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Nociceptive behavior induced by mustard oil injection into the temporomandibular joint is blocked by a peripheral non-opioid analgesic and a central opioid analgesic

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ABSTRACT

The aim of this study was to improve the mustard oil (MO) induced temporomandibular joint (TMJ) nociception model and to investigate the potential analgesic activity of systemic dipyrone and tramadol on the nociceptive behavioral responses induced by injection of low concentrations of the MO into the rat TMJ region. TMJ injection of 2.5% MO produced a significant nociceptive behavior expressed by head flinching and orofacial rubbing. This activity was related to the MO injection since mineral oil (vehicle) did not elicit response. Local application of the lidocaine *N*-ethyl bromide quaternary salt, QX-314 (2%) and systemic administration of morphine (4 mg/kg) significantly reduced the MO-induced nociceptive responses, validating the nociceptive character of the behaviors. The pretreatment with systemic dipyrone (19, 57 or 95 mg/kg) as well as tramadol (5, 7.5 or 10 mg/kg) was effective in decreasing the nociceptive behavioral responses induced by the injection of MO into the rat TMJ. In conclusion, TMJ injection of low concentrations of MO in rats produces well defined and quantifiable nociceptive behaviors constituting a reliable behavioral model for studying TMJ pain mechanisms and testing analgesic drugs. The results also suggest that dipyrone and tramadol could be effective analgesic options in the management of TMJ pain.

1. Introduction

Temporomandibular disorders are musculoskeletal pain conditions characterized by pain in the temporomandibular joint (TMJ) and/or the masticatory muscles (LeResche et al., 2003) and they comprise the most common cause of chronic facial pain conditions (Gameiro et al., 2006). In order to better understand the mechanisms underlying TMJ pain and consequently, better manage it, animal models that use chemical irritants to produce TMJ injury and inflammation have been developed (Swift et al., 1998; Ren and Dubner, 1999; Roveroni et al., 2001). However, the current knowledge of pain mechanisms in TMJ disease does not always include overt damage to anatomical structures or extensive inflammation. Such examples of sympathetically maintained pain or localized regions of neurogenic inflammation may constitute significant contributors to pain development (Widmer, 2004; Sato et al., 2007). The TMJ injection of the small-fiber excitant and inflammatory irritant, mustard oil (MO) (Woolf and Wall, 1986; Sunakawa et al., 1999; Laird et al., 2001) represents a rat model of TMJ pain that reflexively evokes activity in the digastric and masseter muscles (Yu et al., 1995; Hu et al., 1996; Yu et al., 1996), produces nociceptive behaviors (Hartwig et al., 2003) and results in a local inflammatory response (Haas et al., 1992; Wong et al., 2001). When applied to the rat pulp the MO elicits an increase in jaw muscle activity (Sunakawa et al., 1999), suggesting the activation of pulp afferent inputs to the central nervous system evoked by MO. Whole cell patch-clamp recordings from trigeminal neurons exposed to MO show that these cells are responsive to the application of this irritant (Jordt et al., 2004), supporting a direct activation of the trigeminal nociceptors by MO. Nociceptor excitation seems to be mediated by the TRPA1 channel, a member of the transient receptor potential family of ion channel proteins (Jordt et al., 2004; Nagata et al., 2005). In addition to transmitting nociception to the central nervous system, nociceptors may also release peptides–such as substance P and calcitonin-gene-related peptide (CGRP)–peripherally to produce vascular leakage and vasodilation, leading to inflammation and tenderness at the site of MO application (Louis et al., 1989). Therefore, the electromyographic activity (Yu et al., 1995) and the quantitative behavioral changes induced by MO inflammation of the TMJ (Hartwig et al., 2003) constitute a potential model to study the pathophysiology of TMJ nociception and to assess experimental TMJ treatments (Noguchi et al., 2005; Bakke et al., 1998a, 1998b).

Successful long-term pain management requires analgesic regimens that can treat pains of multiple origins. Safety and tolerability

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are also a high priority when prescribing chronic therapy (Raffa, 2006). The non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics are often prescribed in an attempt to decrease the pain associated with TMJ (Brazeau et al., 1998). Typical NSAIDs acts as analgesic by preventing the hyperalgesia induced by prostaglandins during inflammation (Ferreira, 1972, 2002). In addition to their capability of blocking prostaglandin production, analgesics of the dipyrone type directly block the sensitization of nociceptors (Lorenzetti and Ferreira, 1985; Tonussi and Ferreira, 1994), thus constituting an alternative in the treatment of ongoing nociceptive primary afferent activity.

Major concerns about long-term use of NSAIDs are the gastrointestinal toxicity (Lazzaroni and Bianchi Porro, 2004) and risk for thrombotic events, such as cardiovascular mortality, non-fatal myocardial infarctions or strokes (Farkouh et al., 2007). Opioid drugs avoid the peripheral toxicity of the NSAIDs, but their long-term use is limited by side effects, such as sedation and tolerance. Tramadol is an atypical weak opioid with a multiple action mechanism, such as inhibition of noradrenaline and serotonin reuptake (Raffa et al., 1992; Reimann and Hennies, 1994; Raffa, 1996; Dayer et al., 1997), and has a better tolerability profile when compared with traditional opioids (Miranda and Pinardi, 1998; Grond and Sablotzki, 2004). It is widely used for the treatment of acute and chronic pain (Cicero et al., 1999), and unlike pure opioids, clinically relevant effects on respiratory or cardiovascular parameters are rare at recommended doses for postoperative pain (Scott and Perry, 2000). According to our knowledge, the effects of tramadol on TMJ pain have not been evaluated. However, some studies have reported good analgesic effects of tramadol in oral surgery and dental pain (Moore and McQuay, 1997; Fricke et al., 2004). Dipyrone is another analgesic option to reduce nociception. This drug is a non-opioid analgesic that acts predominantly on the primary peripheral sensory neurons (Lorenzetti and Ferreira, 1985, 1996). Although the effect of dipyrone on TMJ pain has never been reported, Planas et al. (1998) demonstrated that dipyrone (1 and 2 g) was able to relieve the pain after surgical extraction of the mandibular third molar. Moreover, Bagan et al. (1998) evaluated a total of 125 outpatients with moderate to severe pain after surgical removal of one impacted third molar and verified that dipyrone was efficient in relieving pain intensity in 70% of cases after the end of the first-dose phase. Although dipyrone has been associated with induction of agranulocytosis (Schug and Manopas, 2007), there is no meta-analysis study supporting this effect, and the evidences of agranulocytosis are almost all based on case reports. Therefore, dipyrone continues to be used in many countries for different types of clinical pain, such as tension-type headache (Martinez-Martin et al., 2001; Bigal et al., 2002) and migraine (Fernandes Filho et al., 2006).

Against this background, the aim of the present study was to improve the previously reported MO-induced TMJ nociception model by reducing the concentration of the MO injected and to investigate the potential analgesic activity of systemic dipyrone and tramadol on the nociceptive behavioral responses induced by TMJ application of the MO. The nociceptive character of the behavior elicited by MO injection in the TMJ was ascertained by testing the sensitivity of this behavior to local lidocaine and systemic morphine.

2. Methods

2.1. Animals

This study was conducted in 84 male (n=6/group) Wistar rats (200–300 g) housed in standard clear plastic cages with soft bedding (5/cage) with free access to food and water *ad libitum*. They were maintained in a temperature-controlled room (23±1 °C) with a 12/12 h light–dark cycle with lights on at 6:00 A.M. for at least 1 week prior to the experiments. Experimental protocols were approved by the Committee on Animal Research of the University of Campinas and conformed to IASP guidelines for the study of pain in animals (Zimmermann, 1983).

2.2. TMJ injection

Animals were briefly anesthetized by inhalation of halothane and the posteroinferior border of the zygomatic arch was palpated. The needle was inserted immediately below this point and was advanced in an anterior direction until reaching the posterolateral aspect of the condyle. TMJ injections were performed via a 30-gauge needle introduced into the left TMJ at the moment of the injection. A cannula consisting of a polyethylene tube was connected to the needle and also to a Hamilton syringe (50 μL). Volume per injection was 50 μL. Each animal regained consciousness approximately 30 s after discontinuing the anesthetic and was returned to the test chamber. After the conclusion of each experiment, animals were anesthetized with an intraperitoneal injection of a mixture of urethane (1 g/kg) and α-chloralose (50 mg/kg). The Evans blue dye (0.1%, 5 mg/kg) was then administered systemically to visualize the MO-induced plasma extravasation of Evans blue dye bound to plasma protein upon postmortem examination of the injected TMJs. The correct site of injection was indicated by the observation that the plasma extravasation induced by the TMJ injections was restricted to the TMJ region.

2.3. General procedures

Each animal was placed in a test chamber (30×30×30 cm mirrored-wood chamber with a glass at the front side) for a 15-min habituation period. Each animal was used for and was sacrificed at the end of the experiment. Testing sessions took place during the light phase (between 7:00 AM and 12:00 PM) in a quiet room maintained at 23 °C.

2.4. Measurement of behavioral nociceptive responses

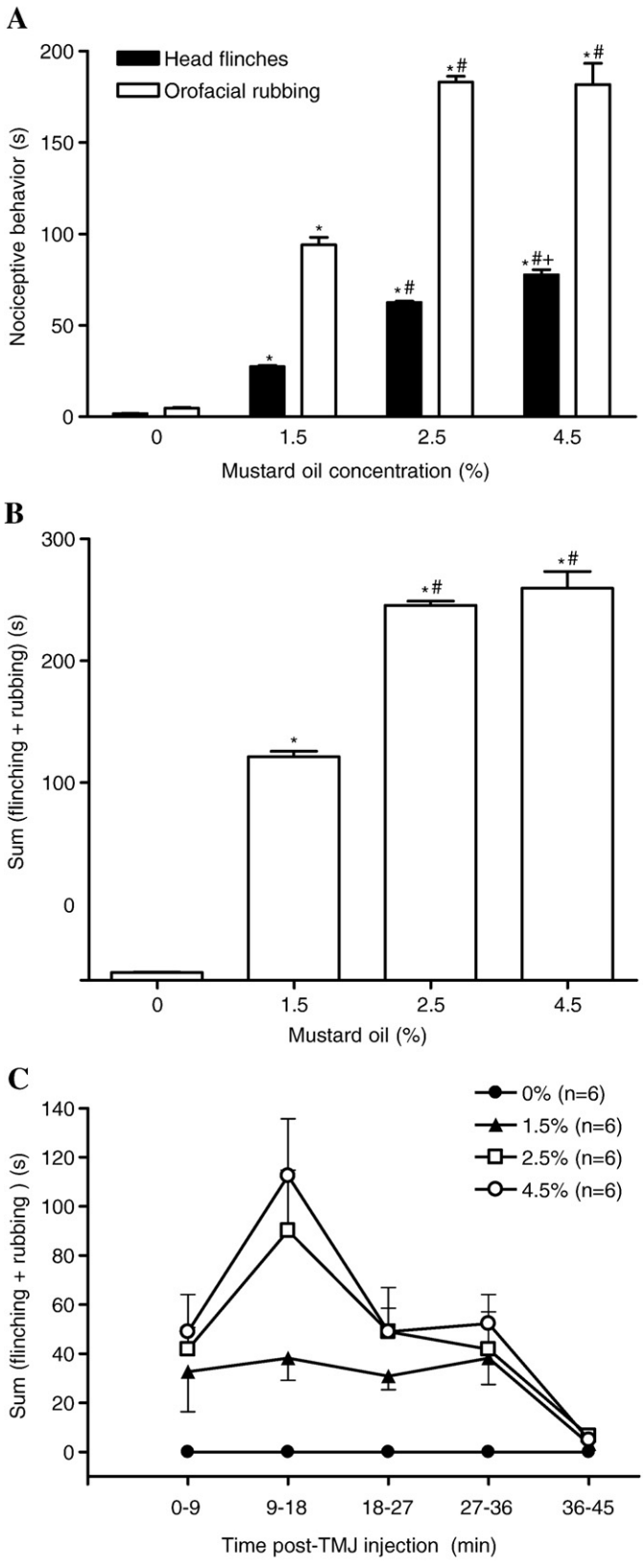
Following the TMJ injection each rat was returned to the test chamber for an observation period of 45 min. Rats immediately recovered from the anesthesia after the TMJ injection. The recording time was divided into blocks of 3 min and was quantified by (1) the time (seconds) that the animal spent rubbing the orofacial region asymmetrically with the ipsilateral fore or hindpaw and (2) the number of flinches with the head in an intermittent and reflexive way characterized by high-frequency shakes of the head as previously described (Roveroni et al., 2001). Considering that the flinching of the head behavior followed a uniform pattern of 1 s in duration, each flinching was expressed as 1 s when the combination (sum) of both behaviors was considered. It is important to point out that the sum of the nociceptive behaviors provides a better measure of pain intensity than any single behavior (Roveroni et al., 2001). The analysis of the behaviors was made by an investigator who was blind to the rat's group assignment.

2.5. Drugs and doses

Increasing concentrations (1.5, 2.5, 4.5% — n=6/dose) of MO or mineral oil (n=6) were injected into the TMJ region. Lidocaine *N*-ethyl bromide quaternary salt (2%) (QX-314, Research Biochemical Inc.) (n=6) or saline (n=6) was co-applied with 2.5% mustard oil. Morphine sulfate (4 mg/kg) (n=6), dipyrone (19, 57, 95 mg/kg — n=6/dose), tramadol (5, 7.5, 10 mg/kg — n=6/dose, Hong Xie et al., 2008) or saline (n=6) were given intraperitoneally in a volume of 10 ml/kg, 30 min prior (Clavelou et al., 1989) to the TMJ injection. Except for the MO that was dissolved in mineral oil, all drugs were dissolved *n* 0.9% saline.

2.6. Statistical analysis

The TMJ mustard oil nociceptive behaviors were evaluated separately first. Considering that the flinching of the head behavior



followed a uniform pattern of 1 s in duration, each flinching was expressed as 1 s. The different TMJ MO nociceptive behaviors were also evaluated by the sum of the behavioral responses measured for 45 min. Data with homogeneity of variance were analyzed using the *t*-test or one way analysis of variance (ANOVA) and multiple post-hoc comparisons were performed using the Tukey test. A probability level of less than 0.05 was considered to indicate statistical significance. Data are presented in figures and text as means±SEM.

3. Results

3.1. Mustard oil-induction of nociceptive response

Injection into the TMJ of increasing concentrations of MO significantly increased ($p<0.05$, Tukey test) the behavior characterized by flinching the head and rubbing the orofacial region evoked by the concentration of 1.5% (Fig. 1A). For head flinching behavior, the responses evoked by 1.5, 2.5 and 4.5% of MO differed from each other and for the orofacial rubbing the responses evoked by 2.5 and 4.5% of MO were also significantly different from the response elicited by 1.5% of the MO.

The graph shown in Fig. 1B illustrates the responses induced by the injection of 1.5, 2.5 and 4.5% of MO into the TMJ region of rats when the sum of these responses was used to evaluate them. The maximum in the response amplitude was achieved with the concentration of 2.5%. The injection of vehicle (mineral oil) alone had no effect.

The Fig. 1C illustrates the time-course of the dose-dependent increase in the sum of flinching and rubbing behavior of the rats injected with MO on the TMJ region. TMJ injection of MO significantly increases the nociceptive behavior evoked by the concentration of 1.5%. There was a positive relationship between the amplitude of the nociceptive response and the MO concentrations. The nociceptive behavior elicited by MO was significantly different among the groups, except between 2.5 and 4.5% ($p<0.05$, Tukey test). MO evoked nociceptive response that lasted for approximately 40 min and returned to the control level by the end of the experiment.

3.2. Effects of peripheral QX-314 and systemic morphine on the MO-induced nociceptive behavior

Co-application of the local anesthetic 2% QX-314 with 2.5% MO significantly reduced the flinching and rubbing behavior response induced by MO ($p<0.001$, *t*-test, Fig. 2A) confirming its nociceptive character in comparison with the control (local saline+MO). Given that QX-314 does not cross the blood–brain barrier, this finding also confirmed that MO-induced nociception results from a peripheral action of MO. The intraperitoneal administration of 4 mg/kg of morphine 30 min prior to the 2.5% MO injection into the TMJ region of rats also significantly reduced the MO-induced flinching and rubbing ($p<0.001$, *t*-test, Fig. 2A) comparison with the control (systemic saline+MO). Injection of the QX-314 and morphine also significantly reduced the nociceptive behavior expressed by the sum of face rubbing and head flinching (Fig. 2B, *t*-test, $p<0.05$).

Fig. 1. Effect of increasing concentrations of TMJ mustard oil (MO) on the duration of head flinching or orofacial rubbing behavior. (A) The injection of MO (1.5, 2.5 and 4.5%) induced a significant dose-dependent increase in the head flinching and orofacial rubbing behaviors. (B) Increase in the sum of flinching and rubbing behavior induced by TMJ injection of MO. The symbol (*) indicates a response significantly greater than that induced by the control (mineral oil vehicle). The symbols (#) and (+) indicate a response significantly greater than that induced by 1.5% and 2.5% of MO, respectively. Each column represents the mean response of six animals. Error bars indicate the SEM. The significance level was set at $p<0.05$ (ANOVA+Tukey). (C) Time-course of increasing concentrations of MO on the sum of head flinches and orofacial rubbing behavior and effect of dipyrone and tramadol. The injection of MO (2.5 and 4.5%) induced a significant time-dependent increase in the sum of nociceptive behaviors ($p<0.05$, Tukey test). Head flinching — degree of freedom=5; *F*-value=90,41, $p<0.001$. Orofacial rubbing — degree of freedom=5; *F*-value=30,13, $p<0.001$.

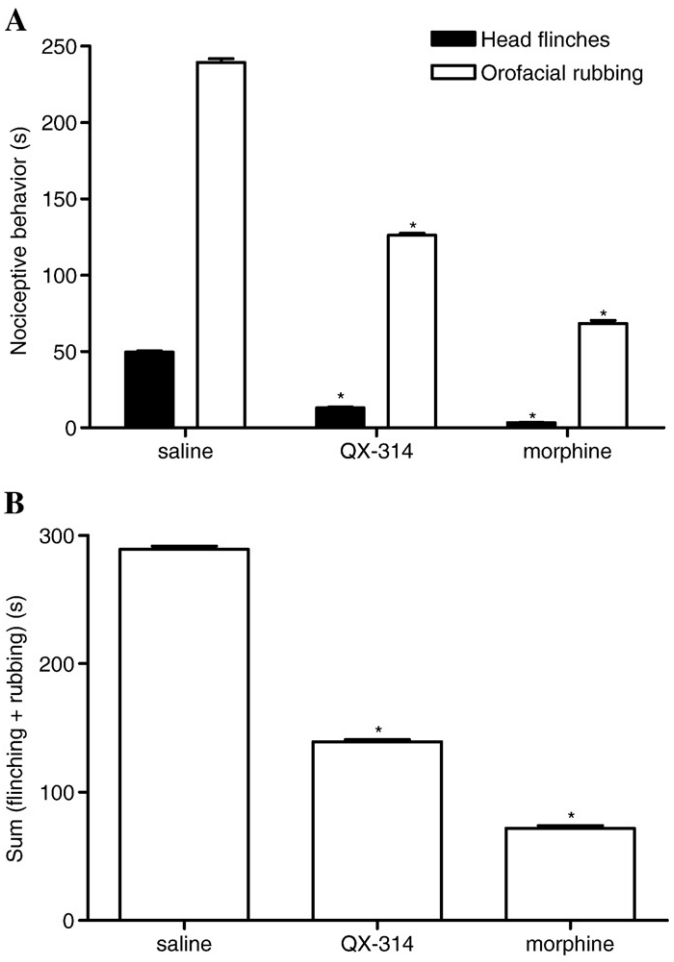


Fig. 2. Effect of QX-314 or systemic morphine on the duration of 2.5% MO-induced head flinching and orofacial rubbing. (A) Co-application of 2% of the quaternary derived lidocaine (QX-314) or the i.p. injection of morphine (4 mg/kg) significantly reduced the head flinching and the orofacial rubbing behaviors. (B) Decrease in the sum of flinching and rubbing behavior induced by MO-TMJ injection elicited by co-application of 2% QX-314 and systemic morphine. The symbol (*) indicates a significantly shorter response than that induced by the control (local or systemic saline + MO injection). Each column represents the mean response of six animals. Error bars indicate the SEM. The significance level was set at $p<0.05$ (*t*-test). *QX-314*: Head flinching — degree of freedom = 5; *T*-value = −19.13. $p<0.001$. Orofacial rubbing — degree of freedom = 5; *T*-value = −16.85. $p<0.001$. *Morphine*: Head flinching — degree of freedom = 5; *T*-value = 29.98. $p<0.001$. Orofacial rubbing — degree of freedom = 5; *T*-value = 29.61. $p<0.001$.

Here it was observed that the behaviors of rubbing the orofacial region and flinching the head were strongly exacerbated by MO and strongly correlated with MO concentration. When the behaviors are evaluated separately, this allows the different components of the pain experience, which might be modulated separately, to be studied; and when evaluated together, they are extremely useful for assessing the full impact of analgesic drugs on nociception. Taking into account the MO nociceptive behavior by summing the flinching and rubbing responses allows a description of the overall changes in behavior that better reflect the pain intensity, as reported earlier for formalin injection into the TMJ (Roveroni et al., 2001), in the next experiments, the sum of nociceptive rubbing and flinching responses induced by MO injection was used as an index of TMJ pain.

3.3. Effects of systemic dipyrone and tramadol on the MO-induced nociceptive behavior

The i.p. administration of dipyrone (19, 57 or 95 mg/kg) 30 min prior to the 2.5% MO injection in the TMJ region produced a dose-dependent reduction of the nociceptive behavior of TMJ–MO injected rats ($p<0.05$, Tukey test). This reduction was significant for all the doses of dipyrone used (Fig. 3). Similar results were obtained by the i.p. administration of tramadol (5, 7.5 and 10 mg/kg) 30 min prior to the injection of 2.5% MO in the rat TMJ. All doses of tramadol significantly reduced the sum of flinching and rubbing behavior elicited by MO (Fig. 4, $p<0.05$, Tukey test).

4. Discussion

Mustard oil (MO) has been used to activate nociceptors in a variety of models. In particular, administration of MO in the TMJ region of rats has been used to study central and peripheral consequences of noxious TMJ manipulation in anesthetized rats (Broton et al., 1988; Sessle and Hu, 1991; Haas et al., 1992; Yu et al., 1995). In this study we modified the behavioral model of TMJ pain induced by local mustard oil injection by decreasing the MO concentration from 20% (Hartwig et al., 2003) to 2.5%. The injection of 2.5% of MO but not of the vehicle in the TMJ region produced a quantitative and stereotyped nociceptive behavior characterized by flinching the head and rubbing the face that lasted for about 45 min. By using this model, we showed the potential efficacious analgesic action of dipyrone and tramadol on the TMJ pain.

4.1. Modification of the MO-induced TMJ nociception model

The mustard oil is an irritant agent that produces nociceptive behavior and inflammation (Hartwig et al., 2003; Yu et al., 1995) attributed to the stimulation of the nociceptive primary afferent fibers (Jordt et al., 2004). However, the concentration of 20% MO usually used to induce increase in electromyographic activity (Yu et al., 1995, Tambeli et al., 2001) or spontaneous nociception elicits a "freezing" behavior during the first hour after the injection. During this period, the rats did not move, explore or groom themselves (Hartwig et al., 2003). Thereafter, rats exhibited an increase in nociceptive behavior during the next 1 h of observation. The "freezing" behavior observed with the high concentration of the MO injected in the TMJ can produce ambiguous changes in the total pain sore, as occurs with orofacial injection of high dosages of

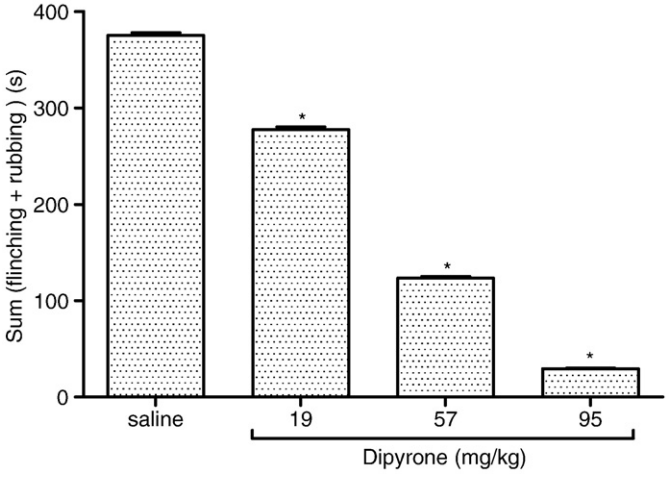


Fig. 3. Effect of systemic dipyrone on the duration of 2.5% MO-induced nociceptive behavior. The i.p. injection of dipyrone (19, 57 or 95 mg/kg) produced a significant dose-dependent reduction in the TMJ nociceptive behavior index expressed by the sum of head flinching and the orofacial rubbing behaviors elicited by TMJ–MO application. The symbol (*) indicates a significantly shorter response than that induced by the control (systemic saline + MO injection). Each column represents the mean response of six animals. Error bars indicate the SEM. The significance level was set at $p<0.05$ (ANOVA + Tukey). Head flinching — degree of freedom = 5; *F*-value = 685,717, $p<0.001$. Orofacial rubbing — degree of freedom = 5; *F*-value = 147,64, $p<0.001$.

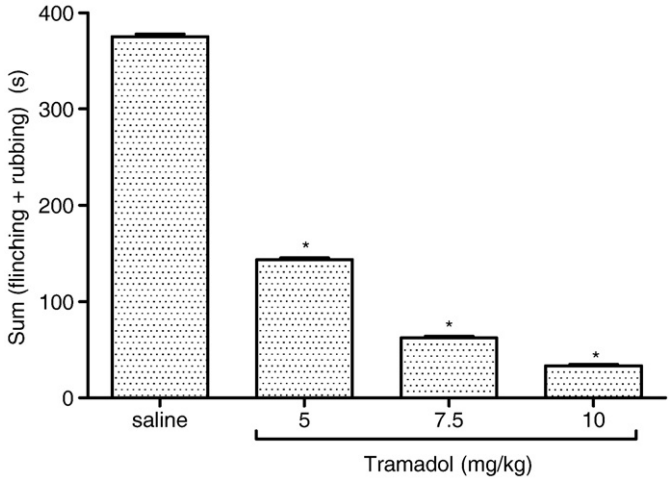


Fig. 4. Effect of systemic tramadol on the duration of 2.5% MO-induced nociceptive behavior. The i.p. injection of tramadol (5, 7.5 or 10 mg/kg) produced a significant dose-dependent reduction in the TMJ nociceptive behavior index expressed by the sum of orofacial rubbing and flinching behavior elicited by TMJ–MO application. The symbol (*) indicates a significantly shorter response than that induced by the control (saline+MO injection). Each column represents the mean response of six animals. Error bars indicate the SEM. The significance level was set at $p<0.05$ (ANOVA+Tukey). Head flinching — degree of freedom=5; F-value=433,21, $p<0.001$. Orofacial rubbing — degree of freedom=5; F-value=901,21, $p<0.001$.

formalin (Clavelou et al., 1995). Therefore, a reduction in total pain score may not be sufficient to demonstrate, for example, the antinociceptive properties of weak analgesics.

The observations reported here help to refine the model by showing that a dose-dependent increase in the head flinches and orofacial rubbing will be observed during the first 45 min after the 2.5% MO injection. These behaviors were not observed with the injection of the mineral oil (vehicle) which relates the responses to the MO concentration. This reduction in MO concentration will decrease the time of observation and abolish the "freezing" behavior seen with higher dosages. Moreover, it will also have the effect of minimizing the suffering of the experimental animal.

The head flinches and the orofacial rubbing after TMJ injection of an irritant agent constitute reproducible, quantifiable and well defined behavioral parameters to evaluate the magnitude of nociception (Roveroni et al., 2001). These two behaviors occur in an alternate manner, when orofacial rubbing increases there is less flinching response (Roveroni et al., 2001). Therefore, the sum of these complementary behaviors allows a description of the overall changes in behavior that better reflect the pain intensity, which is in agreement with the idea that the combination of several behaviors provides a better measure of pain intensity than a single behavior (Abbott et al., 1995; Coderre et al., 1993). Indeed, in addition to the increase in the flinching and rubbing behavior elicited by the MO injection into the TMJ of rats, the sum of the behaviors showed the same pattern of nociceptive dependence on the MO concentration, a result similar to that obtained in the TMJ formalin test developed by Roveroni et al. (2001).

The anesthetic blockade of the MO-induced flinching and rubbing responses by the co-application of the hydrophilic quaternary derived lidocaine (QX-314) indicates the nociceptive character of these behaviors. The systemic morphine administration significantly reduced the MO-induced rubbing and flinching responses at a concentration (4 mg/kg) that did not impair locomotor activity. Injection of the QX-314 and morphine also significantly reduced the nociceptive behavior expressed by the sum of face rubbing and head flinching. These results validate the sum of rubbing and flinching behavior as reliable pain measure of the MO-TMJ nociception.

4.2. The antinociceptive effect of dipyrone and tramadol on MO-TMJ nociception

Pharmacological intervention in TMJ pain was also tested in this study, since pharmacotherapy is often the primary approach to treating inflammatory pain processes that are frequently associated with TMJ (Denucci et al., 1996; Cardelli et al., 2005). To our knowledge, this research was the first to evaluate these drugs in a model of TMJ pain. Dipyrone is a type of pyrazolone derivative sold as a painkiller and widely used in many countries (Brogden, 1986; Bensenor, 2001). The mechanism of action of dipyrone differs from that of classical non-steroidal anti-inflammatory drugs. Although the site of action is peripheral its analgesic effect is not derived from inhibition of prostaglandin synthesis but is exerted via direct blockade of inflammatory hyperalgesia (Lorenzetti and Ferreira, 1985). The hyperalgesia results from excitatory actions of endogenous mediators on the primary afferent terminals, which up-regulate the nociceptor response. When the nociceptor is up-regulated, drugs that block the release of peripheral mediators, such as prostaglandin or sympathomimetic amines are not effective as analgesic agents (Ferreira et al., 1990). Under this circumstance, dipyrone is still capable of reducing the nociception by direct down-regulation of the nociceptor (Ferreira et al., 1990). Therefore, unlike other NSAIDs, dipyrone seems to acts as a classic analgesic. Dogrul et al. (2007) also reported differences in the mechanisms of action between dipyrone and NSAIDs. These authors suggest that the endogenous opioid system could contribute to the peripheral antinociceptive effects of dipyrone, but not to that of diclofenac, ketorolac, lysine acetyl salicylate, or sodium salicylate.

Here, we show that the pretreatment of the rats with systemic dipyrone reduced the nociceptive behavior induced by the injection of MO into the TMJ region. As MO seems to induce nociceptive behavior by directly acting on the nociceptors (Jordt et al., 2004), this result points out an efficacious analgesic action of dipyrone on ongoing TMJ pain.

Tramadol is a centrally acting, synthetic analgesic compound that is structurally related to codeine and morphine. It has a dual action mechanism which includes a weak affinity for mu-opioid receptor and inhibition of serotonin and noradrenaline reuptake (Raffa, 1996; Dayer et al., 1997). Tramadol is an effective and well tolerated agent to reduce pain resulting from trauma, renal or biliary colic and labor, as well as for chronic pain management, and appears to produce less constipation and dependence than equianalgesic doses of strong opioids (Miranda and Pinardi, 1998; Grond and Sablotzki, 2004).

In this study, the systemic administration of tramadol prior to the TMJ mustard oil injection was effective for reducing the nociceptive behaviors of the rats. Therefore, tramadol could be a pharmacological alternative for the treatment of patients in whom NSAIDs are contraindicated.

In summary, this study showed that injection in the rat TMJ of low a concentration of mustard oil constitutes a reliable model for the study of TMJ pain mechanisms and their treatment, since it produces well defined and quantifiable nociceptive behaviors. We suggested that this modification will make the model more sensitive to the study of analgesic drug properties by abolishing the "freezing" behavior seen with higher MO dosages. The study also demonstrated the potential analgesic activity of dipyrone and tramadol in the management of TMJ pain.

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