Ankle Joint Mobilization Affects Postoperative Pain Through Peripheral and Central Adenosine $A_1$ Receptors

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Background. Physical therapists frequently use joint mobilization therapy techniques to treat people with musculoskeletal dysfunction and pain. Several studies suggest that endogenous adenosine may act in an analgesic fashion in various pain states.

Objective. The purpose of this study was to investigate the contribution of the adenosinergic system on the antihyperalgesic effect of ankle joint mobilization (AJM).

Design. This was an experimental study.

Methods. To test the hypothesis that the adenosinergic system is involved in the antihyperalgesic effect of AJM, mice (25–35 g) submitted to plantar incision surgery were used as a model of acute postoperative pain. The mice were subjected to AJM for 9 minutes. Withdrawal frequency to mechanical stimuli was assessed 24 hours after plantar incision surgery and 30 minutes after AJM, adenosine, clonidine, or morphine treatments. The adenosinergic system was assessed by systemic (intraperitoneal), central (intrathecal), and peripheral (intraplantar) administration of caffeine. The participation of the $A_1$ receptor was investigated using a selective adenosine $A_1$ receptor subtype antagonist. In addition, previous data on the involvement of the serotonergic and noradrenergic systems in the antihyperalgesic effect of AJM were confirmed.

Results. Ankle joint mobilization decreased mechanical hyperalgesia, and this effect was reversed by pretreatment of the animals with caffeine given by intraperitoneal, intraplantar, and intrathecal routes. In addition, intraplantar and intrathecal administrations of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, a selective adenosine $A_1$ subtype receptor antagonist) or systemic administration of yohimbine or $p$-chlorophenylalanine methyl ester hydrochloride (PCPA) blocked the antihyperalgesia induced by AJM.

Limitations. The results are limited to animal models and cannot be generalized to acute pain in humans.

Conclusions. This study demonstrated the involvement of the adenosinergic system in the antihyperalgesic effect of AJM in a rodent model of pain and provides a possible mechanism basis for AJM-induced relief of acute pain.
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Physical therapists frequently use manual physical therapy techniques to treat people with musculoskeletal dysfunction and pain. Evidence for the efficacy of lower-limb mobilization is particularly scarce, as the majority of the studies of peripheral joints have used an upper-limb model.1,2 Although the scientific literature has begun to characterize the effects of spinal manual physical therapy,3,4 there are only a few studies that have investigated the hypoalgesic effects of peripheral joint mobilization techniques.5 There is consequently an urgent need for further lower-limb ankle joint mobilization (AJM) studies. In the last decade, clinical and experimental studies on joint mobilization therapy have indicated the possible neurophysiological effects for analgesia induced by mobilization.6–8

A synopsis of current evidence for the initial mechanism of action of joint mobilization therapy indicates, in part, a neurophysiological basis.9 A clinical study on patients with osteoarthritis showed that knee joint mobilization induces an increased threshold for mechanical pressure, both locally and distant from the treated joint.8 Sluka and Wright10 reported that knee joint mobilization reversed mechanical hyperalgesia induced by intra-articular injection of capsaicin in the ankle joint. Martins et al11 have shown that AJM suppressed pain behavior caused by a neuropathic pain model and attenuated astroglial activation in the spinal cord of rats. In addition, Martins et al12 recently demonstrated that 9 minutes of AJM, administered once a day over 5 days, reduced postoperative pain; this effect was prevented by systemic and local administrations of naloxone, but not by fucoidin.

A recent animal study showed that the analgesia produced by joint mobilization involves serotonin and norepinephrine receptors in the spinal cord, thereby supporting a role for descending pain modulatory systems.6 The descending pain inhibitory pathway in rodents and humans is activated by the perception of pain in the brain. Endorphins, endocannabinoids, serotonin, norepinephrine, and adenosine play important roles in this pathway.13 Adenosine regulates pain transmission in the spinal cord and in the periphery, and a number of agents can alter the extracellular availability of adenosine and subsequently modulate pain transmission, particularly by activation of adenosine A1 receptors (A1Rs).14,15 Of note, adenine nucleotides are frequently released following tissue stimulation and have pain-related functions in animals.15

Goldman and coauthors16 uncovered a role for peripheral adenosine receptor activation in the antinociceptive effects of acupuncture. It has been hypothesized that nonallopathic treatments of pain (eg, chiropractic manipulations, massage), modalities that involve the mechanical manipulation of joints and muscles, also might be associated with an efflux of cytosolic adenosine triphosphate (ATP) that is sufficient to elevate extracellular adenosine.16

Based on this evidence, we asked whether AJM could increase endogenous adenosine levels and produce antihyperalgesia through signaling via A1Rs. The present study investigated the relative contributions of central (spinal) and peripheral (paw) A1Rs subtype on the antihyperalgesic effect of AJM. In addition, we confirmed and extended previous data from the literature by demonstrating that AJM activates the descending inhibitory pathways (serotonin and noradrenaline) in a model of postoperative pain in mice.
Materials and Method

Animals

All experiments were conducted using male Swiss mice (25–35 g), housed at 22±2°C, under a 12 hours light/12 hours dark cycle (lights on at 6:00 am) and with free access to food and water. Animals were acclimatized to the laboratory for at least 1 hour before testing and were used only once throughout the experiments. The experiments were performed after approval of the protocol (PP00622) by the Institutional Ethics Committee of the Federal University of Santa Catarina and were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals.

Postoperative Pain Model

The postoperative pain model was carried out according to the procedure described for rats17 and adapted for mice.12,18 Mice were anesthetized with 2% isoflurane via a nose cone. After antiseptic preparation of the right hind paw with 10% povidone-iodine solution, a 5-mm longitudinal incision was made with a number 11 blade through the skin and fascia of the plantar side of the foot. The incision was 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. The skin was apposed with a single mattress suture of 6.0 nylon.

Intrathecal Injections

Intrathecal injections were given to fully conscious mice using the method previously described by Hylden and Wilcox.19 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 intrathecal positioning of the needle tip was confirmed by a characteristic tail-flick response in the animal. A 5-μL volume containing the test agent was slowly injected with a 25-μL Hamilton microsyringe (Hamilton, Birmingham, United Kingdom). In addition, a constant volume of 5 μL of saline or vehicle (saline + 5% dimethyl sulfoxide [DMSO]) was injected simultaneously. Intrathecal injections were given over a period of 5 seconds.19

AJM Treatment

The AJM treatment was carried out according to the procedure described for humans and adapted for mice.12 The knee joint was stabilized, and the ankle joint was rhythmically flexed and extended to the end of the range of movement, as previously described.12 The mice were anesthetized with 1% to 2% isoflurane prior to and for the duration of the joint mobilization procedure via a nose cone. The treatment group received 3 applications of mobilization, each of 3 minutes’ duration, and were separated by 30 seconds of rest, described here as 9 minutes of AJM. Recently, our group has demonstrated that this time frame is optimal for producing antihyperalgesia in this model.12

Placebo AJM

Mice were lightly anesthetized with 2% isoflurane, and the ankle was maintained in a neutral position using the same hand contact and positioning as in the actual treatment technique.12

Behavioral Measurement: Mechanical Hyperalgesia

Animals were tested for withdrawal thresholds to mechanical stimuli (von Frey filaments) applied to the plantar aspect of the hind paw.20,21 The mice were acclimated in individual clear boxes (9 × 7 × 11 cm³) on an elevated wire mesh platform to

The Bottom Line

What do we already know about this topic?

The available evidence suggests that manual therapy, including the ankle joint mobilization (AJM), is effective in the treatment of musculoskeletal disorders. Despite the literature supporting its effectiveness, the mechanism of action of manual therapy is not well established.

What new information does this study offer?

This study provides the first evidence showing the important role of the adenosinergic system in the antihyperalgesic effect of AJM. Thus, the antihyperalgesic effect of AJM appears to involve the activation of both central and peripheral adenosine A1 receptors.

If you’re a patient, what might these findings mean for you?

This study shows that caffeine, one of the world’s most commonly consumed dietary ingredients, can inhibit the analgesic effect of AJM. Thus, physical therapists may advise their patients to avoid the use of caffeine before physical therapy sessions in order to improve the effectiveness of AJM.
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allow access to the ventral surface of the hind paws. The right hind paw was stimulated with a constant pressure of a 0.4-g von Frey filament (Stoelting, Chicago, Illinois). The withdrawal frequency was measured as the number of times (out of 10) that the animal withdrew the paw after the application of the 0.4-g filament. The results were expressed as the percentage of withdrawal frequency.21

In all experiments below, the animals were submitted to plantar incision surgery (operated), and the tests were performed before (as baseline) in all animals (data not shown), 30 minutes after placebo AJM or 9 minutes of AJM; antagonist + 9 minutes of AJM or antagonist + placebo AJM (see experiments 1, 2, and 3). The withdrawal response before surgery procedure was 20% to 30%, as observed in our previous study.12 The plantar incision procedure induced hyperalgesia, as noted by the high percentage (80%-100%) of withdrawal responses, 24 hours22 after surgery.

Experiment 1: Involvement of the Adenosinergic Receptors in the Antihyperalgesic Effect of AJM

Systemic administration of caffeine. The role of the adenosinergic receptors on the antihyperalgesic action of AJM was investigated by treating the mice with saline (10 mL/kg, intraperitoneal) or caffeine (a nonselective adenosine receptor antagonist, 10 mg/kg, intraperitoneal)23 20 minutes before placebo AJM. Mechanical hyperalgesia was evaluated 30 minutes after placebo AJM or 9 minutes of AJM. Placebo AJM animals were subjected to 9 minutes of anesthesia and were assessed over the same time intervals. For this experiment, the following groups (n=8) were used: (1) operated + saline + placebo AJM, (2) operated + saline + placebo AJM, (3) operated + caffeine + placebo AJM, and (4) operated + caffeine + 9 minutes of AJM.

In the positive control experiment, mice were pretreated with an intraperitoneal injection of saline (10 mL/kg) or caffeine (10 mg/kg), and after 20 minutes they received an intraperitoneal injection of saline (10 mL/kg) or adenosine (30 mg/kg, a nonselective adenosine receptor agonist).15 For this experiment, the following groups (n=8) were used: (1) operated + saline + saline, (2) operated + caffeine + saline, (3) operated + saline + adenosine, and (4) operated + caffeine + adenosine.

Peripheral (paw) administration of caffeine. In an attempt to investigate the involvement of peripheral adenosine receptors on the antihyperalgesia induced by 9 minutes of AJM, the animals received an intrathecal injection of saline (5 μL/site) or caffeine (150 nmol/site). After 15 minutes, the animals received placebo AJM or 9 minutes of AJM. Mechnical hyperalgesia was evaluated 30 minutes after placebo AJM or 9 minutes of AJM. In this experiment, the following groups (n=8) were used: (1) operated + saline + placebo AJM, (2) operated + caffeine + placebo AJM, (3) operated + saline + 9 minutes of AJM, and (4) operated + caffeine + 9 minutes of AJM.

In the positive control experiment, mice were pretreated with an intrathecal injection of saline (20 μL/site) or caffeine (150 nmol/site). After 15 minutes, the animals received an intraperitoneal injection of saline (10 mL/kg) or adenosine (30 mg/kg). In this experiment, the following groups (n=8) were used: (1) operated + saline + saline, (2) operated + caffeine + saline, (3) operated + saline + adenosine, and (4) operated + caffeine + adenosine.

Central (spinal) administration of caffeine. In another set of experiments, to evaluate the involvement of spinal adenosine receptors on the antihyperalgesia induced by 9 minutes of AJM, the animals received an intrathecal injection of saline (5 μL/site) or caffeine (150 nmol/site). After 15 minutes, the animals received placebo AJM or 9 minutes of AJM. Mechanical hyperalgesia was evaluated 30 minutes after placebo AJM or 9 minutes of AJM. In this experiment, the following groups (n=8) were used: (1) operated + saline + placebo AJM, (2) operated + caffeine + placebo AJM, (3) operated + saline + 9 minutes of AJM, and (4) operated + caffeine + 9 minutes of AJM.

In the positive control experiment, mice were pretreated with an intrathecal injection of saline (20 μL/site) or caffeine (150 nmol/site). After 15 minutes, the animals received an intrathecal injection of saline (20 μL/site) or caffeine (150 nmol/site). In this experiment, the following groups (n=8) were used: (1) operated + saline + saline, (2) operated + caffeine + saline, (3) operated + saline + adenosine, and (4) operated + caffeine + adenosine.

Experiment 2: Involvement of Adenosine A1 Subtype Receptor in the Antihyperalgesic Effect of AJM

Peripheral (paw) administration of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX). We investigated the involvement of peripheral A1Rs on the antihyperalgesia induced by 9 minutes of AJM. The animals received an intraplantar injection of vehicle (saline solution with 5% DMSO) or DPCPX (10 nmol, a selective adenosine A1 receptor antagonist)24 in the right hind paw. After 15 minutes, the animals...
received placebo AJM or 9 minutes of AJM. Mechanical hyperalgesia was evaluated 30 minutes after placebo AJM or 9 minutes of AJM. In this experiment, the following groups (n=8) were used: (1) operated + vehicle + placebo AJM, (2) operated + DPCPX + placebo AJM, (3) operated + vehicle + 9 minutes of AJM, and (4) operated + DPCPX + 9 minutes of AJM.

In the positive control experiment, mice were pretreated with an intraplantar injection of vehicle (20 μL/paw) or DPCPX (10 nmol/paw). After 15 minutes, the animals received an intraperitoneal injection of vehicle (10 mL/kg) or adenosine (30 mg/kg). In this experiment, the following groups (n=8) were used: (1) operated + vehicle + saline, (2) operated + DPCPX + saline, (3) operated + vehicle + adenosine, and (4) operated + DPCPX + adenosine.

Central (spinal) administration of DPCPX. To determine the involvement of spinal A1Rs on the antihyperalgesic pathways induced by AJM, the animals received an intrathecal injection of vehicle (5 μL/site) or DPCPX (10 nmol/site). After 15 minutes, the animals received placebo AJM or 9 minutes of AJM. Mechanical hyperalgesia was evaluated 30 minutes after placebo AJM or 9 minutes of AJM. In this experiment, the following groups (n=8) were used: (1) operated + vehicle + placebo AJM, (2) operated + DPCPX + placebo AJM, (3) operated + vehicle + 9 minutes of AJM, and (4) operated + DPCPX + 9 minutes of AJM.

In the positive control experiment, mice were pretreated with an intrathecal injection of vehicle (20 μL/site) or DPCPX (10 nmol/site). After 15 minutes, the animals received an intraperitoneal injection of saline (10 mL/kg) or adenosine (30 mg/kg). In this experiment, the following groups (n=8) were used: (1) operated + vehicle + saline, (2) operated + DPCPX + saline, (3) operated + vehicle + adenosine, and (4) operated + DPCPX + adenosine.

Experiment 3: Involvement of Descending Monoaminergic Systems in the Antihyperalgesic Effect of AJM

Some studies indicate that the antinociception induced by adenosine depends on the spinal cord noradrenergic system. Thus, to confirm and extend previous data from the literature, we verified whether 9 minutes of AJM activates the descending inhibition pathways (serotonin and noradrenaline) to produce analgesia in the postoperative pain model. For this purpose, mice were pretreated with yohimbine (an α2-adrenoceptor antagonist) or β-chlorophenylalanine methyl ester hydrochloride (PCPA, an inhibitor of serotonin synthesis), as described below.

Involvement of the noradrenergic pathways: systemic administration of PCPA. To assess the possible contribution of endogenous serotonin on the antihyperalgesic effect of 9 minutes of AJM, the animals were pretreated with an intraperitoneal injection of saline (10 mL/kg) or PCPA (100 mg/kg, an inhibitor of serotonin synthesis) once a day, for 4 consecutive days, 3 days before and 24 hours after plantar incision surgery. Twenty minutes after the last administration, placebo AJM or 9 minutes of AJM were performed, and after 30 minutes, mechanical hyperalgesia was assessed. In this experiment, the following groups (n=8) were used: (1) operated + saline + placebo AJM, (2) operated + PCPA + placebo AJM, (3) operated + saline + 9 minutes of AJM, and (4) operated + PCPA + 9 minutes of AJM.

In the positive control experiment, mice were pretreated with an intraperitoneal injection of saline (10 mL/kg) or PCPA (100 mg/kg). After 20 minutes, they received saline (10 mL/kg, subcutaneous) or morphine (5 mg/kg, subcutaneous). In this experiment, the following groups (n=8) were used: (1) operated + saline + saline + saline, (2) operated + PCPA + saline, (3) operated + saline + morpaine, and (4) operated + PCPA + morphine.

Drugs

The following substances were used: DMSO (solvent used to dissolve DPCPX, experiment 2), morphine...
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hydrochloride (a nonselective opioid receptor agonist, experiment 3) (Merck, Darmstadt, Germany), caffeine (a nonselective adenosine receptors antagonist, experiment 1), clonidine hydrochloride (a selective α2-adrenergic receptors agonist, experiment 3), yohimbine hydrochloride (a selective α2-adrenergic receptors antagonist, experiment 3), PCPA (experiment 3) (Sigma Chemical Co, St Louis, Missouri), adenosine (an agonist with higher affinity for A1Rs and A2A adenosine receptors, experiments 1 and 2), and DPCPX (experiment 2) (Tocris Bioscience, Ellisville, Missouri). The DPCPX was dissolved in saline with 5% DMSO. The final concentration of DMSO did not exceed 5% and did not cause any effect. Other substances were dissolved in saline. When drugs were administered by intraperitoneal route, a constant volume of 10 mL/kg body weight was injected. When drugs were administered by intrathecal or intraplantar routes, a volume of 5 μL or 20 μL was injected, respectively. Appropriate control-treated groups also were assessed simultaneously. The doses of all substances used were chosen based on data in the literature15,23–25,27 or were selected from preliminary experiments conducted in our laboratory.

Data Analysis

Behavioral tests were analyzed using one-way analysis of variance following the Student Newman-Keuls test. Results were presented as the mean ± standard error of the mean for each group. *P < .05 was considered significant.

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Results

The Adenosinergic System Mediates the Antihyperalgesic Effect of AJM

The fact that adenine nucleotides are frequently released following tissue stimulation31 and have pain-related functions in animals, and that Goldman and coauthors16 discovered that peripheral adenosine receptor activation mediates the antinociceptive effects of acupuncture, raises the question of whether the antihyperalgesic effect of AJM can be mediated via activation of adenosine receptors. To our knowledge, the adenosinergic system has not previously been implicated in mobilization-induced antihyperalgesia. Caffeine administration without 9 minutes of AJM had no effect on the withdrawal frequency, and the thresholds were similar to those of the intrathecal, intraplantar injections of saline without 9 minutes of AJM groups (Figs. 1A, 1C, and 1E) and operated + saline + saline groups (Figs. 1B, 1D, and 1F). The pretreatment intrathecal, intrathecal, and intraplantar of mice with caffeine prevented the decrease in withdrawal frequency resulting from the 9 minutes of AJM (P < .05) or adenosine treatment (P < .05) (Figs. 1A, 1C, and 1E). Thus, these results suggest that the central and peripheral activation of adenosine receptors contributes to the antihyperalgesic effect of 9 minutes of AJM in the postoperative pain model.

Peripheral and Central A1Rs Mediate the Antihyperalgesic Effect of AJM

We determined the role of A1Rs on the antihyperalgesic effect of 9 minutes of AJM in the postoperative pain model. We administered the selective A1Rs antagonist DPCPX in 2 different sites: intrathecal and intraplantar (Figs. 2A–D).

The pretreatment with DPCPX had no effect on withdrawal frequency, and the thresholds were similar to those of intrathecal and intraplantar injections of vehicle without AJM for 9 minutes (Figs. 2A and 2C). In addition, the withdrawal frequency values for the groups pretreated with DPCPX via intrathecal and intraplantar routes were significantly higher compared with those of the vehicle + 9 minutes of AJM group 30 minutes after treatment (Figs. 2A and 2C). Spinal and plantar administration of DPCPX, 15 minutes before AJM for 9 minutes, prevented the decrease in the withdrawal frequency resulting from the treatment (P < .05) (Figs. 2A and 2C). These findings suggest that central and peripheral activation of A1Rs contributes to the antihyperalgesic effect of 9 minutes of AJM in the postoperative pain model.

Although the doses of caffeine and DPCPX were chosen based on previously published studies,24,25 we tested them against adenosine (an agonist with higher affinity for A1 and A2A receptors)15 in this model to confirm that they were adequate to block systemic, spinal, and peripheral adenosine receptors, respectively (Figs. 1B, 1D, and 1F). Administration of adenosine decreased withdrawal frequency 30 minutes after treatment (Figs. 1B, 1D, and 1F). In addition, pretreatment in different sites with caffeine (intrathecal, intrathecal, or intraplantar) or with DPCPX (intrathecal or intraplantar) completely blocked the effect of adenosine (Figs. 1B, 1D, 1F, 2B, and 2D, respectively).

The Descending Monoaminergic System Mediates the Antihyperalgesic Effect of AJM

Here we investigated whether the descending monoaminergic system was activated by 9 minutes of AJM. The pretreatment with yohimbine or PCPA without AJM had no effect on
Intraperitoneal (panels A and B), intrathecal (panels C and D), and intraplantar (panels E and F) pretreatment with caffeine and the antihyperalgesic effect of 9 minutes of ankle joint mobilization (AJM) or adenosine (30 mg/kg, i.p.) in mice. White bars show the operated (control) + saline (10 mL/kg, i.p.) group or the antihyperalgesic effect of 9 minutes of AJM or adenosine (30 mg/kg, i.p.). Blue bars show the effects of caffeine (10 mg/kg, i.p.; 150 nmol/site, i.t.; or 150 nmol/paw, i.pl.) antagonist injected before 9 minutes of AJM or adenosine (30 mg/kg, i.p.) treatment. Each point represents the mean of 8 animals, and vertical lines show the standard error of the mean. *Significant difference (P < 0.05) compared with operated + saline + placebo AJM groups (panels A, C, and E) or operated + saline + ajm groups (panels B, D, and F). #Significant difference (P < 0.05) compared with operated + saline + 9 minutes of AJM groups (panels A, C, and E) or operated + saline + adenosine groups (panels B, D, and F). Abbreviations: i.p. = intraperitoneal, i.t. = intrathecal, i.pl. = intraplantar.
the withdrawal frequency, and the thresholds were similar to those of the saline without AJM group (Fig. 3A and 3C). The withdrawal frequency values for the group pretreated with yohimbine or PCPA were significantly higher compared with the vehicle + 9 minutes of AJM group 30 minutes after treatment (Figs. 3A and 3C). The intraperitoneal administrations of yohimbine or PCPA 20 minutes before the 9 minutes of AJM prevented the decreased in withdrawal frequency resulting from the treatment (P<.05, Figs. 3A and 3C). The results presented in Figures 3B and 3D showed that the pretreatment of animals with yohimbine or PCPA completely reversed the antihyperalgesia caused by clonidine (P<.05, Fig. 3B) or morphine (P<.05, Fig. 3D), used as a positive control. Thus, we confirmed and largely extended previous data from the literature by demonstrating that 9 minutes of AJM activates serotonin and noradrenaline descending inhibitory pathways to produce analgesia.

Discussion
This study represents the first direct demonstration of the role of the adenosinergic system in supporting the antihyperalgesic effect of AJM. Our findings indicate that the activation of adenosine receptors is central to the mechanistic actions of AJM. Herein, we have reported that the antihyperalgesic effect of AJM is directly related to its ability to activate central and peripheral adenosine A1Rs.
Acute postoperative pain remains a significant medical problem. For those patients undergoing major surgical procedures, ongoing pain or pain at rest and pain during activities are important clinical symptoms. Pain at rest is usually moderate during the first 2 to 3 days after surgery. These pain scores occur even when parenteral treatments are administered. Usually, pain at rest resolves within the first week after surgery. Pain with activities, such as coughing or walking, is severe during the first 2 to 3 days. Pain with activities is moderate or severe for many days and even weeks later. Functional capability is limited during this period as well. Thus, pain can be moderate, and the patient’s ability to cough or the walking distance to evoke this pain is reduced.

Several studies have indicated that effective postoperative analgesia reduces morbidity following surgery, thereby improving patient outcome and reducing clinical expenses. Adequate knowledge regarding treatment of postoperative pain is important to reduce the morbidity and mortality of patients after surgery.
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Surgery. However, recent surveys have demonstrated that about 50% to 70% of patients experience moderate to severe pain after surgery, indicating that despite the development of new therapies and improved analgesic techniques, postoperative pain remains underevaluated and poorly treated. There is now ample evidence that postoperative pain is based on distinct pathophysiologic and pharmacologic mechanisms compared with other pain models. Therefore, it is extremely important to investigate new treatments that are efficient to reduce postoperative pain.

Analgesic Effect Induced by Activation of A1Rs

Prior studies characterize adenosine as an important modulator of the nociceptive processes. It can act as either a pronociceptive or an antinociceptive agent, depending on the level of the nociceptive system (central or peripheral) and on the receptor subtype activated. There is a great body of evidence indicating that the activation of A1Rs produces antinociception in models of postoperative, neuropathic, and inflammatory pain. In addition, mice lacking A1Rs exhibited increased nociceptive responses and failed to show the antinociceptive effects of an A1R agonist, confirming the importance of A1Rs in antinociception. Recently, it has been demonstrated that spinal A1Rs but not A2A receptors play an important role in the maintenance of non-evoked and evoked pain behaviors after an incision. In addition, A1R-induced spinal antinociception is mediated by interactions with peritussis toxin-sensitive G proteins.

Antihyperalgesia Induced by AJM: Interaction Between the Descending Monoaminergic System and A1Rs

The effects of the descending monoaminergic system on nociceptive processing in the dorsal horn are complex. Serotonin, norepinephrine, and dopamine may exert either antinociceptive or pronociceptive effects according to the type of receptor involved, site of action in the dorsal horn, and cross talk between descending and local neurochemical signals, including adenosine, endogenous opioids, and nitric oxide. Recent studies indicate a dependence of antinociception mediated by adenosine on the spinal cord noradrenergic system. For example, the antinociceptive effect of adenosine was blocked by coadministration of the α2-receptor antagonist idazoxan. Adenosine receptor agonists enhanced α2-adrenergic antinociception. Finally, the destruction of spinal noradrenergic terminals with neurotoxins abolishes the antihypersensitivity effects of adenosine and adenosine modulators.

Skyba and coauthors demonstrated that descending pain inhibitory systems could be activated by mobilization, producing analgesia by activation of serotonin and adrenergic receptors in the spinal cord. Here, we largely extend previous data from the literature demonstrating in a postoperative pain model in mice that 9 minutes of AJM activates descending inhibitory pathways using serotonin and norepinephrine to produce analgesia. Another relevant finding of our study is that central (spinal) administration of caffeine or DPCPX before 9 minutes of AJM prevented the decrease in the withdrawal frequency resulting from the treatment. These findings suggest that peripheral activation of A1Rs contributes to the antihyperalgesic effect of AJM on postoperative pain.

Limitations

Further studies are necessary to more directly investigate the supraspinal involvement of the adenosinergic system on the antihyperalgesia induced by AJM in other pain models. Additionally, it will be relevant to assess whether other mobilization techniques that involve minimal joint movement will activate the adenosinergic system. The lack of biochemical analysis may be a

Peripheral A1Rs Mediate the Antihyperalgesic Effect of AJM

Adenine nucleotides are frequently released following tissue stimulation and have pain-related functions in animals. Recently, it has been demonstrated that adenosine mediates acupuncture analgesia via activation of A1Rs. Vibratory stimulation applied to the skin depressed the activity of nociceptive neurons in the lower lumbar segments of cats by release of adenosine. It has been hypothesized that other nonallopathic treatments of pain, such as chiropractic manipulations and massage modalities that involve the mechanical manipulation of joints and muscles, also might be associated with an efflux of cytosolic ATP that is sufficient to elevate extracellular adenosine. As in acupuncture, adenosine may accumulate during joint mobilization and dampen pain, in part through the activation of A1Rs on sensory afferents of ascending nerve tracks. Our results confirm this hypothesis, once peripheral (paw) administration of caffeine or DPCPX before 9 minutes of AJM prevented the decrease in the withdrawal frequency resulting from the treatment. These findings suggest that peripheral activation of A1Rs contributes to the antihyperalgesic effect of AJM on postoperative pain.
limitation of this study and should be considered in future studies.

**Clinical Significance**

Clinical trials and meta-analyses provide increasing evidence to support the use of manual physical therapy in the management of painful spinal conditions. As evidence increases to support the use of manual physical therapy, it is becoming increasingly important to establish the mechanism of action of manual physical therapy techniques. The present study investigated the pharmacological properties of AJM because of the possibility that it represents the neural basis at the central and peripheral levels for the antihyperalgesic effect of AJM in humans. The implication of adenosine in the antihyperalgesia induced by AJM necessitates some new perspectives in evaluating the efficacy of AJM in clinical trials. Caffeine can block adenosine A1Rs in modest doses and can inhibit antihyperalgesia induced by AJM. It is important to note that caffeine (200 mg) has been reported to inhibit the efficacy of transcutaneous electrical nerve stimulation when given prior to the stimulation in a small experimental pain trial in humans. Future studies should determine whether daily caffeine intake alters the effectiveness of AJM in animals and humans.

**Conclusion**

The results of this study demonstrated that AJM decreased injury-induced mechanical hyperalgesia, and this effect was prevented by the pretreatment with caffeine given via intraperitoneal, intrathecal, and intraplantar routes. We also showed that intrathecal and intraplantar administrations of DPCPX blocked the antihyperalgesic effect of AJM. To our knowledge, this is the first direct demonstration of the role of the adenosinergic system in mediating the antihyperalgesic effect of AJM. In addition, we confirmed and largely extended previous data by demonstrating that AJM activates the descending control of pain using serotonin and norepinephrine to produce analgesia.

Mr Martins, Ms Mazzardo-Martins, Mr Cidral-Filho, and Dr Santos provided concept/idea/research design and writing. All authors provided data collection. Mr Martins, Ms Mazzardo-Martins, and Ms Stramoski provided data analysis. Dr Santos provided project management, fund procurement, and facilities/equipment.

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