibular disorders and painful diabetic neuropathy)³⁵. The dysfunctional (aerobic exercise activating pain facilitation rather than inhibition) endogenous analgesia during aerobic exercise is not a characteristic of all chronic pain patients, but limited to those with an evidence of central sensitization (e.g. chronic whiplash, fibromyalgia, chronic fatigue syndrome)³⁶. It is very difficult, however, to estimate the exact participation of peripheral or central neuronal sensitization and impaired descending modulatory mechanisms in the maintenance of painful states.

Exercise is commonly included in rehabilitation programs for patients with chronic pain. It is important to know that not all individuals will be benefited with an exercise program as part of the management program of orofacial pain, specially the chronic ones, since it was showed in the present study that there is an evident EIH profile variations among animals, what could be extrapolated to humans. According to Nijs *et al.*, future researches should examine whether these proposed combinations of drug treatment and graded exercise therapy are able to treat the dysfunctional endogenous analgesia in patients with chronic pain.

Moreover, the combined treatment programs should not only improve endogenous analgesia during exercise, it should benefit the patient at the level of daily functioning and quality of life as well³⁶.

The present study findings may help understand the mechanisms involved in EIH phenomenon. It was concluded that rats with high EIH profile had a significant EIH reduction after injection of naltrexone, but the EIH was just partially reduced, suggesting that others mechanism is involved in the EIH phenomenon. Furthermore, the findings reveal that exercise induces hypoalgesia in healthy rats. More studies are needed to evaluate the phenomenon and the specific mechanisms of HIE in healthy humans and those with chronic pain conditions. Moreover, evaluate the intensity and duration of the exercise that elicit the best EIH effect. Additional research is needed to clarify and expand the understanding of the mechanisms responsible for EIH. The present data may give a direction for further studies in which the researcher blocks specific ORs to analyze if an specific receptor is more involved in the EIH phenomenon.

REFERENCES

1. Benarroch EE: Endogenous opioid systems: current concepts and clinical correlations. *Neurology* 79(8):807-14, 2012
 2. Pradhan AA, Befort K, Nozaki C, Gaveriaux-Ruff C, Kieffer BL: The delta opioid receptor: an evolving target for the treatment of brain disorders. *Trends Pharmacol Sci* 32(10):581-90, 2011
 3. Sauriyal DS, Jaggi AS, Singh N: Extending pharmacological spectrum of opioids beyond analgesia: multifunctional aspects in different pathophysiological states. *Neuropeptides* 45(3):175-88, 2011
 4. Overath CH, Darabaneanu S, Evers MC, Gerber WD, Graf M, Keller A, et al.: Does an aerobic endurance programme have an influence on information processing in migraineurs? *J Headache Pain* 15(1):11, 2014
 5. Deslandes A, Moraes H, Ferreira C, Veiga H, Silveira H, Mouta R, et al.: Exercise and mental health: many reasons to move. *Neuropsychobiology* 59(4):191-8, 2009
 6. Koltyn KF, Brellenthin AG, Cook DB, Sehgal N, Hillard C: Mechanisms of exercise-induced hypoalgesia. *J Pain* 15(12):1294-304, 2014
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7. Matta Mello Portugal E, Cevada T, Sobral Monteiro-Junior R, Teixeira Guimaraes T, da Cruz Rubini E, Lattari E, et al.: Neuroscience of exercise: from neurobiology mechanisms to mental health. *Neuropsychobiology* 68(1):1-14, 2013
 8. Black J, Chesher GB, Starmer GA, Egger G: The painlessness of the long distance runner. *Med J Aust* 1(11):522-3, 1979
 9. Gurevich M, Kohn PM, Davis C: Exercise-induced analgesia and the role of reactivity in pain sensitivity. *J Sports Sci* 12(6):549-59, 1994
 10. Haier RJ, Quaid K, Mills JC: Naloxone alters pain perception after jogging. *Psychiatry Res* 5(2):231-2, 1981
 11. Janal MN: Pain sensitivity, exercise and stoicism. *J R Soc Med* 89(7):376-81, 1996
 12. Janal MN, Colt EW, Clark WC, Glusman M: Pain sensitivity, mood and plasma endocrine levels in man following long-distance running: effects of naloxone. *Pain* 19(1):13-25, 1984
 13. Koltyn KF: Analgesia following exercise: a review. *Sports Med* 29(2):85-98, 2000
 14. O'Connor PJ, Cook DB: Exercise and pain: the neurobiology, measurement, and laboratory study of pain in relation to exercise in humans. *Exerc Sport Sci Rev* 27:119-66, 1999
 15. Kosek E, Lundberg L: Segmental and plurisegmental modulation of pressure pain thresholds during static muscle contractions in healthy individuals. *Eur J Pain* 7(3):251-8, 2003
 16. Hoeger Bement MK, Dicapo J, Rasiarmos R, Hunter SK: Dose response of isometric contractions on pain perception in healthy adults. *Med Sci Sports Exerc* 40(11):1880-9, 2008
 17. Hoeger Bement MK, Weyer A, Hartley S, Yoon T, Hunter SK: Fatiguing exercise attenuates pain-induced corticomotor excitability. *Neurosci Lett* 452(2):209-13, 2009
 18. Hoffman MD, Shepanski MA, Mackenzie SP, Clifford PS: Experimentally induced pain perception is acutely reduced by aerobic exercise in people with chronic low back pain. *J Rehabil Res Dev* 42(2):183-90, 2005
 19. Whiteside A, Hansen S, Chaudhuri A: Exercise lowers pain threshold in chronic fatigue syndrome. *Pain* 109(3):497-9, 2004
 20. Staud R, Robinson ME, Price DD: Isometric exercise has opposite effects on central pain mechanisms in fibromyalgia patients compared to normal controls. *Pain* 118(1-2):176-84, 2005
 21. Kuphal KE, Fibuch EE, Taylor BK: Extended swimming exercise reduces inflammatory and peripheral neuropathic pain in rodents. *J Pain* 8(12):989-97, 2007
 22. Bement MK, Sluka KA: Low-intensity exercise reverses chronic muscle pain in the rat in a naloxone-dependent manner. *Arch Phys Med Rehabil* 86(9):1736-40, 2005
-

23. Cobianchi S, Marinelli S, Florenzano F, Pavone F, Luvisetto S: Short- but not long-lasting treadmill running reduces allodynia and improves functional recovery after peripheral nerve injury. *Neuroscience* 168(1):273-87, 2010
 24. Hutchinson KJ, Gomez-Pinilla F, Crowe MJ, Ying Z, Basso DM: Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats. *Brain* 127(Pt 6):1403-14, 2004
 25. Stagg NJ, Mata HP, Ibrahim MM, Henriksen EJ, Porreca F, Vanderah TW, et al.: Regular exercise reverses sensory hypersensitivity in a rat neuropathic pain model: role of endogenous opioids. *Anesthesiology* 114(4):940-8, 2011
 26. Khan J, Benavent V, Korczeniewska OA, Benoliel R, Eliav E: Exercise-Induced Hypoalgesia Profile in Rats Predicts Neuropathic Pain Intensity Induced by Sciatic Nerve Constriction Injury. *J Pain*, 2014
 27. Galdino G, Romero TR, Silva JF, Aguiar DC, de Paula AM, Cruz JS, et al.: The endocannabinoid system mediates aerobic exercise-induced antinociception in rats. *Neuropharmacology* 77:313-24, 2014
 28. Goldfarb AH, Jamurtas AZ: Beta-endorphin response to exercise. An update. *Sports Med* 24(1):8-16, 1997
 29. Peppin JF, Raffa RB: Delta opioid agonists: a concise update on potential therapeutic applications. *J Clin Pharm Ther* 40(2):155-66, 2015
 30. Cooper K, Carmody J: The characteristics of the opioid-related analgesia induced by the stress of swimming in the mouse. *Neurosci Lett* 31(2):165-70, 1982
 31. Hoffman MD, Shepanski MA, Ruble SB, Valic Z, Buckwalter JB, Clifford PS: Intensity and duration threshold for aerobic exercise-induced analgesia to pressure pain. *Arch Phys Med Rehabil* 85(7):1183-7, 2004
 32. Koltyn KF, Garvin AW, Gardiner RL, Nelson TF: Perception of pain following aerobic exercise. *Med Sci Sports Exerc* 28(11):1418-21, 1996
 33. Le Bars D, Willer JC. Diffuse noxious inhibitory controls (DNIC). In: Basbaum A, Bushnell MC, editors. *Science of Pain*. Oxford (UK): Elsevier; 2009. p. 763-73.
 34. Lemley KJ, Hunter SK, Bement MK: Conditioned pain modulation predicts exercise-induced hypoalgesia in healthy adults. *Med Sci Sports Exerc* 47(1):176-84, 2015
 35. Knauf MT, Koltyn KF: Exercise-induced modulation of pain in adults with and without painful diabetic neuropathy. *J Pain* 15(6):656-63, 2014
 36. Nijs J, Kosek E, Van Oosterwijk J, Meeus M: Dysfunctional endogenous analgesia during exercise in patients with chronic pain: to exercise or not to exercise? *Pain Physician* 15(3 Suppl):ES205-13, 2012
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2.2 ARTICLE 2

THE EFFECT OF SPECIFIC OPIOIDS' RECEPTORS ANTAGONISTS ON EXERCISE-INDUCED HYPOALGESIA IN MALE RATS

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ABSTRACT

Studies demonstrate that Exercise-induced Hypoalgesia (EIH) could be reversed by naloxone/naltrexone but there are no studies that evaluate the role of specific opioid receptors in the EIH levels. The present study aimed to evaluate the role of specific opioids' receptors (μ , κ and δ) antagonists on the EIH levels induced by aerobic exercise in the Rota-RodTM in a hundred and twenty ($n=120$) healthy male adults Sprague-Dawley rats. The rats were divided into four groups (vehicle, μ , κ and δ) and each group was subdivided into high ($67\% \leq \text{EIH} \leq 100\%$), medium ($34\% \leq \text{EIH} \leq 66\%$) or low ($\text{EIH} \leq 33\%$) EIH profile level. After 3 days of habituation, EIH baseline measurements (percentage of withdrawal response to 30 mechanical stimuli with 60g Von Frey monofilaments) at 1, 5, 10 and 20 minutes following exercise were assessed. In the day after the baseline measurements, rats were injected with CTAP (μ receptor antagonist), GNTI (κ receptor antagonist), Naltrindole hydrochloride (δ receptor antagonist) or vehicle (distilled water), then, after 20 minutes (for μ), 15 minutes (for κ) and 10 minutes (for δ), and 10 minutes (for vehicle); EIH measurements were obtained again, just once. Data were analyzed with repeated-measurements ANOVA followed by post-hoc Fisher test. Alpha was set at 0.05 for all analyses. Injection of vehicle (distilled water) did not reduce the EIH ($p=0.904$), differently; κ ($p=0.002$) and δ ($p=0.000$) antagonist's drugs significantly reduced the EIH 1 minute following exercise when compared to baseline. It was concluded that rats with high EIH profile had a significant EIH reduction after injection of some specific opioid receptors antagonists (δ and κ). The EIH effect, however, was just partially reduced, suggesting that others mechanism and/or opioids receptors interactions are involved in the EIH phenomenon. The present study findings may help understanding the mechanisms involved in this phenomenon. Furthermore it may support the development of new drugs targeting to the mechanisms found to be more associated with the hypoalgesia induced by exercise.

KEYWORDS: Exercise-Induced Hypoalgesia. Opioid receptor. Opioid receptor antagonist. Pain behavior.

INTRODUCTION

Pain is a complex sensory and emotional experience often initiated by peripheral nociceptors. Peripherally generated signals may undergo modulation in the Central Nervous System (CNS). Similar external stimuli can evoke different pain experiences. The perception of pain varies among subjects and is affected by emotional status, concurrent painful stimuli and by exercise or physical activity¹.

The opioid system is involved in modulating pain and reward. Opioid receptors (OR) are a group of G-protein coupled receptors divided into three families: the MOR, DOR, and KORs. Three classes of endogenous opioid peptides, beta-endorphin, dynorphin, and enkephalin activate these receptors. The selectivity and distribution of the opioid peptide and receptor systems suggests that the DOR and MOR have relatively high binding affinity for the endogenous opioids beta-endorphin and leu-enkephalin, and dynorphin has affinity for the KOR². Opioids are well known to have potent central analgesic actions, playing a central role in pain control^{3,4,5}. The ORs are located on the central and the peripheral neurons, neuroendocrine, immune, and mucosal cells and on many other organ systems⁵. In the past two decades, researches in pharmacological and genetic area have clarified the specific role of each opioid receptor in many aspects of opioid-related behaviors, physiology and disorders⁴. In recent years, a great number of studies have shown the role of opioids in the pathophysiology of diseases as well as in biological phenomenon of therapeutic interest as seen in Exercise-induced Hypoalgesia (EIH).

Aerobic exercise is commonly used as part of treatment and rehabilitation programs for patients with chronic pain, including fibromyalgia, chronic neck pain, osteoarthritis, rheumatoid arthritis, and chronic low back pain. Nowadays, chronic musculoskeletal orofacial pain has the aerobic exercise as part of its management⁶. It has been demonstrated that the aerobic exercise may increase or optimize level of several neurotransmitters such as serotonin, dopamine, acetylcholine and norepinephrine, activate the endocannabinoid system and endogenous opioid system^{7,8}. Exercise-induced hypoalgesia has been reported in the literature since the early 1980s^{9,10,11,12,13,14,15}. Kosek *et al*, observed that isometric muscle contractions were associated with increased generalized pressure pain thresholds¹⁶. Normal controls have shown increased pain thresholds after aerobic exercise for both pressure^{17,18,19,20} and temperature²¹.

The most attractive evidence to support hypoalgesia following exercise and the involvement of the endogenous opioid system on it have been provided by animal

experimentation¹⁴. Furthermore, in animal models, exercise diminishes measures of inflammatory pain²², chronic muscle pain²³ and chronic neuropathic pain^{24,25}. A single episode of exercise increases the production of endogenous opioids, leading to transient antinociception in both animals and humans¹⁴. The mechanisms underlying EIH have proven to be complex, and the exact mechanism(s) responsible for EIH are not entirely clear at this time^{14,8}. Studies demonstrate that exercise effects could be reversed by naloxone, a non-specific opioid antagonist^{11,13,26} but to the best of our knowledge, there are no studies that evaluate the role of specific opioid receptors antagonist in the EIH profile levels developed after an aerobic exercise program.

The present study performed a recently developed model for EIH in rats¹ to assess the EIH profile levels of the rats and to evaluate the role of specific opioids' receptors (mu, kappa and delta) on the EIH profile levels produced by aerobic exercise in male rats. This study allows an evaluation of any association between the effect of exercise on the rat's ability to modulate pain and activation of a specific opioid receptor. Therefore it is hypothesized that rats with high levels of EIH would have more reduction on hypoalgesia post exercise after injection of specific OR's antagonist drug.

MATERIAL AND METHODS

Animals

Healthy adults male Sprague-Dawley rats, weighing 250-300g, were evaluated in the study (n=120). Animals were sourced from a single breeder and housed in the on-site animal facility under veterinary supervision. They were fed a standard rodent chow, given *ad libitum* access to reverse osmosis water, and maintained on a 12hr day/night cycle. To familiarize the rats to the environment and to the exercise program, they were habituated daily during three consecutive days in the lab environment and for the Rota-RodTM (Stoelting Inc., Wood Dale, IL, USA) (equipment used for the rat's aerobic exercise). Habituation for exercise involved slowly and gentles exposure to the exercise equipment. Once the rat was successful habituated to the Rota-RodTM in motion, subsequent habituation days reinforced a 180 seconds exercise routine. Across the three days, the rats learned to walk for 180 seconds in the Rota-RodTM without failure or verbal protest. The Institutional Animal Care and Use Committee of the

Rutgers University – School of Dental Medicine, approved the experimental procedures and protocols used in the present study.

Exercise-induced Hypoalgesia (EIH) evaluation

The EIH test is typically performed applying a noxious stimulus before and following exercise, creating a percentage of response. In the present study, EIH was evaluated using same methodology of Khan *et al.* 2014¹. The EIH calculation is showed in the Figure 1.

The noxious stimulus performed was 30 successive mechanical stimuli using a 60g force von Frey monofilament, applied in the left rat's mid-plantar hind paw at a rate of approximately 1 stimulus per second. A positive response was recorded when the rat withdrew the paw following the stimulus. When the filament passively elevated the paw (without paw withdrawal by the rat) the stimulus was considered a non-response.

The percentage of response out of 30 were calculated before exercise (baseline), and re-evaluated within 1 minute, 5, 10, and 20 minutes following the cessation of exercise.

The higher the EIH score, the higher the analgesic effect of the exercise. An EIH score of 100% means that the rat, after exercising for 3 minutes had a decrease in the percentage of response for the mechanical stimuli of 100% when comparing to the percentage of response before exercise. High EIH means more efficient EIH and low means less efficient EIH.

After the EIH calculation and according to the EIH score obtained, the rats were divided into thirds¹ as high ($67\% \leq \text{EIH} \leq 100\%$), medium ($34\% \leq \text{EIH} \leq 66\%$) and low ($\text{EIH} \leq 33\%$) EIH responders.

$$\text{EIH} = 100 \times (\text{baseline \% response} - 1 \text{ minute \% response}) / \text{baseline \% response}$$

Figure 1: EIH equation

Experimental conditions

The same researcher performed all the tests at the same time of the day (morning) for all the groups. For exercise, the rats were placed on a rotating surface (Rota-RodTM, Stoelting Inc., Wood Dale, IL, USA) and were required to walk against the motion of the rotating drum with the speed accelerating from 8 to 16 rpm over 100 seconds and maintaining the 16rpm for the remainder of the 180 seconds. After 3 days of habituation, the baseline measurements for

EIH were obtained. In the day after, the injections were administered and measurements for EIH were recorded again.

All drugs injected were purchased from Sigma-Aldrich Company (St Louis, MO) and dissolved in distilled water (vehicle) at the time of the experiment. The drugs used were: C6352 (CTAP), G3416 (GNTI - 5'-Guanidinonaltrindole di (trifluoroacetate) salt hydrate), N115 (Naltrindole hydrochloride). The control group was labeled as vehicle and the rats of this group were injected only with distilled water. All the drugs were administered intraperitoneally (i.p.) in a volume of 0.25ml/rat with a 1ml syringe. The solutions were prepared few minutes before injection.. Drugs administration protocol is described in Table 1. The drug dose selection was based on selected papers^{27,28,29} that used these antagonists' drugs for experiments involving pain and opioid system block.

Rats were injected only one time and measurements of EIH before and after injection were recorded only once too. The study design is showed in the Figure 2.

Figure 2: Study design

Table 1 - Drugs administration protocol

<i>Groups</i>	<i>Dosage</i>	<i>Time waiting to start EIH test after injection</i>
Vehicle (distilled water)	0.25ml/rat	10 min
MOR antagonist (CTAP)	0.025mg/ml/rat ²⁷	20 min
KOR antagonist (G3416)	1.4mg/ml/rat ²⁹	15 min
DOR antagonist (Naltrindole hydrochloride)	1.25mg/ml/rat ²⁸	10 min

MOR – mu opioid receptor; KOR – kappa opioid receptor; DOR – delta opioid receptor

Data Analysis

The study sample size was calculated with the help of the Office of Research, Rutgers School of Dental Medicine. For the purposes of this study, alpha was set at 5% for all analyses. Statistical analyses were performed with Stat View 5.0 software (SAS Institute Inc. NC, USA). Measurements were performed before and after injection. It was compared the EIH score obtained after injection with the one obtained before injection, for all time points, for each group of rats previously categorized as high, medium and low EIH. This comparison was obtained with Repeated-Measurements Analysis of Variance (RMANOVA). Factorial analysis of variance (ANOVA) was performed to compare groups at the same time point. All statistical tests were followed by post-hoc Fisher test. All results are presented as mean $\pm$ standard error of mean ($\pm$ SEM).

RESULTS

Results obtained before injection

The EIH mean scores of the rats after 1, 5, 10 and 20 minutes following exercise, in percentage, are described in Table 2. The minus score in the low EIH group represents more response out of 30 stimulus after exercise than the amount of response at baseline).

EIH	1 minute	5 minutes	10 minutes	20 minutes
High	79%	48%	44%	29%
Medium	53%	36%	46%	41%
Low	-6%	-5%	11%	-4%

Chart 1: EIH mean score of the rats within 1, 5, 10 and 20 minutes following exercise, in percentage.

In the **high EIH group**, the percentage of response to the 30 successive stimuli within 1 minute following exercise decreased significantly ($p<0.05$) when compared to baseline. After 5, 10 and 20 minutes of exercise, it was still significantly different from baseline ($p<0.05$) but increasing. In the **medium EIH group**, the percentage of response significantly decreased compared to baseline after 1, 5, 10 and 20 minutes following exercise ($p<0.05$). The **low EIH group** had no statistical difference when compared to baseline ($p>0.05$) (Figure 3).

Results obtained after injection

The rats were injected with three different opioid's receptors antagonists and one group was injected with distilled water. In the **high EIH group**, at time point of **1 minute following exercise**, the injection of vehicle did not reduce the EIH of the rats ($p=0.904$) differently, KOR ($p=0.002$) and DOR ($p=0.000$) antagonist's drugs, significantly affected the EIH results at 1 minute following exercise, reducing it comparing to the same time point EIH before injection (Figure 3). In the medium and low EIH rats, there was no significant difference in the EIH before and after injection for all drugs at 1 minute following exercise. At 5 minutes following exercise there was a significant reduction of the EIH in the medium EIH rats injected with DOR ($p=0.034$) antagonist's drug. At 10 minutes following exercise, there was a significant EIH reduction in the high EIH rats injected with KOR ($p=0.012$) and DOR ($p=0.002$) antagonist's drugs. At 20 minutes following exercise no significant difference was found in EIH values before and after injection in the high, medium and low group for all the drugs injected (Figure 4).

Figure 3: Percentage of change from baseline (before and after injection) in the response to mechanical stimuli (EIH score) 1 minute following exercise, in the High EIH rats (n=10 for each group) (* $p < 0.05$)

Figure 4: Percentage of change from baseline (before and after injection) in the response to mechanical stimuli (EIH score) 5 and 10 minutes following exercise, in the High and Medium EIH rats (n=10 for each group) (* $p < 0.05$)

DISCUSSION

The present study shows interesting results about the role of specific ORs in the hypoalgesic effect following aerobic exercise. The most significant effect was found in rats that had a high EIH profile at baseline, injected with DOR and KOR antagonists. Although the association between inefficient pain modulation and chronic pain has been demonstrated in various pathological and experimental conditions^{30,31}, the association between pain sensitivity and pain modulation profile in pain free subjects is not yet outlined¹. In the present study, the pain modulation profile provided by aerobic exercise was an important point, making the study unique, comparing to all studies about EIH and rats.

It is well known in the EIH field that injection of naloxone or naltrexone, both non-specific OR antagonist, decreases the analgesic effect of exercise^{11,13,14,26}. Interestingly, in the present study, this reversion in the analgesic effect was observed only in the High EIH profile rats, and happened mainly by injections of KOR and DOR's antagonists. After the injections of KOR and DOR antagonists, the rats had a statistical significant increase in the nociceptive response immediately following exercise, being the DOR the most important receptor playing a role in the EIH, since the antagonist drug reduced the EIH close to 0%, which means no EIH effect. No significant difference was found when comparing the reduction in the EIH results within 1 minute following exercise between Kappa and Delta groups.

Delta opioid receptors are located throughout the pain transmission (periphery, dorsal root ganglia, spinal cord, ascending neuronal tracts, rostral ventromedial medulla, periaqueductal gray and other brain regions) and modulatory (descending neuronal tracts, involving primarily norepinephrine and serotonin) pathways³². The mechanisms of delta agonist hypoalgesia have recently been extensively overviewed and the stimulation of DOR seems to strongly reduces pain³³. The development of highly selective delta opioid agonists made this receptor as a promising target to treat chronic pain and mood disorders⁴.

In the present study, after injection of DOR's antagonist, the medium and low EIH rats had no significant decrease following 1 minute of exercise, as the opioid system had no activation, following exercise in this medium and low profile rats. A major factor contributing to delta opioid hypoalgesia is the expression level of the receptor protein at the cell surface of neurons, and at the different sites of the pain-processing pathways³³. Sequence variants within the *Oprdl* gene, which encode the DOR, may influence the DOR expression across individuals, and may contribute to inter-individual differences in responses to DOR agonist and antagonists' drugs³⁴. The selectivity and distribution of the opioid peptide and receptor

systems suggests that the DOR and MOR has relatively high binding affinity for the endogenous opioids beta-endorphin, and it is well known that beta-endorphin is produced during exercise^{17,14,35}. In the present study, what could be happened is that the injection of DOR antagonist prevented the beta-endorphin of binding to the receptor and, consequently prevented the activation of the opioid system effects following exercise.

It is possible that the EIH level depends of the DOR's expression in the individual. The identification of natural genetic variants affecting extracellular domains of the DOR and altering pain perception, opens the possibility of individual variability in responses to delta agonists³³. It is possible that the rats with high hypoalgesia following exercise in the present study had higher expression of delta receptors in the pain-processing pathways.

In the present study, KOR antagonist, as well as DOR antagonist, significantly reduced the EIH effect in the rats. Systemic KOR agonists also produce robust analgesia³⁶. KORs are located in the spinal cord and brain stem, and part of their analgesic effect is due to the direct inhibition of pain pathways³⁷. Pharmacologic, molecular biology and thermodynamic studies have suggested subtypes of each of the major opioid receptor types: at least three for MOR, two for DOR and three or more for KOR³⁸. The blocking of KOR in the present study attenuated the hypoalgesic effect of aerobic exercise less than when the DOR was blocked, even though it was not significant statistically, but the higher variation of kappa opioid receptors could explain this difference in the results for KOR and DOR, since GNTI (KOR antagonist used in the present study) do not blocks all variants of KOR.

It is well known that stress activates different intrinsic pain inhibitory mechanisms, leading to hypoalgesia in humans and antinociception in rodents, a phenomenon referred as stress-induced hypoalgesia/antinociception (SIA)^{39, 40}. Some studies show that SIA and endogenous opioid system are related^{39,41}. Depending on the nature of stressors, different pain inhibitory mechanisms can be activated. In general, less severe stressors are thought to activate the endogenous opioid system and elicit the opioid-mediated form of stress-induced hypoalgesia³⁹.

In the present study, in order to not have the SIA mechanism activated while exercising, rats were habituated to the Rota-RodTM as proposed by Khan et al¹. Prior to testing, rats were habituated to walk against the rotating drum for 3 consecutive days. Across the 3 days, all the rats were able to walk for 180 seconds without failure. The habituation process is a proven manner of control the SIA in the rats while researching about EIH¹.

The results of the present study show that some specific opioid system's receptors (delta and kappa) have an important role in the analgesic effect of aerobic exercise. Since the

results showed that the EIH is partially reduced and not totally blocked after DOR and KOR antagonists injections, it is suggested that maybe this is not the only mechanism involved in EIH and maybe there is important interactions among opioid receptors when EIH phenomenon occurs.

The present study has limitations regarding the drug dosing and dose association. The use of a single fixed dose of antagonists is always a problem in pharmacological testing. A dose-response curve was not performed previously. Dose was set based on previous literature^{27, 28, 29}, but the ideal methods is to stratify the dose of the substances to be used in a pilot study and use the most effective dose in the study itself. Further studies with new doses of OR's antagonists and drug/dose association need to be performed since some opioids receptors in known to interact with each other³⁸.

It was concluded that rats with high EIH profile had a significant EIH reduction after injection of some specific opioid receptors antagonists (delta and kappa), but the EIH effect was just partially reduced, suggesting that others mechanism and/or opioids receptors interactions are involved in the EIH phenomenon. The present data may give a direction for further studies about development of new drugs for chronic pain treatment or complementary therapy. DOR agonists could improve pharmacological and psychological approaches to treat chronic pain. Increasing understanding of the phenomena of EIH would support therapeutic use of exercise to relieve pain.

REFERENCES

1. Khan J, Benavent V, Korczeniewska OA, Benoliel R, Eliav E: Exercise-Induced Hypoalgesia Profile in Rats Predicts Neuropathic Pain Intensity Induced by Sciatic Nerve Constriction Injury. *J Pain*, 2014
 2. Mollereau C, Parmentier M, Mailleux P, Butour JL, Moisand C, Chalon P, et al.: ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. *FEBS Lett* 341(1):33-8, 1994
 3. Benarroch EE: Endogenous opioid systems: current concepts and clinical correlations. *Neurology* 79(8):807-14, 2012
 4. Pradhan AA, Befort K, Nozaki C, Gaveriaux-Ruff C, Kieffer BL: The delta opioid receptor: an evolving target for the treatment of brain disorders. *Trends Pharmacol Sci* 32(10):581-90, 2011
-

5. Sauriyal DS, Jaggi AS, Singh N: Extending pharmacological spectrum of opioids beyond analgesia: multifunctional aspects in different pathophysiological states. *Neuropeptides* 45(3):175-88, 2011
 6. Overath CH, Darabaneanu S, Evers MC, Gerber WD, Graf M, Keller A, et al.: Does an aerobic endurance programme have an influence on information processing in migraineurs? *J Headache Pain* 15(1):11, 2014
 7. Deslandes A, Moraes H, Ferreira C, Veiga H, Silveira H, Mouta R, et al.: Exercise and mental health: many reasons to move. *Neuropsychobiology* 59(4):191-8, 2009
 8. Matta Mello Portugal E, Cevada T, Sobral Monteiro-Junior R, Teixeira Guimaraes T, da Cruz Rubini E, Lattari E, et al.: Neuroscience of exercise: from neurobiology mechanisms to mental health. *Neuropsychobiology* 68(1):1-14, 2013
 9. Black J, Chesher GB, Starmer GA, Egger G: The painlessness of the long distance runner. *Med J Aust* 1(11):522-3, 1979
 10. Gurevich M, Kohn PM, Davis C: Exercise-induced analgesia and the role of reactivity in pain sensitivity. *J Sports Sci* 12(6):549-59, 1994
 11. Haier RJ, Quaid K, Mills JC: Naloxone alters pain perception after jogging. *Psychiatry Res* 5(2):231-2, 1981
 12. Janal MN: Pain sensitivity, exercise and stoicism. *J R Soc Med* 89(7):376-81, 1996
 13. Janal MN, Colt EW, Clark WC, Glusman M: Pain sensitivity, mood and plasma endocrine levels in man following long-distance running: effects of naloxone. *Pain* 19(1):13-25, 1984
 14. Koltyn KF: Analgesia following exercise: a review. *Sports Med* 29(2):85-98, 2000
 15. O'Connor PJ, Cook DB: Exercise and pain: the neurobiology, measurement, and laboratory study of pain in relation to exercise in humans. *Exerc Sport Sci Rev* 27:119-66, 1999
 16. Kosek E, Lundberg L: Segmental and plurisegmental modulation of pressure pain thresholds during static muscle contractions in healthy individuals. *Eur J Pain* 7(3):251-8, 2003
 17. Hoeger Bement MK, Dicapo J, Rasiarmos R, Hunter SK: Dose response of isometric contractions on pain perception in healthy adults. *Med Sci Sports Exerc* 40(11):1880-9, 2008
 18. Hoeger Bement MK, Weyer A, Hartley S, Yoon T, Hunter SK: Fatiguing exercise attenuates pain-induced corticomotor excitability. *Neurosci Lett* 452(2):209-13, 2009
 19. Hoffman MD, Shepanski MA, Mackenzie SP, Clifford PS: Experimentally induced pain perception is acutely reduced by aerobic exercise in people with chronic low back pain. *J Rehabil Res Dev* 42(2):183-90, 2005
-

20. Whiteside A, Hansen S, Chaudhuri A: Exercise lowers pain threshold in chronic fatigue syndrome. *Pain* 109(3):497-9, 2004
 21. Staud R, Robinson ME, Price DD: Isometric exercise has opposite effects on central pain mechanisms in fibromyalgia patients compared to normal controls. *Pain* 118(1-2):176-84, 2005
 22. Kuphal KE, Fibuch EE, Taylor BK: Extended swimming exercise reduces inflammatory and peripheral neuropathic pain in rodents. *J Pain* 8(12):989-97, 2007
 23. Bement MK, Sluka KA: Low-intensity exercise reverses chronic muscle pain in the rat in a naloxone-dependent manner. *Arch Phys Med Rehabil* 86(9):1736-40, 2005
 24. Cobiauchi S, Marinelli S, Florenzano F, Pavone F, Luvisetto S: Short- but not long-lasting treadmill running reduces allodynia and improves functional recovery after peripheral nerve injury. *Neuroscience* 168(1):273-87, 2010
 25. Hutchinson KJ, Gomez-Pinilla F, Crowe MJ, Ying Z, Basso DM: Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats. *Brain* 127(Pt 6):1403-14, 2004
 26. Stagg NJ, Mata HP, Ibrahim MM, Henriksen EJ, Porreca F, Vanderah TW, et al.: Regular exercise reverses sensory hypersensitivity in a rat neuropathic pain model: role of endogenous opioids. *Anesthesiology* 114(4):940-8, 2011
 27. Guan Y, Johanek LM, Hartke TV, Shim B, Tao YX, Ringkamp M, et al.: Peripherally acting mu-opioid receptor agonist attenuates neuropathic pain in rats after L5 spinal nerve injury. *Pain* 138(2):318-29, 2008
 28. Ju J, Shin DJ, Na YC, Yoon MH: Role of spinal opioid receptor on the antiallodynic effect of intrathecal nociceptin in neuropathic rat. *Neurosci Lett* 542:118-22, 2013
 29. Obara I, Mika J, Schafer MK, Przewlocka B: Antagonists of the kappa-opioid receptor enhance allodynia in rats and mice after sciatic nerve ligation. *Br J Pharmacol* 140(3):538-46, 2003
 30. Lewis GN, Rice DA, McNair PJ: Conditioned pain modulation in populations with chronic pain: a systematic review and meta-analysis. *J Pain* 13(10):936-44, 2012
 31. Martel MO, Wasan AD, Edwards RR: Sex differences in the stability of conditioned pain modulation (CPM) among patients with chronic pain. *Pain Med* 14(11):1757-68, 2013
 32. Gackenhimer SL, Suter TM, Pintar JE, Quimby SJ, Wheeler WJ, Mitch CH, et al.: Localization of opioid receptor antagonist [3H]-LY255582 binding sites in mouse brain: comparison with the distribution of mu, delta and kappa binding sites. *Neuropeptides* 39(6):559-67, 2005
 33. Gaveriaux-Ruff C, Kieffer BL: Delta opioid receptor analgesia: recent contributions from pharmacology and molecular approaches. *Behav Pharmacol* 22(5-6):405-14, 2011
-

34. Lotsch J, Geisslinger G: Pharmacogenetics of new analgesics. *Br J Pharmacol* 163(3):447-60, 2011
 35. Koltyn KF: Exercise-induced hypoalgesia and intensity of exercise. *Sports Med* 32(8):477-87, 2002
 36. Kolesnikov Y, Jain S, Wilson R, Pasternak GW: Peripheral kappa 1-opioid receptor-mediated analgesia in mice. *Eur J Pharmacol* 310(2-3):141-3, 1996
 37. Simonin F, Gaveriaux-Ruff C, Befort K, Matthes H, Lannes B, Micheletti G, et al.: kappa-Opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology, and expression pattern in the central nervous system. *Proc Natl Acad Sci U S A* 92(15):7006-10, 1995
 38. Peppin JF, Raffa RB: Delta opioid agonists: a concise update on potential therapeutic applications. *J Clin Pharm Ther* 40(2):155-66, 2015
 39. Butler RK, Finn DP: Stress-induced analgesia. *Prog Neurobiol* 88(3):184-202, 2009
 40. Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, et al.: An endocannabinoid mechanism for stress-induced analgesia. *Nature* 435(7045):1108-12, 2005
 41. Parikh D, Hamid A, Friedman TC, Nguyen K, Tseng A, Marquez P, et al.: Stress-induced analgesia and endogenous opioid peptides: the importance of stress duration. *Eur J Pharmacol* 650(2-3):563-7, 2011
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3 Discussion

3 DISCUSSION

Both articles presented in this thesis aimed to evaluate the role of non-specific and specific opioids' receptors antagonists on the EIH levels produced by aerobic exercise in male rats. It is believed that, based on several studies, the endogenous opioid system is not the only, but the most important circuit activated following exercise. This study allowed an evaluation of any association between the effects of exercise on the rat's ability to modulate pain.

According to the International Association for the Study of Pain (IASP), pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is always subjective. Each individual learns to use the word "pain" through past experiences, related to injury in the early life. It is also always unpleasant and, therefore, also an emotional experience.

The sensory experience of pain is most commonly started by activation of primary afferent nociceptors. Nociceptors are free nerve ending afferents that deliver impulses through A-delta and C fibers to the CNS. The nociceptors will respond to a variety of stimuli. The noxious stimulus is converted to electrical signal (nerve impulse) and then, the transmission process of the noxious stimulus starts. The noxious stimulus travels to higher centers in brain. When the stimulus reaches the CNS, a synapse with the second order neurons in the CNS happens (De Leeuw and Klasser, 2013). Neurotransmitters released from primary afferent neurons are normally preceded by depolarization of the nerve terminal and calcium entry through voltage-sensitive calcium channels. Drugs may inhibit neurotransmitters' release by a direct effect on calcium channels to reduce calcium entry, or indirectly by increasing the outward potassium current, thus shortening repolarization time and the duration of the action potential. Opioids produce both of these effects because opioid receptors are apparently coupled via G-proteins directly to potassium channels and voltage-sensitive calcium channels. Opioids also interact with other intracellular effector mechanisms, the most important being the adenylate cyclase (second messenger) system (Dickenson, 1994).

During the transmission process, the excitatory and inhibitory mechanisms modulate nociceptive signals. Pain modulation is part of the normal function of CNS, where the pain signal can be enhanced, inhibited or remain the same at the brain stem level. When persistent nociceptive facilitation exceeds the inhibitory capacity, neuroplastic changes can occur peripherally or centrally known as peripheral sensitization and central sensitization

respectively. Pain is interpreted and perceived in the brain (cortex). Perception is the subjective response after processing of signals at higher centers (De Leeuw and Klasser, 2013).

Pain can be classified as acute or chronic. As cited by Iadarola and Caudle, in a paper published in 1997, in *Science* magazine, there is the “good” pain (acute pain) that has an important protective function in the preservation of the organism, and there is the pain that arises when the “good” pain turns into the “bad” pain (chronic pain) that occurs in pathophysiological conditions such as nerve injury, causing debilitating disease (Iadarola; Caudle, 1997). Inflammatory and neuropathic pain are characterized by altered function of the nervous system as a result of persistent pathology or neuroplasticity changes (Sharav and Benoliel, 2008) initiating the “bad”, chronic, pain.

The standard definition of chronic pain, endorsed by IASP, states that it is the pain that persists past the healing phase following an injury. Chronic pain is, therefore, a disease itself and often not a symptom. It has now become clear that the majority of cases of chronic pain can be explained by alterations in CNS processing of incoming messages (Yunus, 2007). Additionally, chronic pain responds to therapy differently from the acute condition and is associated with emotional and social behavioral changes. The inability to perceive chronic pain as a disease may result in repeated and unsuccessful interventions, all in attempt to eradicate the cause of pain (Sharav and Benoliel, 2008).

Orofacial pain disorders are highly prevalent and debilitating conditions involving the head, face, and neck. This condition has been considered by pain experts, as a challenge to the clinician, since the orofacial region is complex and therefore, pain can arise from many sources. According to Sharav, (2005), diagnosis and treatment of chronic orofacial pain have acquired central roles in today’s modern dental practice. The density of anatomical structures, mechanisms of referred pain, and the underlying systemic and psychological pathology complicate diagnosis and treatment (Sharav, 2005).

The cost of chronic pain is in the billions of dollars annually in the United States for health care services, loss of work and work-days, decreased productivity, and disability compensation. New scientific evidence is constantly providing insight into the cause and pathophysiology of orofacial pain. An evidence-based approach to the management of orofacial pain is extremely necessary for the general clinician (De Rossi, 2013).

Turk *et al.*, (2011) stated that pain is one of the most common medical complaints, but despite its prevalence many individuals still suffer with unrelieved or undertreated pain. The author states that although massive efforts coming from basic science and clinical research, inadequate pain management remains a clinical challenge. Moreover, there is a lack of real breakthrough innovation in the field of pain over the past years. Thus, there is a need for new drugs, with new mechanisms of action, which could increase the efficacy of existing therapies and reduce their unwanted effects (Turk *et al.*, 2011). The same can be said about orofacial pain, giving more attention to the chronic state of it. It has been widely accepted that the management of chronic orofacial pain should approach pharmacological and non-pharmacological therapies. Exercise is an easy and low-cost activity, used as part of the management of these chronic pain conditions.

Clinician needs to have a solid knowledge of the pain conditions and pain mechanisms for proper diagnosis and management (Romero-Reyes and Uyanik, 2014). As stated by Glaros and colleagues (2005), patients will benefit from the care of clinicians who assess both biological and behavioral/psychological factors and use both sets of data in generating a treatment plan for patients who report chronic pain (Glaros *et al.*, 2005).

It has been demonstrated that the aerobic exercise may increase or optimize level of several neurotransmitters such as serotonin, dopamine, acetylcholine and norepinephrine, activates the endocannabinoid system and endogenous opioid system (Deslandes *et al.*, 2009; Matta Mello Portugal *et al.*, 2013). Opioid peptides and their receptors have a widespread, but selective distribution in the central and peripheral nervous systems, particularly in circuits involved in pain modulation, reward, responses to stress, and autonomic control (Benarroch, 2012). Researches in pharmacological and genetic area are always trying to clarified the specific role of each opioid receptor in many aspects of opioid-related behaviors, physiology and disorders (Pradhan *et al.*, 2011). The opioid system has an important role in mechanisms of analgesia; reward-mediating food intake and drug addiction; and modulation of emotion and stress responses.

Koltyn, K. F., in 2000, published a review article about analgesia following exercise in humans and animals. According to the author, studies of exercise and analgesia started when researches observed that dancers and athletes could continue strenuous exercise in the face of severe injuries, and later reported that they felt no pain. It has contributed to the notion that exercise can alter pain perception. Over the past 30 years, a number of studies have examined

whether analgesia occurred following exercise. The most common laboratory tests used are noxious stimuli to produce pain, including electrical, ischemic, temperature and pressure stimulation, applied before and following exercise to observe if analgesia occurs, generally, following aerobic exercise (Koltyn, 2000).

Animal studies demonstrate the phenomena of analgesia following exercise and the involvement of the endogenous opioid system. In the review cited above, Koltyn observed that most of the researches investigated whether EIH is mediated by endogenous opioid mechanisms. Swimming is the predominant exercise stimulus used in the animal research (Koltyn, 2000). The author exposed a great number of animal studies involving EIH and swimming. In summary, the author found that most of the researches uses a forced swimming protocol. A question has arisen regarding whether the analgesia produced following swimming is a result of the stressful nature of forced swimming itself or secondary to changes in body temperature while swimming. According to the author, and based on animal research, it appears that multiple analgesia systems exist (opioid and non-opioid) and that properties of the exercise stressor are important in determining which system is activated during exercise (Koltyn 2000, 2002).

In the present study, the Low EIH profile rats had, after injection of naltrexone, an increased pain perception, leading to a thinking of increasing in pain response after blocking the opioid system. This can be a reflection of how some animals have the pain modulation system altered even being healthy subjects. Exercise-related pain may occur from injuries during exercise. Also, exercise can acutely exacerbate pre-existing pain from medical conditions. However, even healthy people may experience pain from exercise (Dannecker and Koltyn, 2014).

The difference between the EIH before and after injection in the Low EIH profile rats in the present study was not significant, but still it is a tendency to extrapolate this result to humans, as a predictive data to calculate if the patient can or cannot have a benefit from exercise. It has been proposed that, since exercise itself may be painful, it can inhibit other pain sensations and activates descending pain inhibitory system, working in accordance to CPM, even though EIH is not the same as CPM. Recently, Vaegter, Handber and Graven-Nielsen (2015), conducted a study to compare CPM and EIH in chronic musculoskeletal pain patients with high widespread pain sensitivity, compared with those with low widespread pain sensitivity. They found that, even though the groups had similar age, gender distribution,

duration of pain, number of pain sites, clinical pain intensity and mood, the CPM and EIH responses were partly impaired in patients with high pain sensitivity. At baseline, temporal summation (an increase in pain perception upon repetitive stimulus applied at constant intensity) of pain was more pronounced in patients with high pain sensitivity and it was further facilitated after aerobic exercise. Furthermore, the CPM response was predicted by clinical peak pain intensity, which highlights the importance of ongoing pain in the process of pain inhibition. The EIH response after aerobic exercise was predicted by the CPM response after cold test. Pain tolerance and widespread pressure pain threshold were increased in response to the cold test and exercises in both groups. The findings of the present study have important clinical implications as the effect of management strategies, like aerobic exercise, may differ depending on the degree of widespread pain sensitivity. This may also be the case for other pain management strategies, e.g. pharmacological treatment utilizing the pain inhibitory systems (Vaegter *et al.* 2015).

Ellingson *et al.*, (2013) performed a study with sixteen women to investigate the role of conditioned pain modulation (CPM) as a potential mechanism of EIH. Women completed a pain testing during three sessions: painful exercise, non-painful exercise, and quiet rest. Intensity and unpleasantness ratings to noxious heat stimuli were assessed at baseline, during and following each session. Results showed that the analgesic response was greater following painful exercise than non-painful exercise. They concluded that EIH might involve the triggering of CPM via exercise-induced muscle pain. The greater the exercise-induced leg muscle pain, the greater the analgesic effect (Ellingson *et al.*, 2013).

CPM and EIH are not identical phenomena (Vaegter *et al.*, 2014), however they may share same mechanisms including activation of the opioid descending inhibitory pathways. It is assumed that the conditioning painful stimulus delivered to induce CPM and the exercise in EIH activates similar inhibitory pain modulatory systems. There is association between EIH profile and pain sensitivity in healthy pain-free subjects (Khan *et al.*, 2014). However, regarding the effect of exercise on patients with faulty modulation systems, study should be done.

The endogenous opioid system is diverse in both distribution (central and peripheral) and function. Multiple opioid receptors and their cognate ligands mediate or modulate, either directly or indirectly, a wide spectrum of physiological processes. This includes afferent sensory transmission (via ascending pathways) and efferent modulation (via descending

pathways) of the pain sensation/perception system, and key roles in the gastrointestinal, endocrine, respiratory and immune systems; the stress response; and the homeostasis of mood (anxiety/depression). The complexity of activities is orchestrated by the subset of opioid receptors. Among these, the delta opioid receptor is the least explored and targeted for therapeutic utility (Peppin and Raffa, 2015). It now appears that delta opioid agonists might be more studied and the results of our study showed that. DOR can be a target to chronic pain conditions as a hypoalgesia inducer during aerobic exercise.

Selective KOR antagonists may have therapeutic potential against conditions such as depression and anxiety disorders. The best-established agents in this class are nor-BNI, GNTI and JDTic. In vitro, these compounds are potent and selective antagonists at the KOR with much lower potency at MOR or DOR. They are therefore generally considered k-selective, but little evidence is available on binding and activity at other receptors, ion channels, transporters and enzymes. The pharmacodynamics of these compounds in vivo differs dramatically from other opioid antagonists. The KOR antagonism may be delayed by hours or days, compared to minutes for competitive antagonists such as naloxone (Munro *et al.*, 2013). In the present study, KOR antagonist used was GNTI. The EIH test started 15 minutes after GNTI injection. The results affecting EIH after the KOR antagonist injection was significant but did not blocked the EIH phenomenon in 100%, as almost happened to DOR antagonist. However, we observed that, at 10 minutes following exercise, there was a significant reduction of the EIH in the high EIH rats injected with KOR ($p=0.012$) antagonist's drugs. This may be due to what was cited above, that GNTI could start acting later than 15 minutes. Other transient effects of GNTI are of rapid onset and brief duration such as scratching (Munro *et al.*, 2013), which could distract the rat from the Von Frey test (used for EIH calculation) leading him to respond differently for the stimuli.

In the present study, the rats' ability to modulate pain via EIH was evaluated with the percentage of responses (paw withdrawal from the stimuli) to successive mechanical stimuli using a 60g force von Frey monofilament. Stimuli were applied in the left rat's mid-plantar hind paw. Each rat was exposed to 30 successive stimuli at the rate of approximately 1 stimulus per second and the percentage of response was calculated for each time studied. The percentage of response were calculated at a baseline before exercise, and re-evaluated within 1 minute, and again at 5, 10, and 20 minutes following the cessation of exercise. The most important results for EIH before injection in the present study were obtained within 1 minute following the 3 minutes of exercise in the Rota-Rod. Our findings suggest that there is a

minimum duration of exercise required eliciting EIH in rats and the results suggested that there is evidence that the EIH effect lasts more than 20 minutes following exercise. Its results and conclusions obtained with our study is in accordance to Hoffman *et al.*, (2004) and Koltyn *et al.*, (1996) studies in which the alterations in pain perception after exercise appeared to be transient. The author found that EIH lasted at least 15 minutes after aerobic exercise (Hoffman *et al.*, 2004; Koltyn *et al.*, 1996). In Hoffman *et al.*, (2004) study, the results showed an analgesic effect at 5 minutes after exercise, but the effect was not significant 30 minutes after aerobic exercise. The magnitude and duration of the analgesic effect from exercise varies among studies. Although O'Conner and Cook (1999) stated in their review article that the effect of exercise appears to be most reliable following high intensity exercise. Moreover, there has not been a systematic investigation of the duration and intensity of aerobic exercise that is necessary to produce an acute analgesic effect (O'Connor and Cook, 1999).

It is important to understand whether exercise can be used to control pain in people with chronic pain. It is not clear if these people have the same analgesic response to exercises as do healthy individuals. The present study was performed using healthy male rats (animal model), that not received a noxious stimulus. It is difficult to extrapolate the results for healthy humans (male or female). It is unclear whether individuals with chronic pain would experience similar results with exercise as healthy young adults. It would be important to determine if the events from EIH observed in the present study could happen the same way on a population with pain, especially chronic pain conditions.

The present study findings may help understand the mechanisms involved in EIH phenomenon. Finally, although there is EIH, it would be interesting study the intensities and durations and types of exercises that elicit EIH. Additional research is needed to clarify and expand the understanding of the mechanisms responsible for EIH and to determine how it can be used in chronic pain conditions such as low back pain, arthritic pain, fibromyalgia, headache and orofacial pain.

4 Conclusion

4 CONCLUSIONS

Based on the results presented in the articles, it can be concluded that:

1. Opioid system is activated following aerobic exercise in male rats;
 2. EIH effect is just partially reduced after opioid system blocking, suggesting that others mechanisms are involved in the EIH phenomenon;
 3. Male rats with High EIH profile had more pronounced reduction on hypoalgesia following exercise after injection of non-specific OR's antagonist drug (naltrexone hydrochloride);
 4. Rats with High EIH profile are more affected by blocking DOR and KOR with specific antagonists drugs.
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References

REFERENCES

- Allegri M, Clark MR, De Andres J, Jensen TS: Acute and chronic pain: where we are and where we have to go. *Minerva Anesthesiol* 78(2):222-35, 2012
- Bement MK, Sluka KA: Low-intensity exercise reverses chronic muscle pain in the rat in a naloxone-dependent manner. *Arch Phys Med Rehabil* 86(9):1736-40, 2005
- Benarroch EE: Endogenous opioid systems: current concepts and clinical correlations. *Neurology* 79(8):807-14, 2012
- Black J, Chesher GB, Starmer GA, Egger G: The painlessness of the long distance runner. *Med J Aust* 1(11):522-3, 1979
- Dannecker EA, Koltyn KF: Pain during and within hours after exercise in healthy adults. *Sports Med* 44(7):921-42, 2014
- de Leeuw R, Klasser GD. Orofacial Pain: Guidelines for assessment, diagnosis, and management. American Academy of Orofacial Pain. 5th ed. Chicago Quintessence Publishing; 2013.
- De Rossi SS: Orofacial pain: a primer. *Dent Clin North Am* 57(3):383-92, 2013
- Deslandes A, Moraes H, Ferreira C, Veiga H, Silveira H, Mouta R, et al.: Exercise and mental health: many reasons to move. *Neuropsychobiology* 59(4):191-8, 2009
- Dietis N, Rowbotham DJ, Lambert DG: Opioid receptor subtypes: fact or artifact? *Br J Anaesth* 107(1):8-18, 2011
- Dietrich A, McDaniel WF: Endocannabinoids and exercise. *Br J Sports Med* 38(5):536-41, 2004
- Dinler M, Diracoglu D, Kasikcioglu E, Sayli O, Akin A, Aksoy C, et al.: Effect of aerobic exercise training on oxygen uptake and kinetics in patients with fibromyalgia. *Rheumatol Int* 30(2):281-4, 2009
- Ellingson LD, Koltyn KF, Kim JS, Cook DB: Does exercise induce hypoalgesia through conditioned pain modulation? *Psychophysiology*, 2013
-

Galdino G, Romero TR, Silva JF, Aguiar DC, de Paula AM, Cruz JS, et al.: The endocannabinoid system mediates aerobic exercise-induced antinociception in rats. *Neuropharmacology* 77:313-24, 2014

Glaros AG, Williams K, Lausten L: The role of parafunctions, emotions and stress in predicting facial pain. *J Am Dent Assoc* 136(4):451-8, 2005

Gurevich M, Kohn PM, Davis C: Exercise-induced analgesia and the role of reactivity in pain sensitivity. *J Sports Sci* 12(6):549-59, 1994

Haier RJ, Quaid K, Mills JC: Naloxone alters pain perception after jogging. *Psychiatry Res* 5(2):231-2, 1981

Hauser W, Klose P, Langhorst J, Moradi B, Steinbach M, Schiltenswolf M, et al.: Efficacy of different types of aerobic exercise in fibromyalgia syndrome: a systematic review and meta-analysis of randomised controlled trials. *Arthritis Res Ther* 12(3):R79, 2010

Hoeger Bement MK, Dicapo J, Rasiarmos R, Hunter SK: Dose response of isometric contractions on pain perception in healthy adults. *Med Sci Sports Exerc* 40(11):1880-9, 2008

Hoeger Bement MK, Weyer A, Hartley S, Yoon T, Hunter SK: Fatiguing exercise attenuates pain-induced corticomotor excitability. *Neurosci Lett* 452(2):209-13, 2009

Hoffman MD, Shepanski MA, Mackenzie SP, Clifford PS: Experimentally induced pain perception is acutely reduced by aerobic exercise in people with chronic low back pain. *J Rehabil Res Dev* 42(2):183-90, 2005

Hoffman MD, Shepanski MA, Ruble SB, Valic Z, Buckwalter JB, Clifford PS: Intensity and duration threshold for aerobic exercise-induced analgesia to pressure pain. *Arch Phys Med Rehabil* 85(7):1183-7, 2004

Iadarola JM, Caudle RM: Good pain, bad pain. *Science* 278(5336):239-40, 1997

Janal MN: Pain sensitivity, exercise and stoicism. *J R Soc Med* 89(7):376-81, 1996

Janal MN, Colt EW, Clark WC, Glusman M: Pain sensitivity, mood and plasma endocrine levels in man following long-distance running: effects of naloxone. *Pain* 19(1):13-25, 1984

- Khan J, Benavent V, Korczeniewska OA, Benoliel R, Eliav E: Exercise-Induced Hypoalgesia Profile in Rats Predicts Neuropathic Pain Intensity Induced by Sciatic Nerve Constriction Injury. *J Pain*, 2014
- Koltyn KF: Analgesia following exercise: a review. *Sports Med* 29(2):85-98, 2000
- Koltyn KF: Exercise-induced hypoalgesia and intensity of exercise. *Sports Med* 32(8):477-87, 2002
- Koltyn KF, Garvin AW, Gardiner RL, Nelson TF: Perception of pain following aerobic exercise. *Med Sci Sports Exerc* 28(11):1418-21, 1996
- Kosek E, Lundberg L: Segmental and plurisegmental modulation of pressure pain thresholds during static muscle contractions in healthy individuals. *Eur J Pain* 7(3):251-8, 2003
- Lovick TA: Integrated activity of cardiovascular and pain regulatory systems: role in adaptive behavioural responses. *Prog Neurobiol* 40(5):631-44, 1993
- Martins DF, Mazzardo-Martins L, Soldi F, Stramosk J, Piovezan AP, Santos AR: High-intensity swimming exercise reduces neuropathic pain in an animal model of complex regional pain syndrome type I: evidence for a role of the adenosinergic system. *Neuroscience* 234:69-76, 2013
- Matta Mello Portugal E, Cevada T, Sobral Monteiro-Junior R, Teixeira Guimaraes T, da Cruz Rubini E, Lattari E, et al.: Neuroscience of exercise: from neurobiology mechanisms to mental health. *Neuropsychobiology* 68(1):1-14, 2013
- Melzack R, Wall PD: Pain mechanisms: a new theory. *Science* 150(3699):971-9, 1965
- Munro TA, Huang XP, Inglese C, Perrone MG, Van't Veer A, Carroll FI, et al.: Selective kappa opioid antagonists nor-BNI, GNTI and JDTC have low affinities for non-opioid receptors and transporters. *PLoS One* 8(8):e70701, 2013
- O'Connor PJ, Cook DB: Exercise and pain: the neurobiology, measurement, and laboratory study of pain in relation to exercise in humans. *Exerc Sport Sci Rev* 27:119-66, 1999
- Overath CH, Darabaneanu S, Evers MC, Gerber WD, Graf M, Keller A, et al.: Does an aerobic endurance programme have an influence on information processing in migraineurs? *J Headache Pain* 15(1):11, 2014
-

Peppin JF, Raffa RB: Delta opioid agonists: a concise update on potential therapeutic applications. *J Clin Pharm Ther* 40(2):155-66, 2015

Pradhan AA, Befort K, Nozaki C, Gaveriaux-Ruff C, Kieffer BL: The delta opioid receptor: an evolving target for the treatment of brain disorders. *Trends Pharmacol Sci* 32(10):581-90, 2011

Romero-Reyes M, Uyanik JM: Orofacial pain management: current perspectives. *J Pain Res* 7:99-115, 2014

Sharav Y: Orofacial pain: How much is it a local phenomenon? *JADA* 136(April):432-3, 2005

Sharav Y, Benoliel R. The diagnostic process. In: Sharav Y, editor. *Orofacial Pain and Headache*: Mosby Elsevier; 2008. p. 1-17.

Stagg NJ, Mata HP, Ibrahim MM, Henriksen EJ, Porreca F, Vanderah TW, et al.: Regular exercise reverses sensory hypersensitivity in a rat neuropathic pain model: role of endogenous opioids. *Anesthesiology* 114(4):940-8, 2011

Staud R, Robinson ME, Price DD: Isometric exercise has opposite effects on central pain mechanisms in fibromyalgia patients compared to normal controls. *Pain* 118(1-2):176-84, 2005

Turk DC, Wilson HD, Cahana A: Treatment of chronic non-cancer pain. *Lancet* 377(9784):2226-35, 2011

Vaegter HB, Handberg G, Graven-Nielsen T: Similarities between exercise-induced hypoalgesia and conditioned pain modulation in humans. *Pain* 155(1):158-67, 2014

Vaegter HB, Handberg G, Graven-Nielsen T, Edwards R: Hypoalgesia After Exercise and Cold Pressor Test are Reduced in Chronic Musculoskeletal Pain Patients with High Pain Sensitivity. *Clin J Pain*, 2015

Whiteside A, Hansen S, Chaudhuri A: Exercise lowers pain threshold in chronic fatigue syndrome. *Pain* 109(3):497-9, 2004

Yunus MB: Fibromyalgia and overlapping disorders: the unifying concept of central sensitivity syndromes. *Semin Arthritis Rheum* 36(6):339-56, 2007