ACTIVATION OF PERIPHERAL NMDA RECEPTORS CONTRIBUTES TO HUMAN PAIN AND RAT AFFERENT DISCHARGES EVOKED BY INJECTION OF GLUTAMATE INTO THE MASSETER MUSCLE

1 Brian E. Cairns, 2 Peter Svensson, 3 Kelun Wang, 4 Steen Hupfeld, 3 Thomas Graven-Nielsen, 5 Barry J. Sessle, 1 Charles B. Berde and 3 Lars Arendt-Nielsen.

1 Department of Anesthesia, Harvard Medical School/Children’s Hospital, Boston Massachusetts, 02115, USA
2 Department of Clinical Oral Physiology, Dental School, Århus University, DK-8000 Århus C, Denmark
3 Laboratory for Experimental Pain Research, Center for Sensory-Motor Interaction, Aalborg University, DK-9220, Denmark
4 Department of Anesthesia, Aalborg Hospital, DK-9000 Aalborg, Denmark
5 Faculty of Dentistry, The University of Toronto, Toronto, Ontario, M5G 1G6 Canada

Running head: Peripheral NMDA Receptors & Glutamate-Evoked Muscle Pain

Abstract: 239 words

Figures: 5

Correspondence should be addressed to:

Professor Lars Arendt-Nielsen, Ph.D., Dr.Med.Sci
Laboratory for Experimental Pain Research
Center for Sensory-Motor Interaction
Aalborg University, Fredrik Bajers Vej 7D3, DK-9220, Denmark
Phone: +45 96358830
Fax: +45 98154008
Email: LAN@smi.auc.dk
Abstract

Peripheral N-methyl-d-aspartate (NMDA) receptors are found in deep tissues and may play a role in deep tissue pain. Injection of the endogenous NMDA receptor agonist glutamate into the masseter muscle excites deep craniofacial afferent fibers in rats and evokes pain in human subjects. It is not clear whether peripheral NMDA receptors play a role in these effects of glutamate. Accordingly, the effect of NMDA on afferent activity as well as the effect of locally administered NMDA receptor antagonists on glutamate-evoked afferent discharges in acutely anesthetized rats and muscle pain in human subjects was examined. Injection of NMDA into the masseter muscle evoked afferent discharges in a concentration-related manner. It was found that the NMDA receptor antagonists 2-amino-5-phosphonvalerate (APV, 10 mM), ketamine (10 mM) and dextromethorphan (40 mM) significantly decreased glutamate-evoked afferent discharges. The effects of APV and ketamine, but not dextromethorphan, were selective for glutamate-evoked afferent discharges and did not affect hypertonic saline-evoked afferent discharges. In human experiments, it was found that 10 mM ketamine decreased glutamate-evoked muscle pain but had no effect on hypertonic saline-evoked muscle pain. These results indicate that injection of glutamate into the masseter muscle evokes afferent discharges in rats and muscle pain in humans in part through activation of peripheral NMDA receptors. It is conceivable that activation of peripheral NMDA receptors may contribute to masticatory muscle pain and that peripherally-acting NMDA receptor antagonists could prove to be effective analgesics for this type of pain.
Introduction:

The excitatory amino acid glutamate is considered important for nociceptive transmission in the central nervous system (CNS) in part through its activation of N-methyl-d-aspartate (NMDA) receptors, and NMDA receptor activation in the CNS has been shown to play a critical role in the perception of pain arising from deep tissues such as muscles and joints (Cairns et al. 2001c; Ren and Dubner 1999; Schaible et al. 2002; Sessle 2000; Woolf and Thompson 1991). Clinically available NMDA receptor antagonists such as ketamine and dextromethorphan have been demonstrated to be efficacious for the treatment of deep tissue pain in both clinical and experimental research (Gordon et al. 1999; Graven-Nielsen et al. 2000; Henriksson and Sorensen 2002; Mathisen et al. 1995; Nikolajsen et al. 1996). However, their use is often limited by central side effects that include blurred vision, sedation and distorted sensory perception.

It has been speculated that peripheral NMDA receptors may also play a role in cutaneous and deep tissue pain and as such may be a useful target for the development of novel analgesic drugs (Alfredson et al. 2001; Alfredson and Lorentzon 2002; Cairns et al. 1998; Carlton and Coggeshall 1999; Carlton et al. 1995, 2003; Lawand et al. 1997; McRoberts et al. 2001; You et al. 2002). Indeed, it has been recognized that NMDA receptors are also found in deep tissues in association with the terminal endings of nerve fibers (Alfredson et al. 2001; Alfredson and Lorentzon 2002; McRoberts et al. 2001). Further, injection of glutamate into deep craniofacial tissues such as the temporomandibular (jaw) joint or masseter (jaw-closer) muscle excites nociceptive afferent fibers (Cairns et al. 2001 a, b, 2002). Parallel experiments in human subjects indicate that glutamate
injection into the masseter muscle evokes an aching-like pain which lasts for 5-10 min post-injection (Cairns et al. 2001a, 2003b; Svensson et al. 2003). Nevertheless, afferent fiber discharges and muscle pain are only evoked after relatively high concentrations of glutamate (> 100 mM) are injected into deep craniofacial tissues, which may mean that non-specific mechanisms, rather than NMDA receptor activation per se, are responsible for these effects of glutamate. The present investigation compared the effectiveness of NMDA receptor antagonists in attenuating glutamate- and hypertonic saline-evoked masseter muscle afferent fiber discharges in rats and masseter muscle pain in humans, to determine whether injection of glutamate into the masseter muscle activates peripheral NMDA receptors.
Materials and Methods:

Rat Experiments

Animal Preparation: Adult Sprague-Dawley rats of either sex (weight: 225-385 g) were prepared for acute in vivo recording of trigeminal afferent discharges (Cairns et al. 2001a,b, 2002, 2003a). Gas anesthesia (O2: 0.3-0.4 l/min; isoflurane 2.0-2.5%) was administered through a tracheal cannula. Heart rate and body core temperature were continuously monitored throughout the whole experiment and kept within the physiological range of 330-430/min and 36.8-37.1 °C, respectively. The Boston Children’s Hospital Animal Care and Use Committee approved all surgeries and procedures.

Stimulation and Recording Techniques: Single trigeminal afferent unit activity within the trigeminal ganglion was recorded by a parylene-coated tungsten microelectrode (2 MΩ, A-M Systems Inc., Carlsborg, USA). A blunt probe was applied as a mechanical search stimulus to the skin over the masseter muscle while the electrode was slowly lowered in an attempt to identify trigeminal afferent fibers with a muscle mechanoreceptive field. When a unit was found that appeared to respond to mechanical stimulation of the masseter muscle, the skin overlying the mechanoreceptive field was pulled gently away from contact with the muscle, and brush, pinch, and pressure stimuli were applied directly to the skin surface. If the unit did not respond to any of these cutaneous stimuli, then the mechanoreceptive field was considered to lie within the muscle. Semmes-Weinstein monofilaments (Stoelting, Wood Dale II, USA) were used to estimate the mechanical activation threshold of afferent fibers.
To confirm that masseter muscle afferent fibers in the current study projected to the caudal brainstem, constant-current electrical stimuli (50 µsec biphasic pulse, range 10-80 µA, 0.5 Hz) were applied to a stimulating electrode (2MΩ, parylene-coated tungsten electrode, A-M Systems Inc., Carlsborg, USA) lowered into the ipsilateral caudal brainstem to evoke antidromic action potentials (Cairns et al. 2001a, b, 2002, 2003a). Antidromic action potentials were collided with orthodromic action potentials evoked by mechanical stimulation of the masseter muscle to confirm the projection of the masseter muscle afferent fiber to the caudal brainstem. At the end of the experiment, the distance between the stimulating and recording electrodes was measured, and divided by the latency of the antidromically-evoked response of an afferent fiber to give an estimation of the conduction velocity (CV) of the recorded afferent fiber.

After the above characterization of each afferent fiber was completed, the needle tip of a catheter (a 27-gauge needle connected by polyethylene tubing to a Hamilton syringe, 50 µl) was carefully inserted into the afferent receptive field in the masseter muscle (Cairns et al. 2001a, b, 2002). This catheter was used to inject 10µl of NMDA (0.5, 5, 50, or 500 mM) in order to assess if a concentration-response relationship exists. The catheter was also used to inject 10µl of glutamate (500 mM) or hypertonic saline (1.0 M) alone or in combination with the NMDA receptor antagonists 2-amino-5-phosphonvalerate (APV; 1, 10 or 100 mM), ketamine (1, 10 or 20 mM) or dextromethorphan (1, 10 or 40 mM). All solutions were adjusted to ~ pH 7.0 prior to injection. Chemicals were obtained from Sigma (St. Louis, MO).
Baseline primary afferent fiber activity was recorded for 10 min prior to injection of any substance into the masseter muscle. NMDA, glutamate or hypertonic saline was then slowly injected into the masseter muscle (over a 5 sec period) and any resulting afferent activity monitored for 10 min after the injection. A second injection into the masseter muscle of glutamate or hypertonic saline alone or combined with the NMDA receptor antagonists was made 30 min after the initial injection, and any resulting afferent activity again monitored for 10 min after the injection.

Data Analysis: The activity of identified afferent fibers was amplified (gain: 1000x; bandwidth 30-1,000 Hz) and fed into a computer equipped with a CED 1401 Plus board and analysis software (Spike 2; Cambridge Electronic Design, Cambridge, U.K.). Cumulative afferent discharges were calculated by subtracting the summed action potential discharges for 10 min after injection from the baseline (spontaneous) afferent discharges 10 min prior to injection. The cumulative afferent discharges evoked by the second injection were divided by the cumulative afferent discharges evoked by the first injection to yield relative cumulative activity.

Human Experiments

Subjects: Fourteen male human volunteers (mean age ± SD: 27±5 years) took part in a double-blind, crossover experiment to investigate whether 10 mM ketamine would selectively block glutamate-evoked masseter muscle pain as opposed to pain evoked by hypertonic saline. The subjects had no signs or symptoms of temporomandibular
disorders (Dworkin and LeResche 1992) and were recruited from university students. The study protocol was approved by the local ethics committee in Denmark (Counties of Nordjylland and Viborg) and followed the guidelines set out by the Helsinki Declaration.

*Pressure pain thresholds:* A pressure algometer (1 cm² probe area, Somedic, Sweden) was used to measure pressure pain thresholds by applying it to the masseter muscle at 30 kPa/s while the subjects kept their jaw at rest (Svensson et al. 2003). A window display on the pressure algometer aided in the application of pressure at a constant rate. Pressure pain thresholds were determined for both the left and right masseter muscles prior to injection of any solutions. At the end of the experimental session the pressure pain threshold for the left masseter muscle was again determined, and then ketamine (10 mM, 0.2 ml, Ketalar, Park Davis) alone was injected into the left masseter muscle. Five minutes after injection of ketamine, the pressure pain threshold for the left masseter muscle was again determined.

Sterile solutions of glutamate (0.2 ml; 1.0 M; pH = 7.2) or buffered hypertonic saline (0.2 ml; 1.0 M; pH = 7.4) alone or in combination with ketamine (10 mM; Ketalar, Park Davis) were injected over a 10 sec period with a 27-gauge hypodermic needle and disposable syringe into the right masseter muscle midway between its upper and lower border and 1 cm posterior to its anterior border (Svensson et al. 1995). Ketamine alone was injected into the left masseter muscle to test its effect on pressure pain threshold. In all experiments, subjects were given standardized instructions and both subjects and examiners were unaware of which solution was about to be injected. During each
session, two injections into the masseter muscle were made 30 min apart. All subjects participated in 4 sessions; each session was separated by a minimum of 1 week.

The subjects were instructed to continuously rate the pain evoked by each injection of glutamate or hypertonic saline on an electronic visual analogue scale (VAS). A computer sampled the VAS signals every 2 sec. The lower endpoint of the VAS was labeled "no pain at all" and the upper endpoint labeled "the most pain imaginable". Peak pain was measured as the peak VAS score, the area under the VAS curve was used to obtain a measure of the overall amount of pain, and the onset to offset of pain was determined from the VAS profiles and used as a measure of pain duration.

**Statistics**

In rats, since relative cumulative activity data were not normally distributed, a Kruskal-Wallis ANOVA on ranks and post-hoc Dunn’s test were used to assess statistical differences. The Mann-Whitney test was employed to compare mechanical thresholds and chemically-evoked responses between males and female rats. A Spearman correlation was performed to assess the relationship between mechanical threshold and afferent CV, CV and cumulative afferent discharges as well as NMDA, glutamate or hypertonic saline-evoked cumulative afferent discharges and mechanical threshold. Rat afferent fiber data (force, pressure, CV, discharges) in the text is the median and interquartile range (square brackets).
In the human experiments pressure pain thresholds before and after injection of ketamine alone were analyzed with a paired t-test and the peak, duration and overall pain were analyzed with repeated measures ANOVA and Student-Neuman-Keuls test. In all tests, the level of significance was set at P < 0.05.

Results:

Rat Experiments

The characteristics of 187 individual masseter muscle afferent fibers (n=101 rats), which projected to the caudal brainstem and responded to an initial injection of glutamate, NMDA or hypertonic saline, are consistent with our previous findings (Cairns et al. 2001 a, b, 2002, 2003a). All of these fibers had conduction velocities below 25 m/sec. Only 6% (n = 11) were C fibers (< 2.5 m/sec), 59% (n=111) were slow Aδ fibers (≥ 2.5- < 10 m/sec) and the remaining 35% (n = 65) were fast Aδ fibers (≥ 10 m/sec; Figure 1). All fibers were mechanoreceptive (Force: 123 [82-282] mN, Pressure: 196 [118-340] kPa, CV: 6.3 [4.8-12] m/sec) and most of the afferent fibers (90%) exhibited slowly adapting responses to sustained mechanical stimulation prior to injection of compounds into their receptive fields. There was a significant inverse correlation between mechanical threshold and afferent CV (r= -0.32, P < 0.05). The mechanical threshold of 33% of these afferent fibers exceeded 280 kPa (n=62, Force: 744 [302-744] mN, Pressure: 468 [383-469] kPa, CV: 5.4 [4.4-6.9] m/sec), which suggests that these fibers were nociceptors since this value exceeds the mean pressure pain threshold of male human subjects in this study (see below). There was no significant difference in mechanical threshold when afferent fibers recorded in male (n=94, Force: 148 [83-743] mN,

In afferent fibers that were excited by an initial injection of 500 mM glutamate (n=102), there was a significant inverse relationship between CV and glutamate-evoked cumulative afferent discharges (r= -0.43, P < 0.05). Similarly, in afferent fibers that were excited by an initial injection of 1.0 M saline (n=40), which was used as a control, there was also a significant inverse relationship between CV and hypertonic saline evoked cumulative afferent discharges (r= -0.38, P < 0.05; Figure 1). Only 10 afferent fibers that were excited by 500 mM NMDA were examined, and thus the inverse relationship between CV and NMDA-evoked cumulative afferent discharges (n=10; r= -0.515, P = 0.116) was not significant.

The mechanical threshold of 30, 40 and 40% of afferent fibers that responded to 500 mM glutamate (n=31, Force: 744 [282-744] mN, Pressure: 468 [288-469] kPa, CV: 5.3 [4.3-6.4] m/sec), 1.0 M saline (n=16, Force: 744 [743-1234] mN, Pressure: 468 [468-594] kPa, CV: 5.1 [4.4-6.5] m/sec) and 500 mM NMDA (n=4, Force: 513 [282-744] mN, Pressure: 378 [288-468] kPa, CV: 5.3 [4.7-8.2] m/sec), respectively, exceeded 280 kPa. There was, however, no significant relationship between glutamate, NMDA or hypertonic saline-evoked cumulative afferent discharges and mechanical threshold.
Consistent with our previous results (Cairns et al. 2001a, b, 2002, 2003a), glutamate-evoked cumulative afferent discharges were significantly larger in female (n=51, Discharges: 55 [22-291] spikes, CV: 6.1 [4.4-11.3] m/sec,) than in male (n=51, Discharges: 23 [8-91] spikes, CV: 7.0 [4.8-12.4] m/sec) rats (P< 0.05). There were no significant sex-related differences in the cumulative afferent discharges evoked by either 1.0 M saline or 500 mM NMDA.

Injection of NMDA, which is a selective agonist for the NMDA receptor, into the masseter muscle evoked afferent discharges in a concentration-related manner (Figure 2). A comparison of glutamate- and NMDA-evoked masseter muscle afferent fiber discharges indicates that 5 (n=10), 50 (n=14) or 500 (n=10) mM NMDA-evoked afferent discharges were not significantly different from afferent discharges evoked by injection of 500 mM glutamate (n=102; Figure 2). Previous work indicates that 100 mM glutamate does not evoke significant afferent discharges (Cairns et al. 2001a, 2002). Thus, NMDA appears to be a more potent excitant of masseter muscle afferent fibers than glutamate.

Repeated injection of glutamate alone (n=12) evoked reproducible afferent discharges (Figure 3). The mechanical threshold of 25% of these afferent fibers exceeded 280 kPa. Significant attenuation of glutamate-evoked afferent discharges was achieved by co-injection of 10 mM (n=10) and 100 mM (n=10) APV, but not 1 mM (n=10) APV (Figure 3). The mechanical threshold of 20%, 10% and 10% of afferent fibers examined with 1, 10 and 100 mM APV, respectively, exceeded 280 kPa. Significant attenuation of
glutamate-evoked afferent discharges was also achieved by co-injection of 10 mM (n=11) and 20 mM (n=10) ketamine, but not 1 mM (n=10) ketamine (Figure 3). The mechanical threshold of 70%, 50% and 60% of afferent fibers examined with 1, 10 and 20 mM ketamine, respectively, exceeded 280 kPa. Significant attenuation of glutamate evoked afferent discharges occurred after co-injection of 40 mM dextromethorphan (n=10) but not after 1 mM (n=10) or 10 (n=10) mM dextromethorphan. The mechanical threshold of 30, 50% and 60% of afferent fibers examined with 1, 10 and 40 mM dextromethorphan, respectively, exceeded 280 kPa.

To determine whether the effects of these antagonists were selective for glutamate-evoked afferent discharges, control experiments that employed repeated injection of hypertonic saline were performed. Repeated injection of hypertonic saline (n=10) also evoked reproducible afferent discharges (Figure 4). The mechanical threshold of 30% of these afferent fibers exceeded 280 kPa. Hypertonic saline-evoked discharges were not significantly affected by co-injection of 10 mM APV (n=10) or 10 mM ketamine (n=10), however, they were significantly attenuated by 40 mM dextromethorphan (n=10; Figure 4). The mechanical threshold of 60%, 70% and 40% of afferent fibers in the APV, ketamine and dextromethorphan groups, respectively, exceeded 280 kPa.

**Human Experiments**

Masseter muscle pressure pain thresholds (mean ± SD) were bilaterally symmetrical (right: 276 ± 77, left: 279 ±70 kPa) in the 14 men. Five minutes after injection of 10 mM
ketamine alone into the left masseter muscle, the pressure pain threshold in the left
masseter muscle (280 ± 63 kPa) was not significantly altered.

Repeated injections of glutamate (1.0 M) or hypertonic saline (1.0 M) into the right
masseter muscle evoked reproducible pain responses (Figure 5A). Co-injection of 10
mM ketamine with glutamate significantly attenuated the intensity of glutamate-evoked
pain; this attenuation was reflected as a significant decrease in the relative VAS peak and
area (Figure 5B). Co-injection of 10 mM ketamine with hypertonic saline had no
significant effect on the intensity of hypertonic saline-evoke pain.
Discussion

In the present study, slowly conducting masseter muscle afferent fibers that project to the caudal brainstem were excited by injection of concentrations of glutamate or hypertonic saline into the masseter muscle that cause pain in humans (Cairns et al. 2001a, 2003b; Svensson et al. 1995, 1998, 2003) and 33% of these afferent fibers had mechanical thresholds at or above the human pressure pain threshold. Thus, many of the masseter muscle afferent fibers examined in the present study were likely nociceptors. Injection of NMDA into the masseter muscle of isoflurane anesthetized rats was found to potently excite afferent fibers, while local injection of NMDA receptor antagonists was observed to almost entirely abolish glutamate-evoked afferent discharges in a concentration-related manner. Since isoflurane is a putative NMDA receptor antagonist (Hollmann et al. 2001; Ming et al. 2002; Nishikawa and MacIver 2000), our results may actually underestimate the contribution of peripheral NMDA receptor activation to glutamate-evoked afferent discharges. Thus, not only are these findings consistent with the idea that masseter muscle afferent fibers are excited by activation of peripheral NMDA receptors, they also suggest that peripheral non-NMDA receptor activation may be less important for excitation of muscle afferent fibers than for excitation of other deep tissue afferent fibers such as those in the temporomandibular and knee joints (Cairns et al. 1998; Lawand et al. 1997). In human subjects, glutamate-evoked masseter muscle pain was also significantly decreased by co-injection of the NMDA receptor antagonist ketamine. Therefore, the present results indicate that glutamate injection into the masseter muscle evokes pain in human subjects through activation of peripheral NMDA receptors and
suggest that this pain is mediated, in part, through the activation of peripheral NMDA receptors associated with masseter muscle nociceptors.

In the current study, hypertonic saline injections were employed as a control nociceptive stimulus to test for non-specific inhibitory actions of the NMDA receptor antagonists, based on the assumption that hypertonic saline would not activate peripheral NMDA receptors. Although it remains unclear exactly how hypertonic saline does evoke afferent discharges or muscle pain, it has been theorized that the osmotic strength of hypertonic saline solutions shrinks the terminal endings of sensory fibers, an effect which may excite the afferent fibers directly through an opening of stretch-insensitive sodium channels or indirectly through local release of excitatory peptides (Garland et al. 1995; Schumacher et al. 2000). A recent report, which found that injection of hypertonic saline into the calf muscle also elevated muscle glutamate concentrations, calls into question the assumption that intramuscular injection of hypertonic saline would not activate the NMDA receptor (Tegeder et al. 2002). However, in this study of Tegeder et al. (2002) there was a poor temporal relationship between hypertonic saline-evoked pain and increased glutamate levels, which did not become significantly elevated until 20 min after the muscle pain had subsided. Thus, in support of the assumption that hypertonic saline would not activate peripheral NMDA receptors, it appears unlikely based on the results of Tegeder et al. (2002) that elevation of glutamate levels contributes significantly to hypertonic saline-evoked muscle pain.
Ketamine and dextromethorphan, when given systemically, are thought to be efficacious for the treatment of deep tissue pain in humans due to their ability to non-competitively inhibit NMDA receptor activation in the CNS (Gordon et al. 1999; Graven-Nielsen et al. 2000; Henriksson and Sorensen 2002; Mathisen et al. 1995; Nikolajsen et al. 1996; Ren and Dubner 1999; Sessle 2000; Woolf and Thompson 1991). However, at the concentrations employed in this study, ketamine and dextromethorphan could also have decreased glutamate-evoked afferent discharges through non-specific mechanisms, such as sodium or calcium channel blockade (Brau et al. 1997; Netzer et al. 1993). It was found that 10 mM concentrations of ketamine had no significant effect on hypertonic saline-evoked afferent discharges, which suggests that ketamine’s inhibitory actions are selective for glutamate-evoked afferent discharge and therefore unlikely to be mediated through non-specific mechanisms. In contrast, dextromethorphan, which is a relatively weak NMDA receptor antagonist (Church et al. 1994; Netzer et al. 1993), significantly suppressed hypertonic saline-evoked afferent discharges at concentrations required to suppress glutamate-evoked afferent discharges, which suggests that part of the inhibitory effect of dextromethorphan was likely due to a non-specific mechanism. Since 10 mM ketamine selectively inhibited glutamate-evoked afferent discharges in rats, this concentration of ketamine was employed in the parallel human experimental pain studies. In human subjects, 10 mM ketamine was found to also significantly attenuate glutamate-evoked muscle pain intensity. However, 10 mM ketamine did not significantly affect hypertonic saline-evoked muscle pain intensity or masseter muscle pressure pain thresholds, which indicates that systemic or local non-specific effects ketamine did not contribute to its attenuation of glutamate-evoked muscle pain in human subjects. Taken
together, these findings suggest that ketamine attenuates glutamate-evoked muscle pain intensity through blockade of peripheral NMDA receptors.

Although CNS actions of glutamate are well known and its role in pain well documented (see above), deep tissue pain in humans has also been associated with a local elevation in glutamate levels in peripheral tissues. In patients suffering from inflamed knee joints, an association has been made between pain and elevated glutamate concentrations in synovial fluid (McNearney et al. 2000). Calf muscle pain provoked by additional flexions of the foot after prolonged eccentric contractions of the calf muscles has also been associated with increased glutamate levels in these muscles (Tegeder et al. 2002). Glutamate levels are also elevated in the patellar tendon tissues of subjects suffering from “Jumpers knee”, a non-inflammatory knee pain that can develop in athletes who exert very high tensional forces on their knees, such as weightlifters (Alfredson et al. 2001; Alfredson and Lorentzon 2002). NMDA receptors have been identified in association with nerve structures in the patellar tendon in humans (Alfredson et al. 2001) as well as in cutaneous and deep tissues in association with nerve fibers in several animal studies (Carlton and Coggeshall 1999; Carlton et al. 1995; McRoberts et al. 2001; Sahara et al. 1997; Sato et al. 1993). In the present study, the concentration of glutamate injected into the masseter muscle to evoke afferent discharges or induce muscle pain through peripheral NMDA receptor activation approximates the concentration of glutamate that could be released upon afferent excitation from pre-synaptic vesicles in masseter muscle afferent fiber terminals (Riveros et al. 1986). It is, therefore, possible that under conditions that are associated with deep tissue pain, glutamate may be released from
afferent fiber terminals and act on peripheral NMDA receptors to excite nociceptive fibers.

The masseter muscle was used in the current study to investigate peripheral NMDA receptor involvement since it is a common site of myofascial pain, especially in temporomandibular disorders (Drangsholt and LeResche 1999; Dworkin and LeResche 1992; Stohler 1999). Temporomandibular disorders embrace a number of painful conditions in deep craniofacial tissues and are one of the least understood yet most common conditions associated with pain and tenderness in the masticatory muscles (Drangsholt and LeResche 1999; Dworkin and LeResche 1992). Curiously, most temporomandibular disorders appear to lack demonstrable joint or masseter muscle tissue abnormalities (Stohler 1999), and so it is noteworthy that glutamate injection into deep craniofacial tissues does not result in gross signs of inflammation (Fiorentino et al. 1999), but does cause both muscle pain and a more prolonged period (>30 min) of mechanical sensitization, symptoms similar to those of temporomandibular disorders patients (Cairns et al. 2001a, 2002; Svensson et al. 2003). These new findings point to the possibility that activation of peripheral NMDA receptors by elevated muscle glutamate levels may contribute to masticatory muscle pain and that peripherally acting NMDA receptor antagonists could prove to be effective analgesics for this type of pain.

We have consistently found that glutamate evokes greater cumulative afferent discharges in female than in male rats (Cairns et al. 2001a, 2002; Svensson et al. 2003). There is evidence that the female sex hormone estrogen increases the amplitude of NMDA-
receptor mediated excitatory postsynaptic potentials in the hippocampus by enhancing the function of the NMDA receptor (Bi et al. 2000; Foy et al. 1999). Thus, there is a theoretical basis for a sex-related difference in the efficacy and/or potency of NMDA receptor antagonists on peripheral NMDA receptors. However, in the current study the effect of each concentration of the NMDA receptor antagonist was examined on only 5 afferent fibers from each sex, too few to permit statistical analysis of the potential influence of sex on the attenuation of glutamate-evoked afferent discharges by NMDA receptor antagonists in this study. Studies which examine the effect of NMDA receptor antagonists on larger numbers of masseter muscle afferent fibers recorded from male and female rats will be required to answer this question. Further, since the present study tested the effect of NMDA receptor antagonists on men and we have previously documented that injection of glutamate into the masseter muscle is more painful in women than in men (Cairns et al. 2001a, 2003b; Svensson et al. 2003), the efficacy of NMDA receptor antagonists needs to be tested in women in view of the high female predominance of temporomandibular disorders (Dao and LeResche 2000; Huang et al. 2002).

**Acknowledgements:** The Danish Basic Research Foundation and the Boston Children's Hospital Pain Research Endowment Fund supported this research. BJS and BEC are recipients of Canada Research Chairs. Present address of BE Cairns: Faculty of Pharmaceutical Sciences, The University of British Columbia, 2146 East Mall, Vancouver, V6T 1Z3 Canada.
References


Cairns BE, Gambarota G, Dunning PS, Mulkern RV, and Berde CB. Activation of peripheral excitatory amino acid receptors decreases the duration of local anesthesia. *Anesthesiology* 98: 521-529, 2003a.


Cairns BE, Sessle BJ, and Hu JW. Evidence that excitatory amino acid receptors within the temporomandibular joint region are involved in the reflex activation of the jaw muscles. *J Neurosci* 18: 8056-8064, 1998.

Carlton SM and Coggeshall RE. Inflammation-induced changes in peripheral glutamate receptor populations. *Brain Res* 820: 63-70, 1999.


Netzer R, Pflimlin P, and Trube G. Dextromethorphan blocks N-methyl-D-aspartate
induced currents and voltage-operated inward currents in cultured cortical neurons.


effect of ketamine on phantom pain: a central neuropathic disorder maintained by

Nishikawa K and MacIver MB. Excitatory synaptic transmission mediated by NMDA
receptors is more sensitive to isoflurane than are non-NMDA receptor-mediated

Ren K and Dubner R. Central nervous system plasticity and persistent pain. *J Orofac

Riveros N, Fiedler J, Lagos N, Munoz C, and Orrego F. Glutamate in rat brain cortex
synaptic vesicles: influence of the vesicle isolation procedure. *Brain Res* 386: 405-
408, 1986.

Sahara Y, Noro N, Iida Y, Soma K, and Nakamura Y. Glutamate receptor subunits
GluR5 and KA-2 are co-expressed in rat trigeminal ganglion neurons. *J Neurosci*

Sato K, Kiyama H, Park H, and Tohyama M. AMPA, KA and NMDA receptors are


Figure Legends

Figure 1. Relationship between CV and evoked afferent discharges. On the right hand side, peri-stimulus histograms of the response of single C (< 2.5 m/sec), slow Aδ (2.5-10 m/sec) and fast Aδ (> 10 m/sec) fibers to injection of 500 mM glutamate, 1.0 M saline and 500 mM NMDA are illustrated. The sex of the rat and CV for each fiber shown is given in brackets. On the left hand side, each bar indicates median cumulative afferent activity. The largest responses to all three substances were evoked in C fibers. Error bars: interquartile range. * P<0.05 ANOVA on ranks, Dunn’s test. ♀: female rat, ♂: male rat

Figure 2. Concentration-response relationship for NMDA-evoked afferent activity. Each closed circle indicates the median cumulative afferent activity evoked by injection of 0.5 (n=10), 5 (n=10), 50 (n=14) and 500 (n=10) mM NMDA. The open square indicates the median cumulative discharges in response to injection of 500 mM glutamate into the masseter muscle (n=102). Afferent discharges evoked by 500 mM NMDA were significantly greater that those evoked by 0.5 or 5 mM NMDA. Note that afferent discharges evoked by injection of 5, 50 or 500 mM NMDA into the masseter muscle were not significantly different from that evoked by 500 mM glutamate. Error bars: interquartile range, *,# P<0.05 ANOVA on ranks, Dunn’s test compared to 500 mM glutamate and 500 mM NMDA, respectively.
**Figure 3.** Effect of competitive and non-competitive NMDA receptor antagonists on glutamate-evoked masseter afferent discharges.  

**A.** The example illustrates how repeated injection of 0.5 M glutamate at 30 min intervals into the receptive field of a masseter afferent fiber evoked reproducible spike activity.  

**B.** Addition of APV, dextromethorphan or ketamine to the second injection of glutamate had a concentration-related inhibitory effect on the calculated relative cumulative activity.  Points are the median of 10-12 afferent fiber experiments.  Error bars: interquartile range, * P<0.05 ANOVA on ranks, Dunn’s test compared to repeated injection of glutamate alone.

**Figure 4.** Effect of competitive and non-competitive NMDA receptor antagonists on hypertonic saline-evoked masseter afferent discharges.  

**A.** The example illustrates how repeated injection of 1.0 M hypertonic saline at 30 min intervals into the receptive field of a masseter afferent fiber evoked reproducible spike activity.  

**B.** Addition of APV or ketamine (ket) to the second injection of hypertonic saline had no significant effect on relative cumulative activity.  However, addition of 40 mM dextromethorphan to the second injection of hypertonic saline significantly attenuated relative cumulative activity.  Bars indicate the median of 10 afferent fiber experiments.  Error bars: interquartile range, * P<0.05 ANOVA on ranks, Dunn’s test compared to repeated injection of hypertonic saline alone.

**Figure 5.** The effect of ketamine on glutamate- and hypertonic saline-evoked masseter muscle pain in 14 male subjects.  The line graphs in **A.** illustrate the mean VAS scores after injection of glutamate (G), glutamate and ketamine (K), hypertonic saline (S) or
hypertonic saline and ketamine at the concentrations indicated. The bar graphs in B. show the mean relative response (second response divided by first response). Ketamine significantly reduced the glutamate-evoked relative peak VAS score and relative overall area under VAS-time curve. In contrast, ketamine had no significant effect on hypertonic saline-evoked responses. These results indicate that injection of glutamate into the masseter muscle evokes pain, in part, through activation of peripheral NMDA receptors. Error Bars: SE, *: P<0.05 repeated measures ANOVA and Student-Neuman-Keuls test compared to glutamate alone.
Figure 1.

**Glutamate**
- C fiber (♀, 1.6 m/sec)
- slow Aδ fiber (♂, 5.2 m/sec)
- fast Aδ fiber (♂, 16.3 m/sec)

**Hypertonic Saline**
- C fiber (♀, 1.9 m/sec)
- slow Aδ fiber (♂, 5.3 m/sec)
- fast Aδ fiber (♂, 15.0 m/sec)

**NMDA**
- C fiber (♀, 2.4 m/sec)
- slow Aδ fiber (♀, 4.8 m/sec)
- fast Aδ fiber (♂, 14.1 m/sec)
Figure 2.
Figure 3.
Figure 4.

A.

B.
Figure 5.

A.

B.