Intramuscular ketorolac inhibits activation of rat peripheral NMDA receptors

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Submitted 7 December 2011; accepted in final form 5 March 2012

Intramuscular ketorolac inhibits activation of rat peripheral NMDA receptors. J Neurophysiol 107: 3308–3315, 2012. First published March 7, 2012; doi:10.1152/jn.01118.2011.—The nonsteroidal 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 shared by other NSAIDs. This study was undertaken to determine whether intramuscular injection of ketorolac or naproxen at concentrations that do not induce 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 nociceptor 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 αβ-methylene ATP, 5-hydroxytryptamine, or AMPA. The inhibitory effect of ketorolac did not appear to be related to cyclooxygenase inhibition, because the concentration of prostaglandin E2 in the masticatory muscles 10 min 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 similarly 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.

Masseter muscle; N-methyl-D-aspartate receptor; pain; trigeminal

NONSTEROIDAL ANTI-INFLAMMATORY DRUG (NSAID) concentrations that far exceed the median effective concentration 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 concentrations, some NSAIDs, such as 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, also may act to inhibit N-methyl-D-aspartate (NMDA) receptors that are expressed on endings of sensory fibers that innervate skeletal muscle (Dong et al. 2007; Gazerani et al. 2010).

We recently reported that injection of 10 μl of 0.5 mM diclofenac into masticatory muscles of anesthetized rats (which we estimated would 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, because diclofenac did not affect muscle nociceptor discharge evoked by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (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 whether the NSAIDs ketorolac and naproxen could exert peripheral NMDA receptor antagonism at
concentrations that did not result in a nonspecific “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. 2002, 2007, 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 brain stem and upper cervical spinal cord. The dura overlying the brain stem/cervical spinal cord was removed to facilitate placement of a stimulating electrode in contact with the caudal brain stem. 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 subsequently.

Stimulation and recording techniques. Single trigeminal afferent fibers were recorded with a parylene-coated tungsten microelectrode (A-M Systems, Carlsborg, WA) 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 mechanoreceptive 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 brain stem, 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, 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 brain stem 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

Fig. 1. A–D: peristimulus histograms illustrate examples of action potential discharge evoked in response to hypertonic saline (HS), followed 30 min (1,800 s) 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 [conduction velocity (CV) = 11.0 m/s] was 0.97. B: the relative cumulative discharge for this fiber (CV = 6.5 m/s) was 0.00. C: the relative cumulative discharge for this fiber (CV = 11.5 m/s) was 0.82. D: the relative cumulative discharge for this fiber (CV = 9.8 m/s) was 1.10. E: vertical bar chart compares the median (lines indicate interquartile range) relative cumulative discharge (n = 6 nociceptors per treatment group, 24 total). Only co-injection of 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 1-way ANOVA on ranks, Dunn’s post hoc test.
same animal (1 from the temporalis muscle and 1 from the masseter muscle). In these cases, at least 1 h 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; blunt polypropylene tip, diameter 0.5 mm) at 1-min intervals for 10 min. 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. Before injection of any substance into the muscle, 10 min of ongoing nociceptor discharge were recorded as a baseline. At the end of this period, an initial injection was made and nociceptor discharge was monitored for 10 min postinjection. 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 an NSAID was made, and nociceptor discharge was again monitored for 10 min. At the end of each experiment, rats were euthanized with an overdose of pentobarbital sodium (100 mg/kg iv).

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 five nociceptors, we found that αβ-methylene ATP, an ATP P2X<sub>1</sub> 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 by 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. 2003, 2008; Mok et al. 2005).

**Chemicals.** The following excitants were used: 1 M sodium chloride (hypertonic saline), 500 mM NMDA, 500 mM AMPA, 10 mM 5-HT, and 10 mM αβ-methylene ATP. The concentrations of excitants were chosen on the basis of 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 before injection. Chemicals were acquired from Sigma Chemical (St. Louis, MO).

**Assessment of PGE<sub>2</sub> concentration.** Experiments in four male rats were done to determine the concentration of PGE<sub>2</sub> 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 four male rats were done to determine concentration of PGE<sub>2</sub> 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 four 10-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...
KETOROLAC ATTENUATES NOCICEPTOR DISCHARGE

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Effect of ketorolac and naproxen on hypertonic saline-evoked nociceptor discharge. Repeated injection of hypertonic saline resulted in relatively reproducible nociceptor discharge (Fig. 1A). Co-injection of 5 mM ketorolac significantly decreased hypertonic saline-evoked nociceptor discharge (Fig. 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 nonspecific, 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.

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Effect of NMDA, ketorolac, and naproxen on PGE2 concentration. PGE2 concentration assessed 10 min after muscle injections was not significantly affected by intramuscular injection of NMDA, ketorolac, or naproxen sodium (Fig. 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 peristimulus histograms in Fig. 4 illustrate nociceptor discharge evoked by injection of hypertonic saline, αβ-methylene glyoxalate, and NMDA. Co-injection of ketorolac but not naproxen or placebo inhibited both saline and NMDA-evoked nociceptor discharge. This result indicated that ketorolac specifically inhibited NMDA-evoked nociceptor discharge and that ketorolac had no effect on saline-evoked nociceptor discharge.

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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 three 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 (Fig. 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 (Fig. 6A). Co-injection of 0.5 mM, but not 0.05 mM ketorolac, significantly decreased NMDA-evoked nociceptor discharge \((P = 0.044, \text{Kruskal-Wallis 1-way ANOVA on ranks}; P < 0.05, \text{Dunn’s post hoc test})\). There was a significantly greater effect of 0.5 mM ketorolac on NMDA-evoked discharge in female rats than in male rats (Fig. 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, because 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

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**Fig. 4.** A: peristimulus histogram illustrates action potential discharge evoked in an Aδ fiber \((CV = 7.2 \text{ m/s})\) by injection of HS \((0 \text{ s})\) followed by ATP \((1,800 \text{ s})\) into the temporalis muscle. A time-expanded view of the ATP-evoked discharge is shown at right. The relative cumulative discharge for this particular fiber was 0.49. B: peristimulus histogram illustrates action potential discharge evoked in an Aδ fiber \((CV = 7.8 \text{ m/s})\) by injection of HS \((0 \text{ s})\) followed by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; 1,800 s) into the temporalis muscle. A time-expanded view of the AMPA-evoked discharge is shown at right. The relative cumulative discharge for this particular fiber was 0.17. C: peristimulus histogram illustrates action potential discharge evoked in an Aδ fiber \((CV = 7.8 \text{ m/s})\) by injection of serotonin (5-HT) at 0 and 1,800 s into the masseter muscle. A time-expanded view of the 5-HT-evoked discharge is shown at right. The relative cumulative discharge for this particular fiber was 0.57.

**Fig. 5.** Vertical bar chart shows the median (lines indicate interquartile range) relative cumulative nociceptor discharge evoked by injection of ATP, AMPA, or 5-HT alone compared with the relative cumulative discharge evoked when ketorolac \((0.5 \text{ mM})\) was added to the injection \((n = 6 \text{ nociceptors per treatment group, 36 total})\) in male rats. Co-injection of ketorolac had no significant effect on nociceptor discharge evoked by any of the 3 substances.
5-HT$_3$ 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, because injection of NMDA alone did not significantly increase, and neither ketorolac nor naproxen significantly lowered, levels of PGE$_2$, 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. Given that 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, whereas 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 that 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 by naproxen, which may explain the lack of effect of naproxen on hypertonic saline-evoked nociceptor discharge (Fig. 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 40 mM (15 mg/ml) ketorolac or 80 mM (20 mg/ml) lidocaine 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 noncompetitive 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, because 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 nonselective cation channel, further demonstrating the selectivity of ketorolac for peripheral NMDA receptors (Sung et al. 2008). The P2X3 receptor is an ATP-activated nonselective cation channel, further demonstrating the selectivity of ketorolac for 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. Although 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 that 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 disorder-related jaw muscle pain. Importantly, recent evidence suggests that interstitial concentrations of glutamate are elevated in the masseter muscle of myofascial temporomandibular disorder 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, 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.

GRANTS

This research was supported by Canadian Institutes of Health Research Grant MOP 77538 and a Canada Research Chair (to B. E. Cairns).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


KETOROLAC ATTENUATES NOCICEPTOR DISCHARGE


