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MUSCLE PAIN INDUCED BY STATIC CONTRACTION IN RATS IS MODULATED BY PERIPHERAL INFLAMMATORY MECHANISMS

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Abstract—Muscle pain is an important health issue and frequently related to static force exertion. The aim of this study is to evaluate whether peripheral inflammatory mechanisms are involved with static contraction-induced muscle pain in rats. To this end, we developed a model of muscle pain induced by static contraction performed by applying electrical pulses through electrodes inserted into muscle. We also evaluated the involvement of neutrophil migration, bradykinin, sympathetic amines and prostanoids. A single session of sustained static contraction of gastrocnemius muscle induced acute mechanical muscle hyperalgesia without affecting locomotor activity and with no evidence of structural damage in muscle tissue. Static contraction increased levels of creatine kinase but not lactate dehydrogenase, and induced neutrophil migration. Dexamethasone (glucocorticoid anti-inflammatory agent), DALBK (bradykinin B1 antagonist), Atenolol (β1 adrenoceptor antagonist), ICI 118,551 (β2 adrenoceptor antagonist), indomethacin (cyclooxygenase inhibitor), and fucoidan (non-specific selectin inhibitor) all reduced static contraction-induced muscle hyperalgesia; however, the bradykinin B2 antagonist, bradyzide, did not have an effect on static contraction-induced muscle hyperalgesia. Furthermore, an increased hyperalgesic response was observed when the selective bradykinin B1 agonist des-Arg^9-bradykinin was injected into the previously stimulated muscle. Together, these findings demonstrate that static contraction induced mechanical muscle hyperalgesia in gastrocnemius muscle of rats is modulated through peripheral inflammatory mechanisms that are dependent on neutrophil migration, bradykinin, sympathetic amines and prostanoids. Considering the clinical relevance of muscle pain, we propose the present model of static contraction-induced mechanical muscle hyperalgesia as a useful tool for the study of mechanisms underlying static contraction-induced muscle pain.

Key words: static contraction, hyperalgesia, muscle, inflammation, mediators.

INTRODUCTION

Muscle pain is one of the major contributors to years lived with disability in the world (GBD, 2015). Work-related muscle disorders account for 29% of all illnesses (Gerr et al., 2014) that required absence from work in the USA. In Canada, such disorders account for up to 60% of all illnesses (WorkSafeBC, 2009; Silverstein and Evanoff, 2011). Pain has an important socioeconomic impact and represents a cost of more than $600 billion per year in the USA alone (Gaskin and Richard, 2012).

Muscle pain is frequently associated with occupational and daily life activities that require carrying loads and/or static force exertion (Luttmann et al., 2003). In the short-term, this may primarily lead to acute episodes of pain whereas long-term exposure may cause more chronic disorders (Luttmann et al., 2003). Specifically, static contraction (sustained isometric contraction) has been associated with different pain conditions. Sustained activity of the trapezius muscle is one of the mechanisms for work-related neck and shoulder pain (Sjogaard et al., 2000; Boix et al., 2005; Strom et al., 2009a; Eijckelhof et al., 2013; Hanvold et al., 2013). Prolonged static contraction of low back muscle in a sitting posture is related to low back disorders and pain in dental occupations (Valachi and Valachi, 2003). Low-intensity static contraction is the primary cause of back pain during bedrest (Baum and Essfeld, 1999). Static contraction of a muscle in a patient with chronic myalgia – or in patients with fibromyalgia – induces a marked increase in pain (Roe et al., 2000; Umeda et al., 2015). In addition, static contraction is sometimes used in human studies as a model of muscle pain (Elcadi et al., 2013). In spite of its clinical relevance, the mechanism underlying static contraction-induced muscle pain is poorly understood.

Usually, muscle pain is associated with inflammation. Classical mediators, such as bradykinin (Boix et al., 2005), prostaglandins (Hedenberg-Magnusson et al., 2001; Tegeder et al., 2002), ATP (Mense, 2009; Schiavuzzo et al., 2015), pro-inflammatory cytokines

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(Schafers et al., 2003; Loram et al., 2007; Dessem et al., 2010) and 5-hydroxytrypamine (Christidis et al., 2005) are involved with muscle pain. However, the mechanisms underlying muscle pain are dependent on its etiology. It has previously been shown that TRPV1 receptors are involved with eccentric exercise-induced muscle pain but not with carrageenan-induced muscle pain (Fujii et al., 2008). Carrageenan-induced muscle pain promotes a greater neutrophil migration to muscle tissue than eccentric exercise (Fujii et al., 2008; Kanda et al., 2013), while fatigue-induced muscle pain is not associated with this migration (Gregory et al., 2013). Pro-inflammatory cytokines modulate carrageenan but not eccentric exercise-induced muscle pain (Kanda et al., 2013). More specifically, increased levels of IL-6 (interleukin-6) and IL-8 (interleukin-8) are correlated with pain in patients with fibromyalgia (Mendieta et al., 2016). ASIC3 receptors expressed on muscle afferents modulate carrageen-induced muscle pain (Sluka et al., 2007; Walder et al., 2011), but not fatigue-induced pain, which is probably modulated by the ASIC3 expressed on muscle macrophages (Gregory et al., 2016).

Considering the clinical relevance of static contraction-induced muscle pain and evidence suggesting that the underlying mechanisms of muscle pain are dependent on its etiology, the aim of the present study was to evaluate whether peripheral inflammatory mechanisms were involved with static contraction-induced muscle pain. To this end, we developed a model of muscle pain induced by static contraction in rats. In addition, we evaluated the role of neutrophil migration, bradykinin, sympathetic amines and prostanoids on this painful process. We showed that static contraction-induced mechanical muscle hyperalgesia in the gastrocnemius muscle of rats is mediated through peripheral inflammatory mechanisms.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on male Wistar rats (200–250 g) from CEMIB (Multidisciplinary Center for Biological Research) UNICAMP and carried out in accordance with the guidelines on using laboratory animals from IASP (Zimmermann, 1983). The Committee on Animal Research of the State University of Campinas approved all the protocols and procedures (license number 2448-1). Sixty-three experimental groups were used with five animals per group. Sample size was calculated by power analysis (Festing and Altman, 2002). Laboratory animals were housed in plastic cages with soft bedding (five per cage) and were maintained on a 12-h light/dark cycle (lights on at 6:00 a.m.). Food and water were available *ad libitum* and the room was temperature controlled (23 °C). Animals were habituated to the test room for 1 h before testing was begun. Animals were euthanized with CO_2 asphyxiation once experimental procedures were complete.

Muscle hyperalgesia induced by static contraction

Rats were deeply anesthetized with isoflurane (5% for induction and 1.5% for maintenance). Static contraction of the gastrocnemius muscle of the right hind paw was produced by applying electrical pulses through two electrodes (27 gauge) inserted into the belly of the muscle (10 mm apart). The electrical pulses were generated by a Grass S88X stimulator (Grass Technologies, West Warwick, RI, USA) and induced static contraction of the gastrocnemius muscle observed by the maintenance of a plantar flexion without evidence of significant relaxation. The parameters used were monophasic current, repeated pulse, frequency of 50 Hz and pulse duration of 19 ms (protocol taken from Hill et al., 2003, altered and adjusted to the present model). Voltage and duration of electrical stimulation were determined by the development of muscle hyperalgesia. Maximum period of electrical stimulation was 1 h. In the sham group (control), electrodes were placed but no stimulation was administered. To confirm the electrical stimulation parameters, a digital oscilloscope GDS1022 (GW Instek, USA) and a voltimeter were used. All behavioral time points represent time elapsed since the static contraction procedure was complete and as such will be referred to hereafter as post static contraction.

Mechanical nociceptive threshold test

Testing was performed during light phase (9:00 a.m. to 5:00 p.m.) in a quiet room, with controlled temperature (23 °C) (Rosland, 1991). Mechanical muscle hyperalgesia was measured using the Randall–Selitto analgesiometer (Insight, Ribeirao Preto, SP, Brazil) (Randall and Selitto, 1957). The Randall–Selitto rounded tip with a 2-mm diameter was linearly applied to the belly of the gastrocnemius muscle (Fujii et al., 2008; Schiavuzzo et al., 2015) until a response was observed, and the maximal force (grams) was recorded. This diameter shows the nociceptive threshold of deep tissues (Takahashi et al., 2005). Three measures were taken five minutes apart and averaged. The baseline muscle-withdrawal threshold was determined before static contraction. The averaged measurements taken post static contraction was subtracted from the baseline measurements to determine the mechanical muscle hyperalgesia, which is represented by the y-axis. Increases in hyperalgesia are represented with an increase in the y-axis. The tester was blinded to all experimental treatments/groups.

Locomotor activity Tests

To investigate whether static contraction affects locomotor activity, we used the Rotarod and Open-Field Tests. Rotarod testing was performed by placing the animal on a rod of 6-mm diameter (Insight, Brazil). The speed of the rod gradually increased from 5 to 45 rpm. Latency for the rat to fall from the rod was recorded. Four consecutive trials were performed with a 15-min intertrial interval and results were averaged. Animals received training for 2 days for a total of 2 min each day