*Neuroscience* 255 (2013) 110–121

# PERIPHERAL AND SPINAL ACTIVATION OF CANNABINOID RECEPTORS BY JOINT MOBILIZATION ALLEVIATES POSTOPERATIVE PAIN IN MICE

**D. F. MARTINS,** [a,b,c*] **L. MAZZARDO-MARTINS,** [a,b] **F. J. CIDRAL-FILHO,** [a,b] **V. M. GADOTTI** [a†] **AND A. R. S. SANTOS** [a,b*]

[a] *Laboratório de Neurobiologia da Dor e Inflamação, Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Campus Universitário, Trindade, Florianópolis, SC, Brazil*

[b] *Programa de Pós-Graduação em Neurociências, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Campus Universitário, Trindade, Florianópolis, SC, Brazil*

[c] *Curso de Fisioterapia, Universidade do Sul de Santa Catarina, Campus Grande Florianópolis, Palhoça, SC, Brazil*

**Abstract—The present study was undertaken to investigate the relative contribution of cannabinoid receptors (CBRs) subtypes and to analyze cannabimimetic mechanisms involved in the inhibition of anandamide (AEA) and 2-arachidonoyl glycerol degradation on the antihyperalgesic effect of ankle joint mobilization (AJM). Mice (25–35 g) were subjected to plantar incision (PI) and 24 h after surgery animals received the following treatments, AJM for 9 min, AEA (10 mg/kg, intraperitoneal [i.p.]), WIN 55,212-2 (1.5 mg/kg, i.p.), URB937 (0.01–1 mg/kg, i.p.; a fatty acid amide hydrolase [FAAH] inhibitor) or JZL184 (0.016–16 mg/kg, i.p.; a monoacylglycerol lipase [MAGL] inhibitor). Withdrawal frequency to mechanical stimuli was assessed 24 h after PI and at different time intervals after treatments. Receptor specificity was investigated using selective $CB_1R$ (AM281) and $CB_2R$ (AM630) antagonists. In addition, the effect of the FAAH and MAGL inhibitors on the antihyperalgesic action of AJM was investigated. AJM, AEA, WIN 55,212-2, URB937 and JZL184 decreased mechanical hyperalgesia induced by PI. The antihyperalgesic effect of AJM was reversed by pretreatment with AM281 given by intraperitoneal and intrathecal routes, but not intraplantarly. Additionally, intraperitoneal and intraplantar, but not intrathecal administration of AM630 blocked AJM-induced antihyperalgesia. Interestingly, in mice pretreated with FAAH or the MAGL inhibitor the antihyperalgesic effect of AJM was significantly longer. This article presents data addressing the CBR mechanisms underlying the antihyperalgesic activity of joint mobilization as well as of the endocannabinoid catabolic enzyme inhibitors in the mouse postoperative pain model. Joint mobilization and these enzymes offer potential targets to treat postoperative pain.**


**Key words: acute pain, cannabinoid receptors, passive mobilization, rehabilitation, mice.**

## INTRODUCTION

The endocannabinoid system (ES) has emerged as an attractive therapeutic target for pain management in recent years (Vicenzino et al., 1998; Pacher et al., 2006). It consists of two known cannabinoid receptors (CBRs), subtypes $CB_1R$ and $CB_2R$ (Matsuda et al., 1990); a number of endogenous ligands including anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) (Mechoulam et al., 1998); a high-affinity reuptake transport system; and endocannabinoid (ECB) synthesizing and metabolizing enzymes (Beltramo et al., 1997). AEA is hydrolyzed by fatty acid amide hydrolase (FAAH) (Facci et al., 1995), and 2-AG is primarily metabolized by monoacylglycerol lipase (MAGL) (Hanus et al., 1999). Whereas exogenously administered ECBs are rapidly degraded by FAAH and MAGL, pharmacological inhibition of these enzymes results in elevated levels of ECBs in the brain and spinal cord tissues (Facci et al., 1995; Malan et al., 2002), which might represent an alternative approach that can be used to harness the potential therapeutic effects of cannabinoids. Prevention of ECBs metabolism produces behavioral analgesia in models of acute pain (Roques et al., 2012). Targeting FAAH and/or MAGL activity, therefore, presents a promising new therapeutic strategy for the treatment of pain.

Health practitioners commonly use joint mobilization (JM) to treat musculoskeletal pain and dysfunction (Vicenzino et al., 1998). Mobilization-induced analgesia has been demonstrated in several clinical (Zusman et al., 1989; Vicenzino et al., 1998) and pre-clinical studies (Sluka and Wright, 2001; Skyba et al., 2003; Martins et al., 2011). However, the mechanisms through

*Correspondence to: D. F. Martins, Laboratório de Neurociências Experimental (LaNex), Programa de Pós-graduação em Ciências da Saúde (PPGCS), Universidade do Sul de Santa Catarina, Campus Grande Florianópolis, Palhoça, SC, Brazil. Tel: +55-48-3279-1144. A. R. S. Santos, Departamento de Ciências Fisiológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil. Tel: +55-48-3721-4685; fax: +55-48-37219672.

E-mail addresses: daniel.martins4@unisul.br (D. F. Martins), adair.santos@ufsc.br (A. R. S. Santos).

† Present address: Department of Physiology and Pharmacology, Hotchkiss Brain Institute, University of Calgary, Calgary, Canada.

*Abbreviations:* 2-AG, 2-arachidonoyl glycerol; AEA, anandamide; ANOVA, analysis of variance; AJM, ankle joint mobilization; AUC, area under the curve; CBRs, cannabinoid receptors; ECB, endocannabinoid; FAAH, fatty acid amide hydrolase; JM, joint mobilization; MAGL, monoacylglycerol lipase; PI, plantar incision; VFF, von Frey filament.

0306-4522/13 $36.00 
http://dx.doi.org/10.1016/j.neuroscience.2013.09.055

which JM acts are not fully known. Of note, McPartland et al. (2005) observed that manual therapy (MT), including JM increases AEA blood levels in humans. Furthermore, our research group has demonstrated that the pharmacological blockade of both peripheral and spinal adenosine receptors (Martins et al., 2012) and the peripheral opioid receptors (Martins et al., 2012, 2013) also blocks the antihyperalgesic effect of JM in a mouse model of postoperative pain. In addition, a functional interaction between the adenosine, opioid and cannabinoid system has been shown in various pharmacological responses (Tuboly et al., 2008; Parolaro et al., 2010).

Studies have demonstrated that patients can develop persistent or chronic pain following surgical procedures, in addition, nearly half of all surgical patients still have inadequate pain relief; therefore, it is becoming more important to understand the mechanisms involved in postoperative pain in order to better treat it (Brennan, 2011).

The present study was undertaken to explore the relative contributions of central (spinal) and peripheral (paw) CBRs subtypes that mediate the antihyperalgesic effect of ankle joint mobilization (AJM). Additionally, we analyzed cannabimimetic mechanisms involved in the inhibition of AEA and 2-AG degradation through evaluation of the effects of JZL184 (MAGL inhibitor) and URB937 (FAAH inhibitor) on the antihyperalgesic effect of AJM.

## 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 conducted following the protocol approved by the Institutional Animal Care and Use Committee. All experiments were conducted using male Swiss mice (25–35 g), housed at 22 ± 2 °C under a 12-h light/12-h dark cycle (lights on at 6:00 am) and with free access to food and water. All manipulations were carried out between 11:00 am and 3:00 pm. The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the treatments. Control animals received the same vehicle used to dilute the compounds. When drugs were delivered by intraperitoneal (i.p.) route, a constant volume of 10 mL/kg body weight was injected. When drugs were administered by intrathecal (i.t.) or intraplantar (i.pl.) routes, volumes of 5 or 20 μL were injected, respectively. Appropriate vehicle-treated groups were also assessed simultaneously.

### Plantar incision (PI) surgery

Here, we used a pre-clinical model of postoperative pain. The model consists of a small incision to the plantar surface of the right hind paw. Paw incision in rodents induces a variety of nocifensive behaviors that closely resemble the time course of postoperative pain in humans (Brennan et al., 1996). The postoperative pain model was carried out according to the procedure described for rats (Brennan et al., 1996) and adapted for mice (Pogatzki and Raja, 2003). Briefly, mice were anesthetized with 1–2% isoflurane delivered via a nose cone. After sterile preparation of the right hind paw, a 5-mm longitudinal incision was made through skin and fascia of the plantar surface using a number 11 scalpel blade. The incision started 2 mm from the proximal edge of the heel and extended toward the toes. The underlying muscle was elevated with a curved forceps, leaving the muscle origin and insertion intact. After wound homeostasis, the skin was apposed with an 8.0 nylon mattress suture, and the wound was covered with 10% povidone-iodine solution. Control animals underwent a sham procedure and were kept under anesthesia.

### Intrathecal injections

Intrathecal (i.t.) injections were given to fully conscious mice using the method previously described by Hylden and Wilcox (1980). Briefly, the animals were manually restrained, the dorsal fur of each mouse was shaved, the spinal column was arched, and a 30-gauge needle was inserted into the subarachnoid space between the L4 and L5 vertebrae. Correct i.t. positioning of the needle tip was confirmed by a characteristic tail-flick response. A 5 μL volume of solution containing the test agent was slowly injected with a 25 μL Hamilton microsyringe (Hamilton, Birmingham, UK). Intrathecal injections were given over a period of 5 s.

### Ankle JM

The knee joint was stabilized, and the ankle joint was rhythmically flexed and extended to the end of the range of movement, according to a previously reported dosage regime (Skyba et al., 2003; Martins et al., 2011). The treatment with AJM was carried out in animals lightly anesthetized with 1–2% isoflurane prior to and for the duration of the JM. The treatment group received AJM three times, each time with 3 min in duration separated by 30 s of rest. Our group has previously shown this time frame to be optimal for producing antihyperalgesia in this model (Martins et al., 2011, 2012). Placebo AJM mice were lightly anaesthetized with 1–2% isoflurane and the ankle was maintained in a neutral position using the same hand contact and positioning as the treatment technique (Martins et al., 2012).

### Behavioral measurement: Mechanical and thermal hyperalgesia

Animals were tested for withdrawal thresholds to mechanical stimuli (von Frey filaments [VFFs]) applied to the plantar aspect of the right hindpaw (Bortalanza et al., 2002; Bobinski et al., 2011). Mice were acclimated in individual clear boxes ($9 \times 7 \times 11\ cm^3$) on an elevated wire mesh platform to allow access to the ventral surface of the hind paws. The right hind paw was stimulated with a constant pressure of 0.4 g VFF (Stoelting, Chicago, IL, USA). The response frequency to 10 applications was taken as the nociceptive