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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

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Abstract—This study investigated the involvement of the adenosinergic system in antiallodynia induced by exercise in an animal model of complex regional pain syndrome type I (CRPS-I). Furthermore, we analyzed the role of the opioid receptors on exercise-induced analgesia. Ischemia/reperfusion (IR) mice, nonexercised and exercised, received intraperitoneal injections of caffeine (10 mg/kg, a non selective adenosine receptor antagonist), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (0.1 mg/kg, a selective adenosine A_1 receptor antagonist), ZM241385 (3 mg/kg, a selective adenosine A_{2A} receptor antagonist), adenosine deaminase inhibitor erythro-9-(2-hydroxy-3nonyl) adenine [(EHNA), 5 mg/kg, an adenosine deaminase inhibitor] or naloxone (1 mg/kg, a nonselective opioid receptor antagonist). The results showed that high-intensity swimming exercise reduced mechanical allodynia in an animal model of CRPS-I in mice. The antiallodynic effect caused by exercise was reversed by pretreatment with caffeine, naloxone, DPCPX but it was not modified by ZM241385 treatment. In addition, treatment with EHNA, which suppresses the breakdown of adenosine to inosine, enhanced the pain-relieving effects of the high-intensity swimming exercise. This is the first report demonstrating that repeated sessions of high-intensity swimming exercise attenuate mechanical allodynia in an animal model of CRPS-I and that the mechanism involves endogenous adenosine and adenosine A_1 receptors. This study supports the use of high-intensity exercise as an adjunct therapy for CRPS-I treatment.

Key words: adenosine, analgesia, caffeine, CRPS-I, exercise.

INTRODUCTION

Chronic pain is a major health problem and one of the most frequent reasons for seeking medical care (Gureje et al., 1998; Schappert and Burt, 2006). Depending on its origin, chronic pain can be classified as inflammatory or neuropathic. Complex regional pain syndrome-I (CRPS-I) is a severe, disabling, and painful disease that may occur in an extremity after a trauma or injury; clinical features include spontaneous and stimulus-evoked pain, edema, vasomotor and sudomotor disturbances, motor dysfunction, and trophic changes. It is recognized as being difficult to treat, despite various methods of treatment being available, including physiotherapy, calcitonin, corticosteroids, sympathetic blockade, and non-steroidal anti-inflammatory drugs (Hord and Oaklander, 2003).

During exercise, there is a high rate of ATP breakdown, which results in high tissue levels of adenosine (Bockman et al., 1975; Dworak et al., 2007; Roque et al., 2011). Many cell types produce adenosine, including neurons (Phillis, 1989), heart (Bacchus et al., 1982) and skeletal muscle (Bockman et al., 1975). Thus adenosine can overwhelm the purine catabolic pathway in the cell and the excess moves out, accumulating in the extracellular space and blood. High blood and extracellular levels of adenosine give this purine access to extracellular regulatory adenosine receptors, influencing the mechanisms of angiogenesis, blood flow, systolic blood pressure, respiration, plasma norepinephrine and epinephrine levels, through which it may exert various regulative effects on the body's complex adaptation response to exercise. Adenosine has a half-life in the human blood of about 10 s (Klabunde, 1983) and can exert its effect through four distinct adenosine receptors (ARs) (A_1, A_{2A}, A_{2B} and A_3), which are G-protein-coupled receptors. It is reformed into ATP via adenosine kinase, while excess

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Abbreviations: ANOVA, analysis of variance; AR, adenosine receptor; CPIP, chronic post-ischemic pain; CRPS-I, complex regional pain syndrome type I; DMSO, dimethylsulfoxide; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; EHNA, erythro-9-(2-hydroxy-3nonyl) adenine; IMP, inosine monophosphate; IR, ischemia and reperfusion; SEM, standard error of the mean; ZM241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)-phenol.

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amounts are enzymatically converted via adenosine deaminase in the purine catabolic pathway sequence, to inosine, hypoxanthine, xanthine and finally uric acid (Jacobson and Gao, 2006). Furthermore, previous studies have shown that ATP and adenosine regulate pain transmission by acting at supraspinal, spinal and peripheral sites (Sawynok, 1998, 1999, 2013), and may play an important role in inflammatory and neuropathic pain (Dickenson et al., 2000).

Our previous study has shown that high-intensity swimming exercise reduces pain-related behavior by the activation of opioidergic and serotonergic receptors in mice (Mazzardo-Martins et al., 2010). In addition, it has been shown that adenosine A_1R and $A_{2A}R$ subtypes are involved in the antinociception caused by adenosine and inosine (Goldman et al., 2010; Sawynok, 1998; Nascimento et al., 2010). Considering that (1) high-intensity exercise increases the concentration of adenosine and inosine in the blood, and (2) adenosine receptor activation induces analgesia, we hypothesized that exercise-induced analgesia could be mediated by the activation of adenosine receptors. Therefore, the present study investigated, through pharmacological behavioral tests, the involvement of adenosinergic systems in exercise-induced analgesia in an animal model of CRPS-I.

EXPERIMENTAL PROCEDURES

Animals

All animal care and experimental procedures were carried out in accordance with the National Institutes of Health Animal Care Guidelines (NIH publications No. 80-23), and were approved by the Ethics Committee of the Universidade Federal de Santa Catarina – UFSC (protocol number PP00640). The present study employed male Swiss mice (25–35 g) obtained from the UFSC. Animals were housed under a 12-h light/12-h dark cycle (lights on at 6:00 a.m.) in a room with controlled temperature (22 ± 2°C), and given food and water *ad libitum*. Animals were habituated to the laboratory conditions for at least 1 h before testing. Experiments were performed between 8:00 am and 12:00 am. The numbers of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects observed from drug treatments.

Drugs

The following substances were used: dimethylsulfoxide (DMSO) (Merck, Darmstadt, Germany), chloral hydrate (Vetec; São Paulo, Brazil), caffeine, erythro-9-(2-hydroxy-3nonyl) adenine (EHNA) and naloxone hydrochloride (Sigma Chemical Co., USA). Chloral hydrate, caffeine and naloxone were immediately dissolved in saline (0.9% NaCl solution) used as vehicle. 1,3-Dipropyl-8-cyclopentylxanthine (DPCPX), 4-(2-[7-amino-2-(2-furyl)[1,2, 4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)-phenol (ZM241385) (Tocris Bioscience, Ellisville, MO) and EHNA were dissolved in saline with DMSO. The final concentration of DMSO did not exceed 2.5% and did not cause any effect *per se*. In addition, a constant volume of saline or vehicle (saline plus 2.5% DMSO) was also injected simultaneously. The doses of all substances employed were chosen based on literature data (Mazzardo-Martins et al., 2010; Nascimento et al., 2010; Sawynok et al., 2010; Martins et al., 2012) or were selected from preliminary experiments conducted in our laboratory.

Animal model of complex regional pain syndrome type-I

Chronic post-ischemic pain (CPIP) is an animal model of CRPS-I developed using a 3-h ischemia-reperfusion injury of the rodent hind paw. CPIP induction was performed as described previously (Millecamps et al., 2010; Nucci et al., 2012). CPIP mice were generated following exposure to prolonged hind paw ischemia and reperfusion (IR). Mice were anesthetized over a 3-h period with a bolus (7%, 0.6 ml/kg, i.p.) of chloral hydrate and 20% of the initial volume at the end of the first and second hour. After induction of anesthesia, an elastic O-ring for braces (ElásticoLigadura 000-1237, Uniden, SP, Brazil) with 1.2-mm internal diameter was placed around the right hind limb just proximal to the ankle joint. The O-rings were selected to provide a tight-fit that produced ischemia. They were left on the limb for 3 h as initially described with larger O-rings. The O-ring was always positioned at a point on the limb just proximal to the medial malleolus and its application was standardized by sliding it off the outside of a 100 μL pipette tip (that had 4 mm of the larger end cut) after the hind paw was inserted into the pipette as far as possible. Sham mice received exactly the same procedure except that the O-ring was cut so that it only loosely surrounded the ankle and did not occlude blood flow to the right hind paw.

High-intensity swimming exercise protocol

The high-intensity swimming exercise protocol was performed as described previously by Mazzardo-Martins et al. (2010). Our group has previously showed that this protocol increases blood levels of lactate reaching 7.84 ± 1.40 mmol/L at 30 min of exercise at the end of the last day of the exercise protocol (Mazzardo-Martins et al., 2010), indicating that this is a high-intensity exercise. Animals were placed in a plastic box divided in eight compartments (170 x 100 mm), filled with 35 L of water at 37°C. Liquid soap (8 mL) was added to reduce surface tension and to abolish the ''floating'' behavior. After each exercise session, animals were gently dried with a cloth towel. Control (nonexercised) mice were allowed to swim for only 30 s each day and were then gently dried (see protocol in Fig. 1). Mice were randomized to nonexercised or exercised groups. Five days after hind paw ischemia and reperfusion (IR), exercised mice were exposed to water for 30 s twice and for 2 min twice on the sixth day. Mice were thus acclimated to the new environment. On the seventh and eighth days, the animals were submitted to intermittent exercise. They swam for 10 min and had a 5-min rest (3 times, 30 min of exercise) on the seventh day; swam for 15 min and had a 5-min rest on the eight day (2 times, 30 min of exercise). In total, mice performed

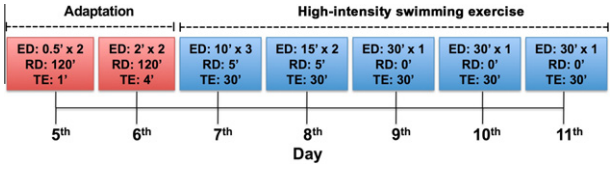

Fig. 1. Timeline of the exercise. In the fifth day after hind paw ischemia and reperfusion, mice exercised were exposed to water for 30 s (0.5 min) twice and for 2 min twice on the sixth day. Mice were thus acclimated to the new environment. On the seventh and eighth days, the animals were submitted to intermittent exercise. They swam for 10 min and had a 5-min rest on the third day; swam for 15 min and had a 5-min rest on the fourth day. In total, mice performed 30 min of swimming exercise. On the ninth, tenth and eleventh days the animals swam continuously for 30 min. ED, exercise duration; RD, rest duration; TE, total exercise.