Glutamate-induced Temporomandibular Joint Pain in Healthy Individuals Is Partially Mediated by Peripheral NMDA Receptors

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Aim: *To determine if glutamate injected into the healthy temporomandibular joint (TMJ) evokes pain through peripheral N-methyl-D-aspartate (NMDA) receptors and if such pain is influenced by sex or sex steroid hormones.* ***Methods:*** *Sixteen healthy men and 36 healthy women were included and subjected to two randomized and double-blind intra-articular injections of the TMJ. Experimental TMJ pain was induced by injection of glutamate (1.0 mol/L) and NMDA block was achieved by co-injection of the NMDA antagonist ketamine (10 mmol/L). The TMJ pain intensity in the joint before and during a 25-minute postinjection period was continuously recorded on an electronic visual analog scale (0 to 10). Estradiol, progesterone, and testosterone levels in serum were analyzed.* ***Results:*** *Glutamate-induced pain showed a median (25/75 percentile) duration of 8.3 (5.2/12.2) minutes. The peak pain intensity was 6.1 (4.2/8.2), the time to peak was 50 (30/95) seconds, and the area under the curve was 59 (29/115) arbitrary units. The women reported higher maximum pain intensity than the men and shorter time to peak. The sex hormone levels were not significantly related to the glutamate-induced TMJ pain. NMDA block significantly reduced the glutamate-induced TMJ pain, mainly in the women. There were no significant correlations between sex hormone levels and the effects of NMDA block for any pain variable.* ***Conclusion:*** *Glutamate evokes immediate pain in the healthy human TMJ that is partly mediated by peripheral NMDA receptors in the TMJ.* J OROFAC PAIN 2010;24:172–180

Key words: glutamate, NMDA, pain, sex hormones, temporomandibular joint

Peripheral glutamate and its receptors have been found to possess modulatory roles in peripheral nociception and sensitization.[1] Glutamate is elevated in synovial fluid from joints with active arthritis[2] and has also been found in higher tissue concentrations in tendonitis.[3,4] Elevated peripheral glutamate may therefore contribute to development and maintenance of pain in inflammatory conditions.

Possible sources of glutamate in peripheral tissues include primary afferents,[5,6] macrophages,[7] Schwann cells,[8] and thrombocytes.[9] Synovial tissue glutamate may thus originate from inflammatory cells or nerve fibers in the inflamed synovial membrane but also from plasma extravasation into the synovial tissues.[2,10–13]

Pain-related effects of glutamate injected into synovial tissues have not been reported in humans but glutamate injected into the rat temporomandibular joint (TMJ) activates and sensitizes nociceptive afferents, facilitates jaw reflexes, and activates nociceptive brainstem neurones.[3,10,14,15] In addition, peripheral application of glutamate may activate or induce peripheral sensitization in a subpopulation of trigeminal nociceptive afferents innervating deep craniofacial tissues.[16] Correspondingly, injection of glutamate into the rat masseter muscle activates and sensitizes masseter nociceptive afferents. This is in part due to activation of peripheral N-methyl-D-aspartate (NMDA) receptors since the effects can be attenuated by co-administration of a NMDA receptor antagonist[17] or mimicked by injection of NMDA into the muscle.[9,18] Glutamate injection into the masseter muscle also evokes pain in healthy human volunteers[5,14,19–23] and this effect is attenuated by co-injection of the NMDA receptor antagonist ketamine, indicating that glutamate-induced muscle pain is, at least partially, mediated through activation of peripheral NMDA receptors.[6]

There seems to be a sex difference in the effects of peripheral NMDA block since ketamine only attenuates the glutamate-evoked muscle pain in men[1,22] despite the fact that women report higher pain intensity than men after glutamate injection into the masseter or trapezius muscle.[1,19] Also, secondary pinprick hyperalgesia is more pronounced in women after subcutaneous glutamate injection.[23] These findings suggest sex differences both in the pain-inducing effects of glutamate as well as in the relative involvement of peripheral NMDA receptor mechanisms. In turn, sex-related differences could be due to influences of sex steroid hormones. Estrogen appears to modulate the mechanoreceptive properties of cutaneous afferent fibers[24,25] and masseter muscle nociceptive afferents.[9,18] In mice, sensory neurons innervating craniofacial tissues express estrogen receptors and the release of neuropeptides by trigeminal neurons is influenced by sex hormone levels.[26] These findings suggest an estrogen-mediated modulation of glutamate receptors on cutaneous and musculoskeletal afferent fibers.

No information is available on the effects of glutamate injected into human articular tissues. Therefore, the aim of this study was to determine if glutamate injected into the healthy human TMJ evokes pain through peripheral NMDA receptors and if such pain is influenced by sex or sex steroid hormones.

Materials and Methods

Healthy Individuals

A total of 52 healthy individuals were included; 16 men with a median (25/75 percentile) age of 30 (24/41) years and 36 women with a median age of 25 (23/29) years. None of the women were considered postmenopausal (age > 50 years and estradiol levels < 50 pmol/L). The study was approved by the local ethics committee (Regional Board at the Karolinska Institutet: 495/03) and conducted in accordance with the Helsinki Declaration. All volunteers read and signed informed consent forms.

Inclusion criterion was a statement from the subject where he or she claimed to be healthy. Exclusion criteria were age below 20 or above 70 years, ongoing or past (< 1 year) TMJ symptoms, pregnancy, serious systemic disease, chronic administration of psychiatric or analgesic medication, current malignancies, history of TMJ surgery or trauma within the past two years, disease that may cause orofacial pain, skin infection over the TMJ, or high blood pressure.

Experimental Protocol

The subjects were subjected to two randomized and double-blind intra-articular injections of the TMJ, 30 minutes apart in a single session. Experimental TMJ pain was induced by injection of a 0.2 mL sterile solution of monosodium glutamate (1.0 mol/L; prepared by the Department of Pharmacy, Aalborg Hospital, Aalborg, Denmark) into the upper joint compartment through a disposable needle (diameter = 0.65 mm, length = 30 mm). The concentration and volume used were chosen with reference to recent studies of glutamate injections into the masseter muscle[1,27] and the authors' own tests of TMJ injections where 0.2 mL and 0.5 mL 1.0 mol/L glutamate were injected into the TMJ of two volunteers who compared the elicited pain intensity and duration between the two dosages. Based on these tests, the dose of 0.2 mL 1.0 mol/L glutamate was chosen since 0.5 mL 1.0 mol/L was considered as too painful. The contribution of NMDA receptors to the experimentally induced TMJ pain was tested by injection of a combination of glutamate (1.0 mol/L) and the NMDA receptor antagonist ketamine (10 mmol/L, Ketalar, Pfizer). The side to be used for injection and the order of injections (glutamate – glutamate+ketamine or glutamate+ketamine – glutamate) were randomized.

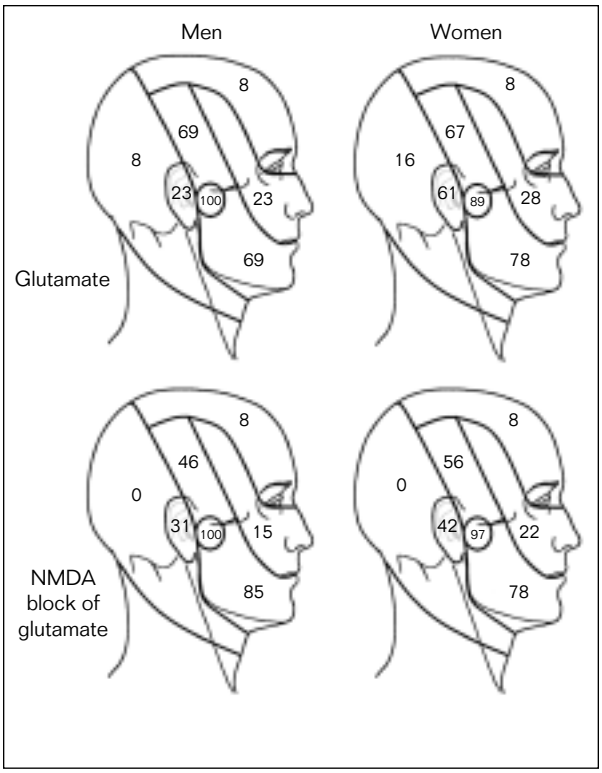

Fig 1 Percentage of subjects reporting pain in neuroanatomically relevant orofacial and cervical regions after glutamate-induced pain by intra-articular injection and after NMDA receptor block with ketamine of the glutamate-induced pain in 16 men and 36 women. In the women, the reduction of proportion of patients reporting auricular pain on glutamate injection was significantly reduced by NMDA block ($P = .036$).

Assessment of Experimental Pain

Two experienced and calibrated operators performed all clinical assessments and injections. The TMJ pain intensity in the injected joint before and during a 25-minute postinjection period was used as the primary outcome variable. It was continuously recorded by an electronic visual analog scale (VAS, 0 to 10; Aalborg University, Center for Sensory-Motor Interaction, Aalborg, Denmark) connected to a computer for online recording. The VAS end-points were marked with "no pain" and "worst pain intensity ever experienced." In the statistical analyses, the pain was quantified as maximum amplitude of pain intensity (peak pain intensity) after each injection, time to peak (seconds), pain duration (seconds), and area under the VAS curve (arbitrary units).

Tenderness to digital palpation, TMJ and glabella pressure pain thresholds, and maximum mouth opening capacity were assessed before injections and during the 25-minute postinjection period with 5-minute intervals. These assessments were performed in duplicate before the injections in order to establish baseline values (average of the two assessments) and as a single assessment after the injections.

Digital palpation was performed on three aspects of the joint: laterally over the joint with the mouth closed (lateral aspect), laterally over the joint with the mouth wide open (lateroposterior aspect), and the posterior aspect via the acoustic meatus with the mouth closed. Tenderness provoked by digital palpation of the TMJ was scored by the subject as present or absent.

The pressure pain threshold was determined over the palpable lateral pole of the TMJ condyle with the subject's mandible in a resting position and over the glabella on the frontal bone as a reference point.[28] The measurement was made by a handheld electronic pressure algometer (Somedic Production AB), which consisted of a pressure-transducer probe connected to a pistol grip with a display unit. The tip of the pressure transducer has a flat, circular rubber tip with an area of 1.0 cm^2. A linearly increasing pressure rate of 50 kPa/s^2 was[28–30] applied until the subject responded to the first pain sensation by pressing a button on a device connected to the probe that fixed the current pressure pain threshold level on the display. The pressure pain threshold was defined as the minimum pressure needed to evoke a painful sensation recognizable by the subject.

The interincisal distance at maximum voluntary mouth opening was measured with a ruler between the 21 and 31 teeth. The subject also reported presence of pain in the TMJ at any position of the mandible during this movement.

Immediately peak pain intensity was reached after each injection, the subjects were asked to draw the area of perceived pain elicited by the injections on a schematic drawing of the lateral side of the head. The subjects were reminded to record any changes in pain intensity on the VAS during drawing of the pain localization and area.The pain area was then measured using an overlying transparent sheet with a thin grid where the number of squares corresponding to the drawn pain distribution was counted and expressed as arbitrary units. In addition, the distribution of pain was estimated as present or absent in the following orofacial areas, which were related to the areas innervated by the sensory divisions of the trigeminal and cervical nerves: TMJ, mandibular, maxillary, temporal, frontal, and occipital (Fig 1).

Blood Sampling

Blood samples were drawn to determine the serum levels of estradiol (the major estrogen in humans), progesterone, and testosterone according to standard procedures at the Department of Laboratory Medicine, Karolinska University Hospital, Huddinge, Sweden. This laboratory is accredited according to the international ISO 15189 and ISO/IEC 17025 standards. The normal reference range according to the laboratory for estradiol levels in fertile women is 100 – 1500 pmol/L, in postmenopausal women < 50 pmol/L, and in men 50 – 150 pmol/L. The corresponding ranges for progesterone levels in fertile women is > 17 nmol/L in the luteal phase (otherwise < 4.8 nmol/L), in postmenopausal women < 3.0 nmol/L, and in men < 3.0 nmol/L. For testosterone, the normal range in women 20 to 50 years of age is < 2.7 nmol/L, in women > 50 years of age < 2.5 nmol/L, and men 10 – 30 nmol/L.

Statistical Analyses

The software SigmaStat (version 3.1, Sysstat) was used for statistical analyses. Nonparametric statistics were used due to the characteristics of the pain variables. Data are presented as median, 25th, and 75th percentile unless otherwise stated. The interindividual difference in response within each session was tested by the Wilcoxon rank sum test or Friedman ANOVA. Univariate analysis of differences between groups was tested with the Mann-Whitney test and the significance of univariate correlations was tested by the Spearman's ranked correlation test. Two-way repeated measures ANOVA on ranks with type of injection and sex as independent factors was used to determine whether there was an effect of type of injection or sex on pain parameters, ie, the primary outcome. Changes in the spatial distribution of pain in orofacial regions on NMDA block of glutamate-induced TMJ pain were tested with the chi-square test. A probability level less than .05 was considered significant; "ns" reflects no significant difference.

Results

Glutamate-induced Temporomandibular Joint Pain

Glutamate injected into the TMJ elicited a prompt pain response and a pain duration of 8.3 (5.2/12.2) minutes. The peak pain intensity was 6.1 (4.2/8.2), the time to peak was 50 (30/95) seconds and the area under the curve was 59 (29/115) AU (Fig 2). The women reported a significantly higher peak pain intensity than the men ($P = .048$) and a significantly shorter time to peak ($P = .017$) but there were no significant differences between the sexes in pain duration or area under the curve (Table 1).

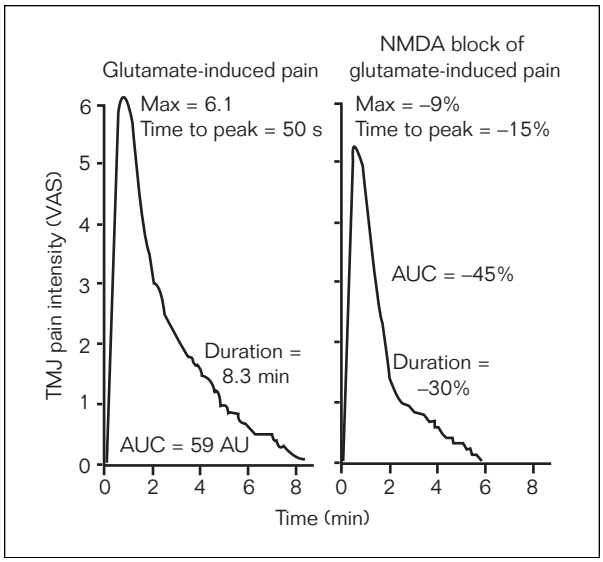

Fig 2 Panel of plots showing TMJ pain intensity on an electronic VAS (0 to 10) in 52 healthy individuals after intra-articular injection of glutamate *(left)* and after NMDA receptor block with ketamine of the glutamate-induced TMJ pain *(right)*. The absolute values for peak pain intensity, time to maximum pain intensity, pain duration, and area under the curve (AUC) are shown for the glutamate-induced pain, whereas the reduction of the glutamate-induced TMJ pain by the NMDA block is shown in percent.

The distribution of the perceived pain in the orofacial and cervical regions is shown in Fig 1. There was no significant difference between the sexes regarding maximum area or total distribution of perceived pain to glutamate injection (Table 1 and Fig 1). The presence of pain in the occipital region after glutamate injection into the TMJ was observed more commonly in females (16%) than in males (8%).

TMJ tenderness to palpation and pain provoked by jaw movement were assessed the first time after 5 minutes. At that time, the proportion of men reporting tenderness to palpation was 64% and the proportion of women 91%. Maximum mouth opening provoked TMJ pain in 29% of the men

Table 1 TMJ Resting Pain Parameters Before and After Intra-articular Injection of Glutamate (1.0 mol/L) or a Combination of Glutamate (1.0 mol/L) and the NMDA Receptor Antagonist Ketamine (10 mmol/L) Into the TMJ

	Men				Women			
			Percentile				Percentile	
	n	Median	25th	75th	n	Median	25th	75th
Glutamate-induced TMJ pain								
Maximum pain intensity (0–10)	15	5.0	4.0	6.3	34	6.6*	4.6	8.5
Time to peak (s)	13	95	50	120	34	50*	30	78
Duration (min)	15	5.9	5.1	10.9	34	9.2	5.6	12.2
AUC (AU)	15	42	28	104	34	75	31	115
Pain drawing area (AU)	13	10	9	21	36	18	11	23
Effect of NMDA block of glutamate-induced pain (relative to glutamate-induced pain)								
Maximum pain intensity (%)	15	93	68	108	34	90†	76	102
Time to peak (%)	13	50	40	85	34	105	52	149
Duration (%)	15	86	53	141	34	64†	42	104
AUC (%)	15	57†	32	92	34	54‡	37	95
Pain drawing area (%)	13	80	67	100	36	82†	51	135

% = values are normalized to the glutamate-induced TMJ resting pain values (100%) for each variable; n = no. of observations; AU = arbitrary units.
*Indicates differences between the sexes, $P < .05$; † indicates differences between glutamate and NMDA-block of glutamate, $P < .05$; ‡ indicates differences between glutamate and NMDA-block of glutamate, $P < .001$.

Table 2 Proportion of Patients with Tenderness to Palpation of the TMJ, TMJ Pain on Mandibular Movement, and Median TMJ and Glabella Pressure Pain Thresholds in Male and Female Healthy Individuals Before (time = 0 min) and Every 5th Minute After Injection of Glutamate (1.0 mol/L) or a Combination of Glutamate (1.0 mol/L) and the NMDA Receptor Antagonist Ketamine (10 mmol/L) into the TMJ

	Minutes after injection									
	Men					Women				
	0	5	10	15	20	0	5	10	15	20
Glutamate-induced TMJ pain										
Pain intensity on digital palpation of the TMJ										
Lateral (%)	7	57	40	20	7	3	71	39	44	36
Lateroposterior (%)	6	40	20	27	13	6	83	39	28	28
Posterior (%)	6	50	20	13	7	3	54	31	19	19
Total (%)	13	64	40	27	20	8	91	61	61	53
TMJ pain on mandibular movement (%)	6	29	13	7	0	8	43	22	19	14
Pressure pain threshold										
TMJ (kPa)	331	308	318	301	305	248*	208	238	201	238
Glabella (kPa)	527	516	532	532	501	364*	360	360	353	313
NMDA block of glutamate-induced pain										
Pain intensity on digital palpation of the TMJ										
Lateral (%)	0	53	27	20	13	19	50	22	19	25
Lateroposterior (%)	0	43	33	27	20	8	53†	22	17	17
Posterior (%)	0	27	13	13	7	6	25†	19	8	19
Total (%)	0	60	33	27	20	22	75	36	28	36
TMJ pain on mandibular movement (%)	0	20	7	7	13	11	25	22	17	14
Pressure pain threshold										
TMJ (kPa)	296	281	284	308	299	243	221	231	231	236
Glabella (kPa)	458	440	444	449	476	362	340	351	348	351

% = values are normalized to the glutamate-induced TMJ resting pain values (100%) for each variable.
*Indicates differences between the sexes, $P < .05$; † indicates differences between glutamate and NMDA-block of glutamate, $P < .05$.

and 43% of the women 5 minutes after the glutamate injection (Table 2). Pressure pain thresholds of neither the TMJ nor glabella changed significantly after glutamate injection (Table 2).

The serum levels of estradiol, progesterone, and testosterone did not significantly influence the glutamate-induced TMJ pain or pressure pain threshold in either gender.

Effects on Glutamate-induced Temporomandibular Joint Pain by NMDA Receptor Block

Tables 1 and 2 show the effect of NMDA receptor block of glutamate-induced TMJ pain. There was no significant difference in any of the investigated variables between individuals who received glutamate as first injection or as second, ie, the order in which the individuals were injected with glutamate or glutamate and ketamine did not influence the results.

NMDA receptor block reduced the glutamate-induced TMJ pain (Fig 2). The peak pain intensity was reduced to 93% (68%/108%) (ns) of the pretreatment value in the men and to 90% (76%/103%) (P = .026) in the women. The glutamate-induced pain duration was reduced to 86% (53%/141%) in the men (ns) and to 64% (42%/104%) in the women (P = .010), whereas the area under the curve was reduced to 57% (32%/92%) in the men (P = .024) and to 54% (37%/95%) in the women (P < .001). The ANOVA of the pain intensity showed a significant effect of type of injection (P = .024) on the area under the curve with no significant interaction between the independent variables type of injection and sex.

The distribution of the perceived pain after NMDA block of glutamate-induced TMJ pain is shown in Fig 1. There was a significant decrease in perceived pain area to 82% of the glutamate-induced pain area by NMDA block in women (P = .015; Table 1). In the women, the reduction of the perceived pain area was positively correlated to the reduction in peak pain intensity (r_s = 0.51, n = 35, P = .002), pain duration (r_s = 0.59, n = 35, P < .001), and area under the curve (r_s = 0.54, n = 35, P < .001).

NMDA receptor block of glutamate-induced TMJ pain reduced the proportion of women with TMJ tenderness to palpation after 5 minutes from 91% to 75%. This reduction was significant for tenderness to lateroposterior (P = .014) and posterior palpation (P = .026; Table 2). However, the proportion of subjects with TMJ movement pain was not significantly reduced by NMDA receptor block. Regarding the pressure pain threshold, neither the TMJ nor glabella pressure pain threshold was significantly related to the effects of NMDA receptor block on glutamate-induced TMJ pain.

There were no significant correlations between sex hormone levels and the effects of NMDA receptor block of glutamate-induced TMJ pain for any pain variable.

Sex Differences Before Injections

The women showed lower TMJ and glabella pressure pain thresholds than the men at baseline recordings (P = .030 and P = .041, respectively; Table 2). The serum levels of estradiol, progesterone, and testosterone were 47 (44/64) pmol/L, 1.4 (1.3/1.7) nmol/L, and 14 (12/16) nmol/L, respectively, for the men and 85 (39/215) pmol/L, 2.2 (1.1/6.4) nmol/L, and 1.1 (0.8/1.5) nmol/L, respectively, for the women. The men had significantly lower levels of progesterone (P = .019) but higher levels of testosterone (P = .003).

Discussion

This study shows that TMJ NMDA receptors in synovial tissues partly mediate glutamate-induced TMJ pain in healthy individuals. The degree of NMDA receptor modulation of glutamate-induced pain seems to some extent to be larger in women, although a sex hormone-dependent influence could not be found in this study.

Peripheral block of the NMDA receptor reduces glutamate-induced TMJ pain with a somewhat larger reduction observed in women as reflected by the univariate differences between injections with and without NMDA receptor block. NMDA receptor block did also reduce glutamate-induced sensitization in women as expressed by the reduction in tenderness to lateroposterior and posterior palpation. These results suggest a possible sex-related difference in the degree of NMDA receptor mediation of TMJ nociception. However, according to the ANOVA analysis, no significant influence of sex was found on pain intensity. There are other studies indicating sex differences regarding peripheral NMDA receptor mechanisms, eg, Dong and coworkers observed a sex-related increase in NMDA receptor expression on masseter muscle afferent neurons in female rats.[9] Also, the magnitude of glutamate-evoked digastric and masseter muscle activity by glutamate injection into the TMJ was greater in female than in male rats.[31] On the other hand, no sex difference in sensitization was observed after glutamate injections into the masseter muscle in healthy individuals.[19] The extent of NMDA receptor involvement in glutamate-induced TMJ pain appears to contrast with recent findings regarding the relative lack of antagonism by ketamine of glutamate-evoked masseter pain in women compared to men.[1,22] However, it is still unclear if these effects reflect species, tissue, or methodological differences.

Glutamate injected into the TMJ in healthy individuals promptly evoked pain of considerable intensity, with significantly higher maximum pain intensity and shorter time to peak pain in women compared to men. These findings are consistent with analogous findings with glutamate injections subcutaneously or into the masseter muscle of healthy human subjects where higher pain intensities were reported by women.[19,23,27] Studies of rat TMJ and masseter nociceptive afferents and jaw reflex effects of glutamate showed that glutamate facilitates the jaw-stretch reflex response in a sex-dependent manner.[13,32] A gender difference in glutamate-induced pain has previously been described in humans.[23,27]

Tenderness to palpation over the TMJ was of short duration but it was present in more than 50% of the women at all time intervals after the glutamate injection, while more than 50% of the males reported tenderness to palpation only at 5 minutes after the injection. The pressure pain threshold was lower in the women than in the men before injections. This difference remained after injections. However, glutamate-induced TMJ pain did not change the absolute pressure pain thresholds, neither over the TMJ nor glabella, which is in agreement with a previous study.[33] The discrepancy between tenderness to palpation and pressure pain threshold indicates that these pain variables are different entities. The TMJ pressure pain threshold is mainly systemically modulated while the tenderness to digital palpation is also indicative of local modulation.[33] The stable pressure pain thresholds after glutamate injections indicate that no substantial central or peripheral sensitization was induced in this experiment in contrast to similar studies of the masseter muscle.[13,19] Pain on jaw opening was reported by less than 50% of the subjects. This is remarkable since clinical inflammatory joint pain usually is provoked by joint movement and this pain entity in particular has been found to be related to the presence of inflammatory mediators in the synovial fluid of the TMJ of patients with chronic arthritis.[33,34] It might be explained by findings[10,13–15,17] that while glutamate may evoke nociceptive responses and pain, it may not induce inflammation.

Very few subjects experienced pain in the occipital region with sensory innervation by the first and second cervical nerves. However, spatial summation of pain after the intra-articular glutamate injection may occur due to the close proximity of the nuclei of the C1-C2 nerves and the subnucleus caudalis of the trigeminal brainstem sensory nuclear complex.

In the present study, no association was observed between sex hormones and glutamate-induced pain or the effect of NMDA block of this pain. Nor were sex hormones related to the observed gender differences. This is in agreement with a recent study where glutamate-induced masseter muscle pain was not related to the estradiol level (Castrillon, personal communication). The authors concluded that sex-related differences in the glutamate-evoked pain and the effectiveness of ketamine to attenuate that pain may not be significantly related to salivary estrogen level. However, studies in rats have shown an estrogen-dependent modulation of NMDA receptor mechanisms.[9,31] The acute character of the pain and the use of healthy human subjects are two possible explanations for the lack of associations between sex hormones and pain in the present study.

Peripheral glutamate levels increase in arthritic conditions, eg, in synovial fluid from patients with rheumatoid arthritis and induce production of tumor necrosis factor,[6] which suggests that glutamate and NMDA receptor mechanisms play a more prominent role for chronic clinical inflammatory pain than acute experimental pain. A recent clinical study that investigated the effect of local NMDA block in patients with TMJ arthralgia reported only limited clinical pain reduction.[35] However, the condition of arthralgia as defined in that study was pain from the joint region which is not necessarily of intra-articular origin but may be related to nearby muscular pain or referred from nearby painful muscles. The influence of the NMDA receptor in inflammatory articular pain conditions of either mono- or polyarthritic character is yet unknown. The potential of NMDA-modulatory drugs in treating TMJ arthritis needs to be determined in future clinical studies.

The present study included an internal control (ie, the effect of ketamine on glutamate-induced TMJ pain as compared to pain caused by glutamate alone in each individual) but not a separate control group. Since no control injection was given in this study, there is a remote possibility that the pain induced was due to pressure by the injected volume and not to the glutamate itself. However, this is unlikely for several reasons. First, the subjects rated the glutamate-induced pain as very high, many as high as maximal. This is even higher than the pain induced by intramuscular injection of glutamate, while intramuscular injection of isotonic saline induces a very low level of pain.[5,19,22,27] Second, injection of a similar volume of isotonic saline into the TMJ of patients with arthralgia increased VAS pain scores by approximately 28%,[34] but the pain

level was still considerably lower than the pain level induced in the healthy subjects without any pain at the start of this study. Compared to glutamate, isotonic saline injection into rat TMJ does not excite TMJ nociceptive afferents or evoke significant jaw reflex responses.[14, 16, 27,31]

In conclusion, this study has shown that glutamate injected in the healthy human TMJ evokes immediate pain that is partly mediated by peripheral NMDA receptors in the TMJ.

Acknowledgments

The authors would like to thank the healthy subjects who participated and Mrs Karin Trollsås for invaluable support in the laboratory. This study was supported by NIDCR grant 1 R01 DE15420.

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