Intramuscular Ketorolac Inhibits Activation of Rat Peripheral NMDA Receptors

Brian E. Cairns¹, Xu-Dong Dong¹, Hayes Wong¹, Peter Svensson²,³

¹Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, V6T 1Z3, Canada.

²Clinical Oral Physiology, Department of Dentistry, Aarhus University, DK-8000 Aarhus C, Denmark.

³MindLab, Center for Functionally Integrative Neuroscience (CFIN), Aarhus University Hospital, DK-8000 Aarhus C

Running title: Ketorolac attenuates nociceptor discharge

Correspondence should be addressed to:
Brian E. Cairns, Ph.D.
Faculty of Pharmaceutical Sciences, The University of British Columbia
2146 East Mall, Vancouver, British Columbia, V6T 1Z3 CANADA
Phone: 1-604-822-7715
Fax: 1-604-822-3035
Email: brcairns@interchange.ubc.ca
The non-steroidal-anti-inflammatory-drug (NSAID) diclofenac has local anesthetic-like and peripheral N-methyl-D-aspartate (NMDA) receptor antagonist characteristics when administered at higher concentrations to masticatory muscle. It is not known if the ability to inhibit NMDA receptors is unique to diclofenac, or is shared by other NSAIDs. This study was undertaken to determine if intramuscular injection of ketorolac or naproxen at concentrations that did not exhibit local anesthetic-like effects could attenuate jaw-closer muscle nociceptor discharge in anesthetized Sprague Dawley rats. It was found that ketorolac (5 mM) inhibited hypertonic saline-evoked nociceptor discharge, which suggests that at this concentration ketorolac has local anesthetic-like properties. A lower concentration of ketorolac (0.5 mM), which did not affect hypertonic saline-evoked discharge, did inhibit nociceptor discharge evoked by NMDA. In contrast, naproxen (5 mM) did not alter hypertonic saline- or NMDA-evoked nociceptor discharge.

Subsequent experiments revealed that ketorolac (0.5 mM) had no effect on nociceptor discharge evoked by α,β-methylene adenosine triphosphate (ATP), 5-hydroxytryptamine or 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA). The inhibitory effect of ketorolac did not appear to be related to cyclooxygenase inhibition, as the concentration of prostaglandin E2 in the masticatory muscles 10 minutes after injection of either NSAID was not significantly decreased. The present study indicates that in vivo, ketorolac, but not naproxen, can antagonize NMDA-evoked nociceptor discharge similar to diclofenac. We speculate that structural similarities between ketorolac and diclofenac could account for the ability of these NSAIDs to inhibit NMDA-evoked
nociceptor discharge. These properties may partly explain the analgesic effect of intramuscularly injected ketorolac in the clinic.

**KEYWORDS**

Masseter Muscle; NMDA receptor; Nociceptor; Pain; Rat; Trigeminal
Concentrations of NSAIDs that far exceed the median effective concentration (EC$_{50}$) for inhibition of cyclooxygenase, a key enzyme in prostaglandin synthesis, are achieved when these drugs are applied topically or injected intramuscularly for treatment of musculoskeletal pain. For example, tissue concentrations in muscle and joints after topical administration of diclofenac are 10-20 times greater than those in the blood (Miyatake et al. 2009; Petersen and Rovati 2009; Zacher et al. 2008). Another NSAID, ketorolac tromethamine, is administered through intramuscular injection at concentrations up to 30 mg/ml (80 mM). The elevated concentrations achieved by local administration of NSAIDs suggest that mechanisms other than cyclooxygenase inhibition may contribute to the analgesic effect of these drugs in muscle tissue. Several lines of evidence indicate that at high concentration, some NSAIDs, like diclofenac and acetylsalicylic acid, can act like local anesthetics and block sodium channels (Brodin and Skoglund 1987; Cairns et al. 2008; Kuo et al. 2000; Riccioppo Neto 1980). It has recently been proposed that at least one NSAID, diclofenac, may act also to inhibit NMDA receptors that are expressed on endings of sensory fibers that innervate skeletal muscle (Dong et al. 2007; Gazerani et al. 2010).

We have recently reported that injection of 10 μl of 0.5 mM diclofenac into masticatory muscles of anesthetized rats (which we estimate will result in muscle concentrations in the 1-10 μM range), could competitively inhibit nociceptor discharge evoked by injection of NMDA (Dong et al. 2009). This action of diclofenac appears selective for the NMDA receptor, as diclofenac did not affect muscle nociceptor
discharge evoked by AMPA, a selective agonist for non-NMDA ionotropic glutamate receptors, or serotonin (5-HT) (Dong et al. 2009). Injection of diclofenac in combination with NMDA or by itself also resulted in a prolonged (> 30 min) increase in nociceptor mechanical threshold that was mimicked by intramuscular injection of the competitive NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid. Addition of prostaglandin E2 (PGE2), a potent algogen and inflammatory mediator, did not alter the effects of diclofenac on NMDA-evoked nociceptor discharge or mechanical threshold, which suggests that inhibition of PGE2 synthesis is not the mechanism by which diclofenac exerts these effects. Combined, these findings lead to the conclusion that diclofenac decreases the excitability of masticatory muscle nociceptors, in part, through inhibition of peripheral NMDA receptors.

It is unclear, at present, whether the ability of diclofenac to competitively inhibit NMDA receptors is a unique feature of the molecular structure of diclofenac, or whether some or all NSAIDs also affect the NMDA receptor. We speculate that the reason diclofenac appears to be capable of interacting with the NMDA receptor is the glutamate-like structural backbone of the diclofenac molecule. Other NSAIDs, such as ketorolac, appear to have a similar structure in their molecules, which may allow them to interact with the NMDA receptor. Selective competitive antagonists for the NMDA receptor are often conformationally constrained amino acid derivatives containing a ω-phosphonic group similar to the structures of diclofenac and ketorolac (43). Naproxen is an NSAID that does not share this structure, and thus would be predicted not to interact with the peripheral NMDA receptor. The aim of the current study was to determine if the
NSAIDs ketorolac and naproxen could exert peripheral NMDA receptor antagonism at concentrations that did not result in a non-specific “local anesthetic-like” effect.

METHODS

Surgical preparation

Adult male and female Sprague-Dawley rats were prepared for acute in vivo recording of trigeminal primary afferent fiber activity under surgical anesthesia as has been previously described in detail (Cairns et al. 2007; Cairns et al. 2002; Cairns et al. 2008; Dong et al. 2007). Briefly, under deep isoflurane anesthesia, each rat's head was placed in a Kopf stereotaxic frame and the skin over the dorsal surface of the skull reflected. A trephination was made on the right side of skull to allow a microelectrode to be lowered through the brain and into the trigeminal ganglion. In addition, an incision was made in the skin overlying the neck to expose the brainstem and upper cervical spinal cord. The dura overlying the brainstem/cervical spinal cord was removed to facilitate placement of a stimulating electrode in contact with the caudal brainstem. Upon completion of all surgical procedures, the isoflurane level was reduced to 2.0-2.5 % to maintain a continued absence of reflex response to noxious toe pinch. Heart rate, mean blood pressure, expired CO₂ and core body temperature were continuously monitored throughout the whole experiment. All procedures were performed in adherence with the principles of the Canadian Council on Animal Care and were approved by the University of British Columbia Animal Care Committee. Efforts were made to minimize animal suffering and to reduce the number of animals used as described in the subsequent sections.
Stimulation and recording techniques

Single trigeminal afferent fibers were recorded with a parylene coated tungsten microelectrode (A-M Systems, Carlsborg, WA, USA) that was lowered into the trigeminal ganglion. Afferent fibers that responded to mechanical stimuli applied with a blunt probe to the jaw-closer (temporalis or masseter) muscle and failed to respond to application of brush, pinch, or pressure stimuli directly to the overlying skin surface were considered to innervate muscle. Confirmation that the mechano-receptive field of the afferent fiber indeed lay within the jaw-closer muscle was further obtained by observing discharge in response to injection of known algogenic chemicals (NMDA, 5-HT, hypertonic saline) into the muscle. All afferent fibers examined in the present study were required to project to the caudal brainstem, since previous work has indicated a strong projection of putative jaw-closer muscle nociceptors to this region (Cairns and Dong 2008; Cairns et al. 2002; Cairns et al. 2008). Orthodromic action potentials evoked by mechanical stimulation of the jaw-closer muscle receptive field were collided with antidromic action potentials evoked by electrical stimulation of the caudal brainstem to confirm this projection. The straight-line distance between the stimulating and recording electrodes was divided by the antidromic latency to permit estimation of the conduction velocity (CV) of each nociceptor. To minimize the number of animals used, where possible, recordings from more than one jaw-closer muscle nociceptor were made in the same animal (one from the temporalis muscle and one from the masseter muscle). In these cases, at least one hour separated the two recording experiments.
Experimental protocol

In all experiments baseline nociceptor mechanical threshold was first obtained by applying a suprathreshold mechanical stimulus with an electronic von Frey hair (Woodland Hills, CA, USA; blunt polypropylene tip, diameter 0.5 mm) at 1-minute intervals for 10 minutes. A catheter needle connected to a Hamilton syringe was then inserted into the mechanoreceptive field of the nociceptor and used to inject compounds at room temperature (21 °C) in a total volume of 10 μl per injection. Prior to injection of any substance into the muscle, 10 minutes of ongoing nociceptor discharge was recorded as a baseline. At the end of this period, an initial injection was made and nociceptor discharge monitored for 10 minutes post injection. The purpose of this initial injection was to confirm that the nociceptor was excited by the algogenic substance being injected. The nociceptor discharge evoked by the first injection also acted as an internal control and could be compared with the discharge evoked by a second injection. Thirty minutes after the first injection, a second injection of the same substance (control) or a mixture of the neurotransmitter and a NSAID was made and nociceptor discharge again monitored for 10 minutes. At the end of each experiment, rats were euthanized with an overdose of pentobarbital (100 mg/kg i.v.).

We have previously found that repeated injection of AMPA did not result in reproducible nociceptor discharge (Dong et al. 2009) and in a pilot study with 5 nociceptors, found that αβ-methylene ATP, an ATP P2X3 receptor agonist, also was incapable of producing reproducible nociceptor discharge. As a result, the paradigm for these agonists was modified so that an initial injection of hypertonic saline (1 M, 10 μl), which served as the internal control, was followed 30 min later by an injection of AMPA.
(500 mM, ) alone or αβ-methylene ATP alone or in combination with the NSAIDs. In previous experiments, hypertonic saline has been shown to reliably evoke jaw-closer muscle nociceptor discharge (Cairns et al. 2008; Cairns et al. 2003; Mok et al. 2005).

Chemicals

The following excitants were used: 1 M sodium chloride (hypertonic saline), 500 mM NMDA, 500mM AMPA, 10 mM 5-HT, and 10 mM αβ-methylene ATP. The concentrations of excitants were chosen based on previous published research (Dong et al. 2009; Oliveira et al. 2005; Tashiro et al. 2007). The NSAIDs naproxen sodium (5 mM) and ketorolac tromethamine (0.05-5 mM) were used in various experiments. Aqueous solutions for intramuscular injection were adjusted to pH 7.0-7.4 prior to injection. Chemicals were acquired from Sigma Chemical Company (St. Louis, MO, USA).

Assessment of Prostaglandin E2 (PGE2) Concentration

Experiments in 4 male rats were done to determine the concentration of PGE2 after injection of saline (10 μl) and NMDA (500 mM; 10 μl) into the right and left temporalis muscle (n=2) and masseter muscle (n=2), respectively. Experiments in an additional 4 male rats were done to determine concentration of PGE2 after injection of saline, ketorolac (0.5 mM) and naproxen (5 mM) into the jaw closer muscles. To reduce the number of rats required in these experiments, each received 4 x10 μl injections, one each into the left and right temporalis and masseter muscles, with the injection sites rotated so that two muscles received saline injections and the contralateral muscles...
received either naproxen or ketorolac. For example, if the saline injections were made into the right temporalis and left masseter, then naproxen could be injected into the left temporalis, and ketorolac could be injected into the right masseter muscle. Ten minutes after injection, the rats were terminated with pentobarbital. Approximately 10 mm$^2$ of muscle tissue was harvested from around the injection site, which was marked on the overlying skin with a black marker. Muscle tissue was place in liquid nitrogen and stored at $-80$ °C. Tissue was homogenized with homogenization buffer (50mM Tris-HCl pH 7.5, 150mM NaCl, 1% Triton, 0.1% Sodium Dodecyl sulphate, 0.5% Sodium deoxycholate). Samples were centrifuged at $4$ °C for 15 minutes at 15000 revolutions per minute. The supernatant of the homogenate was collected and muscle protein concentration was determined using Bradford method (Bradford 1976). PGE$_2$ level in muscle tissue homogenate was measured by enzyme immunoassay (Assay Design, Ann Arbor, USA) according to manufacturers’ instructions. The sensitivity of kit was 8.26 pg/ml. Samples and standards were run in duplicate and were averaged. The concentration of PGE2 was determined per gram of muscle protein.

**Data analysis**

The activity of identified nociceptors was amplified and fed into a computer equipped with a 1401 Plus board and Spike 2 analysis software (Cambridge Electronic Design). Recorded nociceptor activity was stored electronically and analyzed off-line. The evoked response of each nociceptor was calculated by subtracting the total number of the spikes during the 10-minute period before the injection (baseline activity) from the total number of spikes during the 10-minute period after the injection. The relative
cumulative discharge of each nociceptor was calculated by dividing the cumulative
discharge evoked by the second injection by the cumulative discharge evoked by the first
injection (Cairns et al. 2008; Dong et al. 2007). The mean mechanical threshold (g) was
calculated from the average mechanical threshold values over a 10 minute period. PGE2
concentration from the homogenates was normalized to muscle protein concentration.

Statistics

Relative cumulative discharges were not normally distributed and thus significant
differences in the effect of treatment on relative cumulative responses were assessed with
either Mann-Whitney rank sum test for comparison of two medians, or Kruskal-Wallis
one-way Analysis of Variance (ANOVA) on ranks and post-hoc Dunn’s method, for
comparison of multiple medians. Differences in PGE2 concentration between NMDA or
NSAID and saline injected muscles were assessed with a paired t test. For data to which
non-parametric statistics were applied, the median and inter-quartile range was used to
measure central tendency. For all other data, the mean and standard error of the mean
(SEM) were used to measure central tendency. In all tests, the level of significance was
set at $P < 0.05$. 
RESULTS

Baseline Properties of Nociceptors

A total of 80 nociceptors were recorded from 67 male rats. Of these, 79 were A\\\(\delta\\) fibers (8.0 ± 0.3 m/s) and 1 was a C fiber (1.3 m/s). The mean baseline mechanical threshold of the A\\\(\delta\\) nociceptors was 22 ± 3 g, and the baseline mechanical threshold of the single C fiber was 81 g. A total of 15 nociceptors were also recorded from 12 female rats. All nociceptors had conduction velocities in the A\\\(\delta\\) range (6.1 ± 0.7 m/s). The mean baseline mechanical threshold was 33 ± 8 g.

Effect of Ketorolac and Naproxen on Hypertonic Saline-Evoked Nociceptor Discharge

Repeated injection of hypertonic saline resulted in relatively reproducible nociceptor discharge (Figure 1A). Co-injection of 5 mM ketorolac significantly decreased hypertonic saline-evoked nociceptor discharge (Figure 1B). Co-injection of a lower concentration of 0.5 mM ketorolac or 5 mM naproxen sodium had no significant effect of hypertonic saline-evoked nociceptor discharge. This result indicated that ketorolac could exert a non-specific, local anesthetic-like effect at a concentration of 5 mM. As a result, the effect of 0.5 mM ketorolac and 5 mM naproxen on NMDA-evoked nociceptor discharge was examined.

Effect of Ketorolac and Naproxen on NMDA-evoked Nociceptor Discharge

Repeated injection of NMDA resulted in relatively reproducible nociceptor discharge (Figure 2A). Co-injection of 0.5 mM ketorolac significantly decreased NMDA-evoked nociceptor discharge (Figure 2B). Co-injection of a lower concentration
of 0.05 mM ketorolac or 5 mM naproxen sodium had no significant effect of NMDA-evoked nociceptor discharge. This result indicated that ketorolac, but not naproxen, could inhibit activation of peripheral NMDA receptors.

Effect of NMDA, Ketorolac and Naproxen on PGE2 Concentration

PGE2 concentration assessed 10 minutes after muscle injections was not significantly affected by intramuscular injection of NMDA, ketorolac or naproxen sodium (Figure 3). These results suggested that altered PGE2 synthesis was not a major contributor to the effects of naproxen and ketorolac on nociceptor mechanical threshold.

Selectivity of Ketorolac for NMDA-evoked Discharge

The peri-stimulus histograms in Figure 4 illustrate nociceptor discharge evoked by injection of hypertonic saline, αβ-methylene ATP, AMPA and 5-HT into the masticator muscles. Both AMPA and αβ-methylene ATP were less effective than hypertonic saline in evoking nociceptor discharge. Note that all 3 excitants tended to evoke brief discharges. To test whether 0.5 mM ketorolac was selectively inhibiting NMDA-evoked nociceptor discharge, the effect of ketorolac on αβ-methylene ATP, AMPA and 5-HT evoked nociceptor discharge was investigated. Ketorolac had no significant effect on αβ-methylene ATP, AMPA or 5-HT-evoked nociceptor discharge (Figure 5). These findings indicate that ketorolac does not directly interact with P2X3, AMPA or 5-HT3 receptors.
Comparison with Female Rats

In females, repeated injection of NMDA also resulted in relatively reproducible nociceptor discharge (Figure 6A). Co-injection of 0.5 mM, but not 0.05 mM ketorolac significantly decreased NMDA-evoked nociceptor discharge ($p = 0.044$, Kruskal-Wallis One Way Analysis of Variance on Ranks, $P < 0.05$ Dunn’s post-hoc test). There was a significantly greater effect of ketorolac 0.5 mM on NMDA-evoked discharge in female rats than in male rats (Figure 6B).
DISCUSSION

The principal finding of this study was that, at concentrations that were devoid of local anesthetic-like effects, ketorolac, but not naproxen, could inhibit NMDA-evoked nociceptor discharge. This effect of ketorolac appeared to be selective for peripheral NMDA receptors, as ketorolac did not significantly affect masticatory muscle nociceptor discharge evoked by αβ-methylene ATP, AMPA or 5-HT, suggesting that it did not block P2X3, non-NMDA glutamate receptors (GluRs) or 5-HT3 receptors (Ambalavanar et al. 2005; Sahara et al. 1997; Sung et al. 2008). The inhibitory effects of ketorolac on NMDA-evoked discharge do not appear to involve cyclooxygenase inhibition, as injection of NMDA alone did not significantly increase and neither ketorolac nor naproxen significantly lowered levels of PGE2, a potent sensitizer of muscle nociceptors (Dong et al. 2009; Mense 1981). Ketorolac also appeared to exert a greater effect on nociceptor discharge induced by activation of peripheral NMDA receptors in female than in male rats, which indicates that there could be important sex-related differences in the efficacy of this analgesic for muscle pain. As women have been found to be more likely than men to suffer from chronic masticatory muscle pain (LeResche 1997; LeResche et al. 2007; Nilsson et al. 2009), this finding suggests that future studies are warranted to investigate the usefulness of injectable ketorolac to treat masticatory muscle pain associated with a temporomandibular disorder.

Local Anesthetic-like Effects of Ketorolac.

NSAIDs such as acetylsalicylic acid (10-20 mM) and diclofenac (~100 μM) block sodium channels and act like local anesthetics to attenuate action potential...
conduction through nerve fibers in vitro (Brodin and Skoglund 1987; Kuo et al. 2000; Lee et al. 2003; Riccioppo Neto 1980). We have reported that injection of diclofenac (5 mM; 10 μl) into the rat masseter muscle blocks hypertonic saline evoked nociceptor discharge, suggesting that diclofenac can exert a “local anesthetic-like” effect at this high concentration (Cairns et al. 2008). In the present study, ketorolac (5 mM) was also found to exert a similar inhibitory effect on hypertonic saline evoked nociceptor discharge, while naproxen (5 mM) did not. It has been found that a number of drugs, such as the antihistamine diphenhydramine and the anticonvulsant phenytoin, which exert sodium channel blocking ability, share a structure which has two phenyl or similar ring structures joined by either carbon-carbon or carbon-nitrogen bonds (Kuo et al. 2000). This structural similarity is shared by diclofenac and ketorolac, but not naproxen, which may explain the lack of effect of naproxen on hypertonic saline-evoked nociceptor discharge (Figure 7).

Ketorolac tromethamine intramuscular injection for human use has a concentration of up to 80 mM (30 mg/ml), which our present results suggest would be more than sufficient to exert a local anesthetic-like action. We have examined the effect of ketorolac on hypertonic saline-evoked masseter muscle pain in healthy women (Bendixen et al. 2010). Co-injection of either ketorolac 40 mM (15 mg/ml) or lidocaine 80 mM (20 mg/ml) significantly reduced the overall pain ratings of subjects to hypertonic saline injections. These findings indicate that ketorolac can exert a local anesthetic-like effect when high concentrations of the drug are administered to the human masseter muscle and this effect may be responsible, in part, for the relatively infrequent reports of pain at the injection site when this drug is used as an intramuscular analgesic.
Selective Peripheral NMDA Receptor Antagonism.

Ketorolac has been found to significantly attenuate neuronal discharge evoked by microiontophoretic application of NMDA onto spinal cord wide dynamic range (WDR) neurons thought to be involved in the central processing of nociceptive input (Sotgiu et al. 1998). The receptor mechanism underlying this effect of ketorolac on NMDA-evoked spinal cord WDR neuronal discharge has not been determined. However, binding studies indicate that ketorolac (10 μM) could modestly displace both competitive and non-competitive NMDA receptor antagonists, which indicates the potential for a receptor interaction (Jett et al. 1999). We have recently reported that diclofenac, when injected at a concentration of ~0.5 mM into the masticatory muscles of anesthetized rats could selectively inhibit nociceptor discharge evoked by injection of NMDA (Dong et al. 2009). In the present study, the effect of a similar concentration of ketorolac also appeared to be selective for the NMDA receptor, as it did not affect muscle nociceptor discharge evoked by AMPA, a selective agonist for GluRs. It also failed to significantly attenuate 5-HT-evoked nociceptor discharge, which we have shown is mediated primarily through activation of the 5-HT3 receptor, a non-selective cation channel, further demonstrating the selectivity of ketorolac for peripheral NMDA receptors (Sung et al. 2008). The P2X3 receptor is an ATP-activated non-selective cation channel that has been found to be expressed by trigeminal afferent fibers and can be activated to produce nocifensive behaviors (Dessem et al. 2010; Shinoda et al. 2008; Teixeira et al. 2010). Ketorolac had no affect on nociceptor discharge evoked by αβ-methylene ATP, a selective agonist for this receptor. Taken together, these results indicate that like diclofenac, ketorolac is able to selectively antagonize peripheral NMDA receptors in rat
masticatory muscle and suggest that this mechanism may contribute to the local analgesic effect of injected ketorolac (Dong et al. 2009).

Sex-related Differences.

Our results indicate that ketorolac was more effective against NMDA-evoked muscle nociceptor discharge in female than in male rats. While the expression of peripheral NMDA receptors is greater in female than in male rats, sex-related differences in the efficacy of the NMDA receptor antagonists ketamine and ifenprodil to attenuate NMDA-evoked muscle nociceptor discharge have not been found (Dong et al. 2007; McRoberts et al. 2007). This suggests that sex-related differences in the effect of ketorolac may not be due to its NMDA receptor antagonist properties. Weak clinical evidence also supports the idea that ketorolac may be more effective in women. A trend toward better analgesia with oral ketorolac in women than in men was found in a human study examining cold pressor pain tolerance, although overall ketorolac was not better than placebo at attenuating this pain in either sex (Compton et al. 2003). Studies which use pain models in which ketorolac is effective are needed to test the concept that ketorolac may be more effective in women.

Clinical Implications.

NSAIDs are widely used to treat masticatory muscle pain associated with myofascial temporomandibular disorders despite the fact that this pain is often not associated with obvious injury or inflammation (Cairns 2010). The results of the present study suggest that ketorolac injections have several mechanisms other than
cyclooxygenase inhibition which may make this NSAID particularly effective for acute and localized temporomandibular disorders related jaw muscle pain. Importantly, recent evidence suggests that interstitial concentrations of glutamate are elevated in the masseter muscle of myofascial temporomandibular disorders patients and that elevated concentrations of glutamate produce pain and sensitization in humans through activation of peripheral NMDA receptors (Cairns et al. 2006; Castrillon et al. 2008; Castrillon et al. 2010; Svensson et al. 2003). Thus, the ability of ketorolac to inhibit peripheral NMDA receptors at concentrations achievable with the injectable product suggests that this NSAID might have improved efficacy against this type of jaw muscle pain. We propose that clinical trials of ketorolac for local muscle pain related to myofascial temporomandibular disorders are warranted.

Acknowledgements: This research was supported by a Canadian Institutes of Health Research grant (MOP 77538) and a Canada Research Chair (BEC). The authors do not have any conflicts of interest related to this work.
REFERENCES


Cairns BE, Mann MK, Mok E, Dong XD, Svensson P. Diclofenac exerts local
anesthetic-like actions on rat masseter muscle afferent fibers. *Brain Res* 1194:
56-64, 2008.

L. Ketamine attenuates glutamate-induced mechanical sensitization of the

CB, Arendt-Nielsen L. Activation of peripheral NMDA receptors contributes to
human pain and rat afferent discharges evoked by injection of glutamate into the

Castrillon EE, Cairns BE, Ernberg M, Wang K, Sessle BJ, Arendt-Nielsen L,
Svensson P. Glutamate-evoked jaw muscle pain as a model of persistent

Castrillon EE, Ernberg M, Cairns BE, Wang K, Sessle BJ, Arendt-Nielsen L,
Svensson P. Interstitial glutamate concentration is elevated in the masseter
muscle of myofascial temporomandibular disorder patients. *J Orofac Pain* 24:

Compton P, Charuvastra VC, Ling W. Effect of oral ketorolac and gender on human

Dessem D, Ambalavanar R, Evancho M, Moutanni A, Yallampalli C, Bai G.
Eccentric muscle contraction and stretching evoke mechanical hyperalgesia and
modulate CGRP and P2X(3) expression in a functionally relevant manner. *Pain*
Dong X-D, Svensson P, Cairns BE. The analgesic action of topical diclofenac may be mediated through peripheral NMDA receptor antagonism Pain 147: 36-45, 2009.


FIGURE LEGENDS

**Figure 1.** A-D. The peristimulus histograms illustrate examples of action potential discharge evoked in response to hypertonic saline (HS), followed 30 minutes (1800s) later by a second injection of HS alone (A), or in combination with ketorolac (B: K5 = 5 mM, C: K0.5 = 0.5 mM) or naproxen (D: N5 = 5 mM) in male rats. A. The relative cumulative discharge for this fiber (CV: 11.0 m/s) fiber was 0.97. B. The relative cumulative discharge for this fiber (CV: 6.5 m/s) fiber was 0.00. C. The relative cumulative discharge for this fiber (CV: 11.5 m/s) fiber was 0.82. D. The relative cumulative discharge for this fiber (CV: 9.8 m/s) fiber was 1.10. E. The vertical bar chart compares the median (lines: interquartile range) relative cumulative discharge (n=6 nociceptors per treatment group, 24 total). Only co-injection 5 mM ketorolac significantly attenuated HS-evoked nociceptor discharge. These results suggest that ketorolac can exert a local anesthetic action at high concentration. *: P <0.05, Kruskal-Wallis One Way ANOVA on Ranks, Dunn’s post-hoc test.

**Figure 2.** A-D. The peristimulus histograms illustrate examples of action potential discharge evoked in response to NMDA, followed 30 minutes (1800s) later by a second injection of NMDA alone (A), or in combination with ketorolac (B: K0.5 = 0.5 mM, C: K0.5 = 0.05mM) or naproxen (D: N5 = 5.0 mM) in male rats. A. The relative cumulative discharge for this fiber (CV: 8.9 m/s) fiber was 0.83. B. The relative cumulative discharge for this fiber (CV: 8.8 m/s) fiber was 0.46. C. The relative
cumulative discharge for this fiber (CV: 8.6 m/s) fiber was 0.80.  

D.  The relative cumulative discharge for this fiber (CV: 9.2 m/s) fiber was 0.84.  

E.  The vertical bar chart compares the median (lines: interquartile range) relative cumulative discharge (n=5 nociceptors per treatment group, 20 total).  Co-injection of ketorolac (0.5 mM) with NMDA significantly attenuated NMDA-evoked nociceptor discharge.  In contrast, 5mM naproxen had no effect on NMDA-evoked nociceptor discharge.  *:  P < 0.05, Kruskal-Wallis One Way ANOVA on Ranks, Dunn’s post-hoc test.

Figure 3.  A.  The bar graph indicates the mean (±SEM) PGE2 concentrations in the masticatory muscles of 4 male rats.  There was no significant effect of injection of NMDA on PGE2 concentrations after 10 minutes.  B.  The bar graph indicates the mean (±SEM) PGE2 concentrations in the masticatory muscles of 4 additional male rats.  There was no significant effect of injection of either ketorolac (0.5 mM) or naproxen (5 mM) on PGE2 concentrations after 10 minutes.

Figure 4.  A.  The peristimulus histogram illustrates action potential discharge evoked in an Aδ fiber (CV: 7.2 m/s) by injection of hypertonic saline (HS; 0s) followed by ATP (1800s) into the temporalis muscle.  A time expanded view of the ATP-evoked discharge is shown to the right.  The relative cumulative discharge for this particular fiber was 0.49.  B.  The peristimulus histogram illustrates action potential discharge evoked in an Aδ fiber (CV: 7.9 m/s) by injection of HS (0s) followed by AMPA (1800s) into the temporalis muscle.  A time expanded view of the AMPA-evoked discharge is shown to the right.  The relative cumulative discharge for this particular fiber was 0.17.  C.  The
peri-stimulus histogram illustrates action potential discharge evoked in an Aδ fiber (CV: 7.8 m/s) by injection of 5HT at times 0 and 1800 into the masseter muscle. A time expanded view of the 5HT-evoked discharge is shown to the right. The relative cumulative discharge for this particular fiber was 0.57.

**Figure 5.** The vertical bar chart shows the median (lines: interquartile range) relative cumulative nociceptor discharge evoked by injection of ATP, AMPA or 5HT alone compared with the relative cumulative discharge evoked when ketorolac (K0.5 = 0.5 mM) was added to the injection (n=6 nociceptors per treatment group, 36 total) in male rats. Co-injection of ketorolac had no significant effect on nociceptor discharge evoked by any of the 3 substances.

**Figure 6.** A. The vertical bar chart compares the median (lines: interquartile range) relative cumulative discharge evoked by repeated injection of NMDA alone or when ketorolac (K0.5 = 0.5 mM, K0.05 = 0.05 mM) was added to the second injection of NMDA (n=5 nociceptors per treatment group, 15 total) in female rats. Co-injection of ketorolac (0.5 mM) with NMDA significantly attenuated NMDA-evoked nociceptor discharge. *: P < 0.05, Kruskal-Wallis One Way ANOVA on Ranks, Dunn’s post-hoc test. B. The vertical bar chart illustrates the median relative NMDA-evoked discharge in male (n=5 nociceptors per bar) rats compared with female rats. Ketorolac (0.5 mM) exerted a significantly greater suppression of NMDA-evoked discharge in female rats than in male rats. #p<0.05 Mann Whitney rank sum Test.
Figure 7: The structures of glutamate, NMDA, and the NSAIDS diclofenac, ketorolac and naproxen are illustrated. The dark line indicates a glutamate-like (diclofenac) or NMDA-like (ketorolac) structure within the drug molecule which could explain the ability of these NSAIDs to inhibit peripheral NMDA receptor activation. Naproxen lacks this structure and was not found to inhibit peripheral NMDA receptors.
Figure 1
Figure 4

A. HS/ATP

B. HS/AMPA

C. 5HT/5HT
Figure 6

A. Female

B. Relative Discharge

Ketorolac (mM)

- NMDA
- NMDA & K-0.5
- NMDA & K-0.05

Treatment Group

* #