Effect of a Peripheral NMDA Receptor Antagonist on Glutamate-Evoked Masseter Muscle Pain and Mechanical Sensitization in Women

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Aims: *To test the hypothesis that local injection of the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine would significantly attenuate glutamate-evoked masseter mechanical sensitization and muscle pain in healthy young women either taking oral contraceptives (W+OC) or not taking oral contraceptives (W-OC).* ***Methods:*** *Experimental pain was evoked in 47 healthy female subjects (W+OC, n = 25; W-OC, n = 22) by 2 injections of glutamate (0.2 mL, 1 mol/L) into the masseter muscle. A first injection of glutamate alone was followed by a second injection, 35 minutes later, of glutamate combined with ketamine (0, 1, or 10 mmol/L). Evoked pain intensity was scored on a 10-cm electronic visual analog scale (VAS). Distribution of perceived pain was drawn on a lateral view of the face (pain drawing). Masseter muscle pressure pain thresholds (PPT) and pressure-pain tolerances (PPTOL) were determined bilaterally before and at regular time intervals after injections. Analyses of variance (ANOVA) were used to test the data.* ***Results:*** *There were no main effects of ketamine on any of the VAS pain parameters or on the pain drawing (ANOVAs:* P > *.055). Furthermore, there were no differences in PPT, PPTOL, VAS peak pain, duration, overall VAS pain, or pain drawing when W-OC were compared with W+OC (ANOVAs:* P > *.087). Repeated injection of glutamate alone significantly decreased PPT and PPTOL (ANOVAs:* P < *.001); however, this effect was not significantly attenuated by ketamine.* ***Conclusions:*** *Peripherally administered ketamine had no effect on glutamate-evoked masseter muscle pain and sensitization in healthy young women, which contrasts with recent observations in healthy young men. Further studies will be needed to reveal the mechanisms that underlie this apparent sex-related difference in ketamine-mediated analgesia.* J OROFAC PAIN 2007;21:216–224

Key words: glutamate, ketamine, muscle pain, orofacial pain, temporomandibular disorders, trigeminal physiology

The role of peripheral mechanisms in the etiology and pathogenesis of myofascial temporomandibular disorders (TMD), which have a female predominance and are characterized by symptoms of localized ongoing and activity-provoked masticatory muscle pain, remains unclear.[1–3] Some lines of evidence support the concept that a peripheral mechanism involving the elevation of muscle tissue levels of peripherally active neurotransmitters such as serotonin may contribute to the development and maintenance of pain and sensitization in these disorders.[4] A recent review suggested that increased tissue concentrations of the excitatory amino acid (EAA) glutamate, which acts on EAA receptors including N-methyl-D-aspartate (NMDA) receptors, may play a role in certain

musculoskeletal pain conditions and that this may itself involve a peripheral interaction with TRPV1 receptors.[1] For example, elevated glutamate concentrations in deep tissues, such as in the synovial fluid of arthritis sufferers and in the tendon tissues of patients suffering from "jumper's knee" and tennis elbow, are associated with ongoing pain and sensitization.[5–7]

The authors have reported that an experimentally induced increase in glutamate concentration in the human masseter muscle evokes pain, decreases the pressure pain threshold (PPT), and enhances the amplitude of the jaw-stretch reflex.[8–12] Further, it has been found that the intensity of pain evoked by injection of glutamate into the masseter muscle is greater in healthy young women than in healthy young men.[10,11] In healthy young men, pain and mechanical sensitization produced by elevated levels of glutamate in the masseter muscle can be attenuated by local coadministration of the NMDA receptor antagonist ketamine, which suggests that activation of peripheral NMDA receptors mediates these effects.[13,14] These findings are consistent with the authors' data in rats that masseter nociceptive afferent excitability and jaw electromyographic activity reflexively evoked by glutamate application to the masseter muscle are significantly greater in female rats than male rats.[10] These results have led to speculation that sex-related differences in the intensity of glutamate-evoked masseter muscle pain could be mediated through alterations in the activity or number of peripheral NMDA receptors in women. Such findings may have implications for the management of musculoskeletal pain conditions such as TMD, especially in light of the view that manipulation of the peripheral glutamatergic system could lead to advances in the management of maladaptive pain without the risk of serious central nervous system–mediated side effects.[15]

Nonetheless, the involvement of peripheral NMDA receptor mechanisms in glutamate-evoked pain and mechanical sensitization of the masseter muscle has only been demonstrated in men so far.[13,14] One challenge in the investigation of masseter muscle pain and mechanical sensitivity in women derives from the reported variability in their response to noxious stimuli over the natural menstrual cycle.[16–18] A further complication of such studies is the fact that in women who take oral contraceptives (OC), which change the natural fluctuation of sex hormones, eg, progesterone and estrogens, there is the potential for decreased sensitivity to painful stimuli.[19] In previous studies,[10,11] attempts were made to examine the possible effects of OC use, but the sample size was small, and post-hoc tests did not reveal significant differences. As a result, the main aim of the present study was to test the hypothesis that local injection of the NMDA receptor antagonist ketamine would significantly attenuate glutamate-evoked masseter mechanical sensitization and muscle pain in healthy young women either taking OC (W+OC) or not taking OC (W-OC). Because of possible complications in hormonal conditions, an additional goal of the present study was to compare the baseline pain responses of W+OC with those of W-OC to determine whether there are any significant differences between the 2 groups.

Materials and Methods

Volunteers

Healthy female students from the University of Aarhus (n = 47, mean age, 24.2 years) volunteered to participate in this study, which was performed at the Department of Clinical Oral Physiology in the School of Dentistry. All volunteers were paid for their participation. The study was approved by the local ethics committee and conducted in accordance with the Helsinki Declaration. All the volunteers read and signed informed consent forms.

The subjects were divided into 2 groups, W+OC (n = 25) and W-OC (n = 22). All W+OC were taking synthetic estrogens, and the majority (n = 19) were taking them combined with progesterone. W-OC were tested in the early follicular phase 2 to 7 days after menstruation.[20] The exclusion criteria for both groups were signs or symptoms of painful TMD[21]; myositis; contracture or spasms; systemic musculoskeletal pain disorders such as fibromyalgia; symptoms of rheumatoid arthritis[22] or any other concurrent serious systemic diseases; need for chronic administration of psychiatric, analgesic, or other medications that might influence the pain responses; pregnancy; current malignancies; or high blood pressure. Additionally, all volunteers confirmed that they had not taken any analgesics 24 hours prior to the experiment.

Experimental Protocol

The experimental protocol was similar to that used in a previous study in men.[14] Experimental pain was induced deep in the masseter muscle by injections of 0.2 mL of sterile solutions of monosodium glutamate (Ajinomoto) prepared by the Aalborg

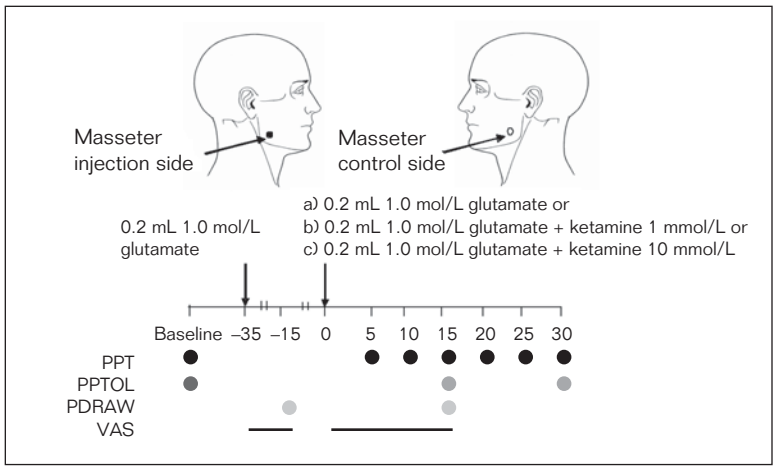

Fig 1 Schematic illustration of the experimental protocol. PPT = pressure pain threshold, PPTOL = pressure pain tolerance, PDRAW = pain drawing, VAS = visual analog scale.

Hospital pharmacy department. The point of injection was midway between the upper and lower border of the masseter muscle and 1 cm posterior to its anterior border over a 10-second period with a 27-gauge hypodermic needle and disposable syringe. Figure 1 illustrates the experimental protocol. Each subject participated in 3 sessions. W-OC were tested in the early follicular phase 2 to 7 days after menstruation,[20] and W+OC were tested at an interval of 1 to 4 weeks between sessions.

The treatment order was randomized between the 3 sessions, and neither the examiners nor the volunteers were aware of the contents of the second injection (double blind). The same masseter muscle was injected twice at each session. The first injection was an internal control with glutamate alone (1.0 mol/L; ~ pH 7.2), and the response to the second injection was normalized with the response of the first injection to control for the intersession variability in raw pain ratings.[14] Thirty-five minutes after the first injection, a second injection, either glutamate alone (treatment 1) or glutamate and ketamine (treatment 2: 1 mmol/L, ~ pH 7.0, Ketalar, Parke-Davis; treatment 3: 10 mmol/L, ~ pH 7.0, Ketalar). It was demonstrated in a previous study that 1.0 mmol/L glutamate evokes muscle pain for up to 15 minutes postinjection.[14]

The concentration of ketamine employed in the present study was based on the authors' previous work, which indicated that a concentration of 10 mmol/L ketamine selectively blocked glutamate-evoked masseter muscle pain in humans and masseter nociceptive afferent discharges in animals.[13,14] In the present study, 1 mmol/L ketamine was tested because it was speculated that women could show a greater sensitivity to the NMDA receptor antagonist. Pain intensity, which was recorded on a visual analog scale (VAS) for 15 minutes after each injection, PPT, and pressure pain tolerance (PPTOL) level were assessed at regular intervals for 30 minutes after each injection (Fig 1).

VAS Recordings

The volunteers continuously scored their pain intensity on a 10-cm electronic VAS for 15 minutes after each injection. The lower extreme was marked "no pain," and the upper extreme was marked "most pain imaginable." The peak pain (the highest VAS score), duration of pain (time required before the VAS scores had returned to "no pain" after injection), and overall pain (area under the VAS time curve) were calculated from the recorded VAS data. The procedure was in accordance with previously described methods.[8,10–13]

Pain Drawings

The volunteers were asked to draw the distribution of perceived pain on a lateral view of the face 15 minutes after the injections. The pain area was digitized (Sigma Scan Pro 4.01.003) and expressed as arbitrary units.[23]

PPT and PPTOL

A pressure algometer (Somedic) was applied to the masseter muscles as previously described[24] to measure PPT, which was defined as the amount of applied pressure (kPa) necessary for a subject to report pain. PPTOL was defined as the maximal pressure (kPa) a subject was willing to accept. The volunteers kept their jaws at rest and did not clench their teeth. The pressure was applied to the muscle at a rate of 30 kPa/s with a 1-cm^2-diameter probe, and the volunteers pushed a button when they reached the PPT or PPTOL. PPT and PPTOL

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Table 1 Absolute Values of the VAS Pain Parameters and Pain Drawing Areas in Response to Injection of Glutamate

	AUC (VAS units × s)		VAS duration		VAS peak pain		Pain drawing area (AU)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
W-OC (n = 22)	974	130	343	31	5.0	0.5	88.5	10.1
W+OC (n = 25)	913	137	321	35	4.6	0.4	107.1	23.3

AU = arbitrary unit; AUC = relative areas under VAS curve.

(kPa) were determined from a single measurement at each time point, except for the PPT at baseline, which was the average of 3 repeated measurements performed prior to the injection of glutamate into the masseter muscle. A template that outlined the outer ear, the corner of the eye, and the mouth was made for all subjects to ensure that the injection and mechanical stimuli were applied to the same location in each session. The side of injection was randomized, and the chosen side for each volunteer was maintained for all 3 sessions. Masseter muscle PPTs and PPTOLs were determined on the injected and noninjected sides prior to injection (baseline) and every 5 minutes (PPT) or 15 minutes (PPTOL) after the injections (Fig 1).

Statistical Analysis

Previous studies in healthy young men indicated that 10 mmol/L ketamine could decrease glutamate-evoked pain by about 50%[13,14]; therefore, the present study was designed to be able to detect a decrease in glutamate-evoked pain of at least 25%. Power analyses indicated that 25 subjects per group would be needed to detect a difference of 25% in the primary outcome parameters, with an interindividual variability of 30% and risk of type I and II errors of 5% and 20%, respectively.

To obtain the relative changes, VAS parameters and pain drawings were normalized by dividing the response obtained after the second injection by the response obtained after the first injection to control the intersession variability in pain ratings. In addition, PPTs and PPTOLs recorded after injections were normalized to their baseline values.[14] One-way and 2-way analyses of variance (ANOVAs) with groups (W-OC, W+OC) as 1 factor and treatment alone, or time and treatment as repeated measures, were used to determine whether there was an effect of ketamine on VAS parameters and pain drawing or PPT and PPTOL, respectively. Tukey tests were used for post-hoc comparison when appropriate. The level of significance was set at $P < .05$.

Results

VAS and Pain Drawing Results

ANOVAs did not indicate any significant differences between the 2 groups in relative changes for the VAS pain parameters or pain drawings. Table 1 shows the average values after injection of glutamate alone for the 2 groups. There were no significant effects of treatment on the VAS pain parameters or pain drawings and no significant interactions between factors (Figs 2 and 3).

PPT and PPTOL

There was no significant difference between the 2 groups for baseline values. The baseline PPTs were 292 ± 15 kPa for the W-OC group and 301 ± 17 kPa for the W+OC group. The baseline PPTOLs were 515 ± 23 kPa for the W-OC group and 518 ± 25 kPa for the W+OC group. For relative changes, there was no significant main effect of groups on PPT or PPTOL. There was no main effect of treatment on PPT or PPTOL (Figs 4 and 5); however, there was a main effect of time (ANOVAs: $P < .001$), with significantly lower PPTs immediately after glutamate injection (5 minutes; Fig 4) and significantly lower PPTOLs 15 and 30 minutes after the injection (Fig 5). There were no significant interactions between factors.

Discussion

The results of the present study indicate that locally administered ketamine had no detectable effects on glutamate-evoked masseter muscle pain or sensitization in healthy young women. This contrasts with recent findings in young healthy men and with data obtained from animals and suggests that different mechanisms may underlie the development of muscle pain in women than in men. In particular, the present findings suggest that non-NMDA receptor mechanisms may be more important in

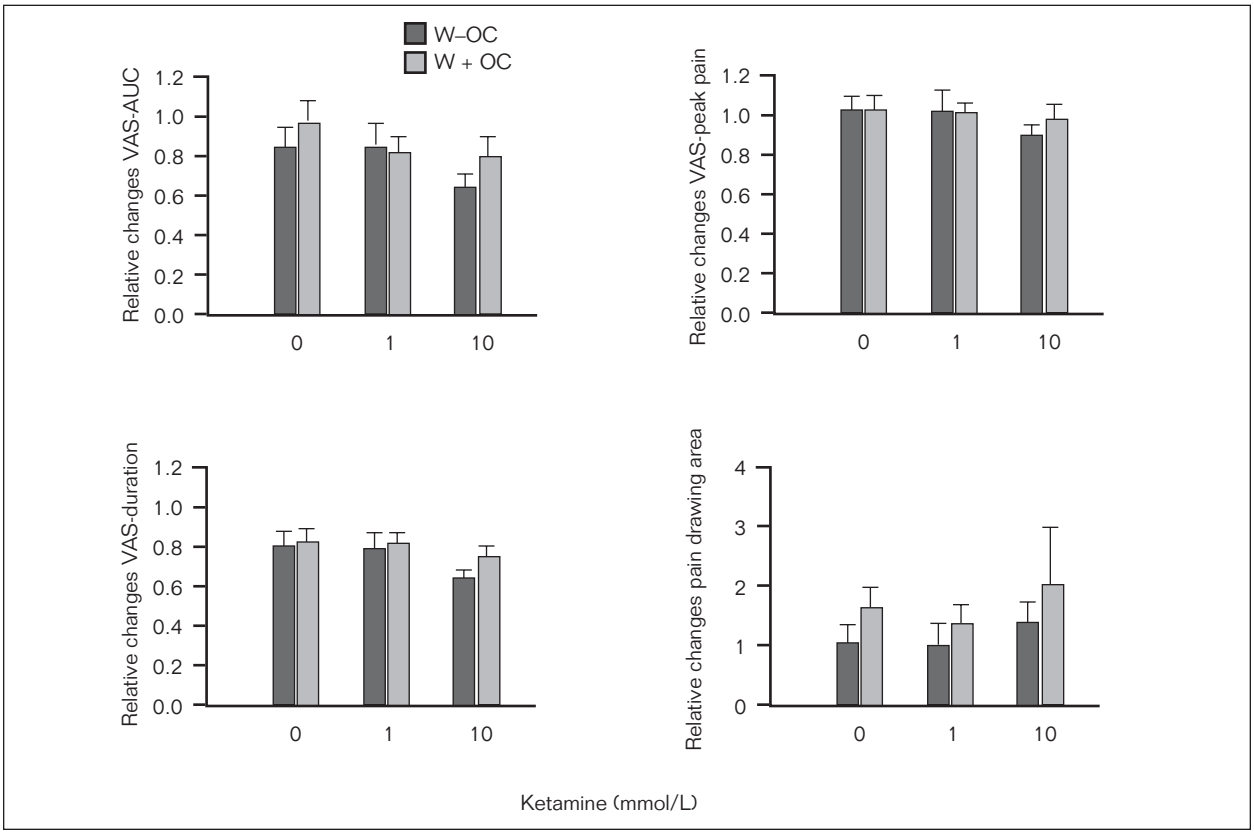

Fig 2 Mean values (n = 47, ± SEM) of the relative pain drawing area in arbitrary units (AU), relative area under VAS curve (VAS-AUC), relative VAS-duration, and relative VAS-peak pain in response to the 3 different types of treatment injections (0, 1, and 10 mmol/L ketamine) into the masseter muscle. There were no significant differences between groups or treatments in the relative changes in any of the VAS parameters or in pain drawing values (ANOVA: $P > .055$).

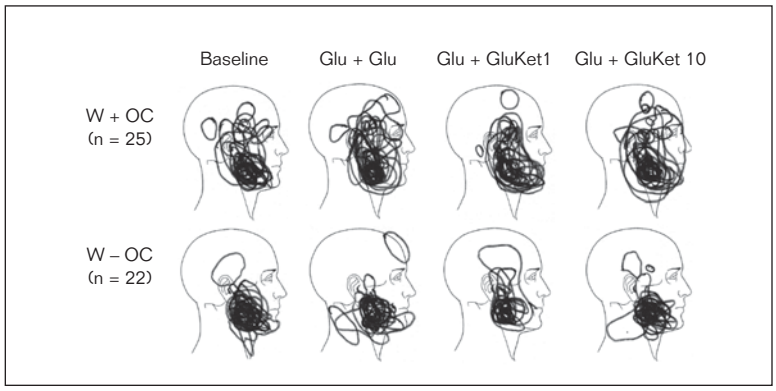

Fig 3 Anatomic distribution of the pain drawings in the sessions with 2 glutamate injections (treatment 1; Glu + Glu), glutamate and ketamine 1 mmol/L (treatment 2; Glu + GluKet 1), and glutamate and ketamine 10 mmol/L (treatment 3; Glu + GluKet 10) in women taking (W+OC) and not taking oral contraceptives (W-OC).

glutamate-related muscle pain in healthy young women than NMDA receptor mechanisms.

Glutamate-Evoked Muscle Pain

The results of the present study show that there were no significant differences in glutamate-evoked pain between W-OC and W+OC at baseline, which is consistent with the authors' previous research.[10] Moreover, the results also suggest that there may be a sex-related difference in the effectiveness of the NMDA receptor antagonist ketamine to block glutamate-evoked muscle pain. In this group of healthy young women, ketamine

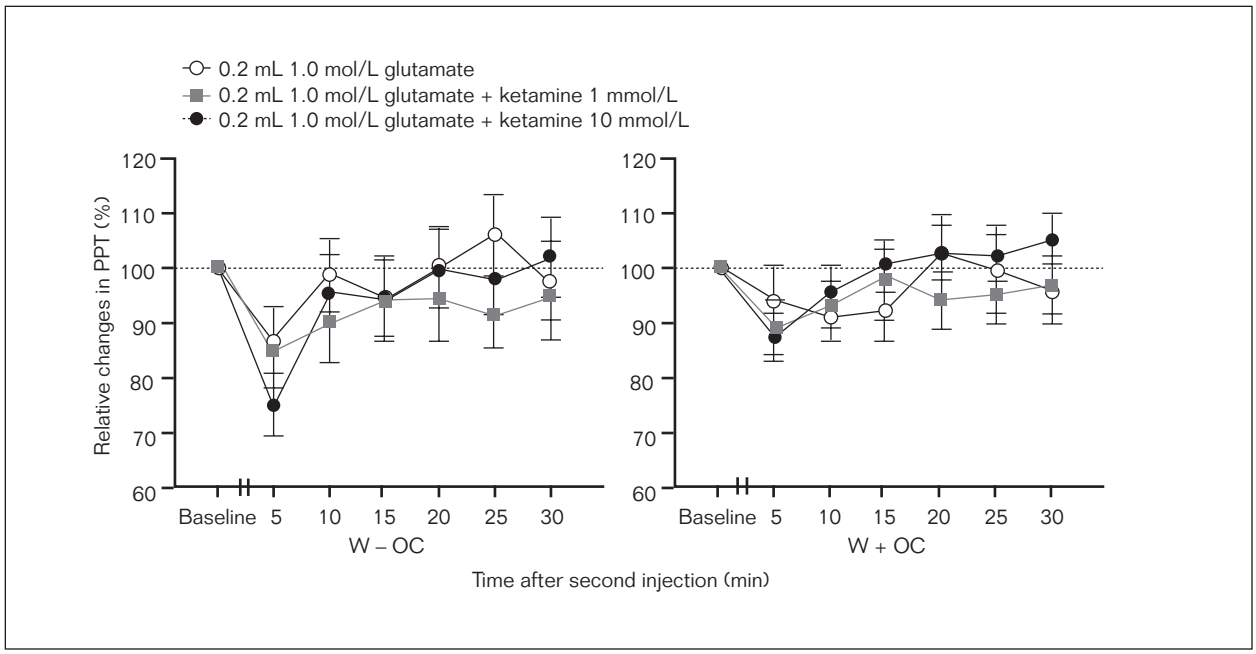

Fig 4 Mean PPTs normalized to baseline values at various time points after the second injection for the W-OC and W+OC groups. There were no significant differences between groups or treatments (ANOVAs: $P > .730$), but there was a significant effect of time (ANOVAs: $P < .001$).

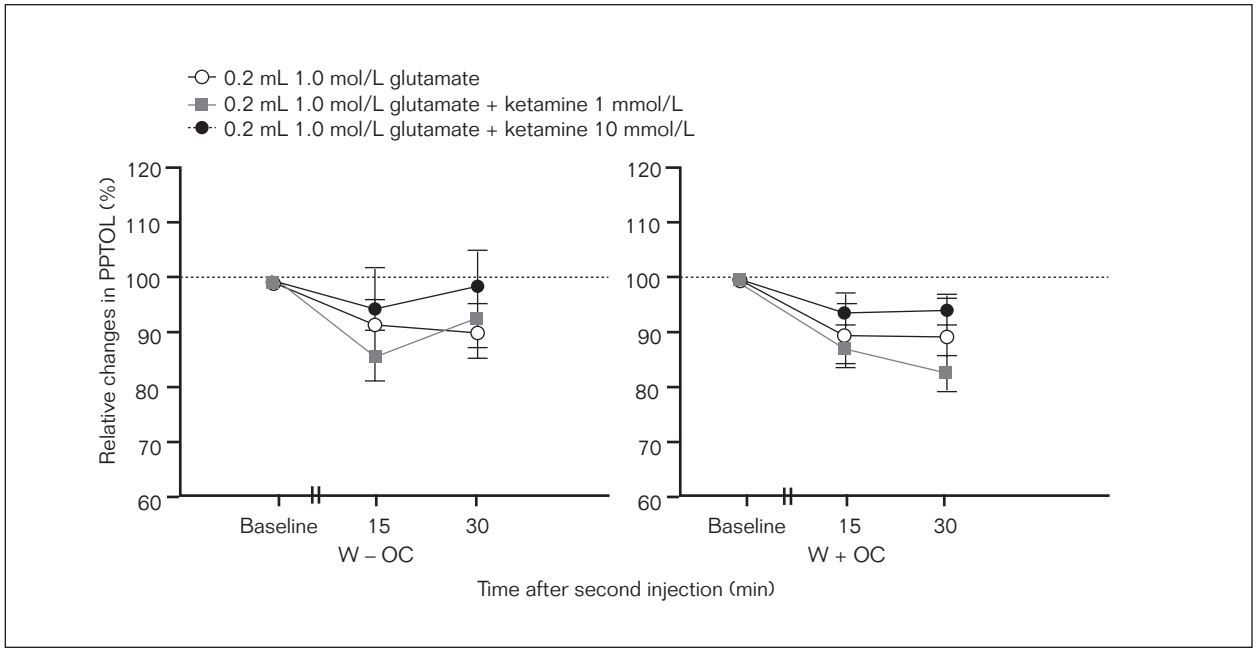

Fig 5 Mean PPTOLs normalized to baseline values 15 and 30 minutes after the second injection. There were no significant differences between groups or treatments (ANOVA: $P > .167$), but there was a significant effect of time both 15 and 30 minutes after the second injection (ANOVA: $P < .001$).

did not affect glutamate-evoked muscle pain (Fig 2). This finding differs from recent results in healthy young men; in that population, coinjection of the same concentration of ketamine under analogous experimental conditions attenuated the glutamate-evoked peak pain, duration of pain, and overall pain by ~50%.[14] This finding also contrasts with the authors' previous findings in rats of sex-dependent, NMDA-related increases in masseter nociceptive afferent activity and jaw electromyographic activity reflexively evoked by glutamate injection into the rat masseter muscle.[10]

It is unlikely that methodologic issues such as sample size or ketamine dose could be the main reason for the lack of ketamine-induced effects in the present study, because a similar methodology was previously employed to study men.[14] Furthermore, a paired design was used in which the female subjects acted as their own controls. However, different investigators and subject populations participated in the 2 studies, which may have led to some minor variations in experimental protocol. Furthermore, it is possible that a higher dose of ketamine would have been effective and that the authors' speculation that women would be more sensitive to administration of ketamine was incorrect. Higher concentrations of ketamine were not used because 10 mmol/L ketamine does not exert nonselective, local anesthetic-like actions, and thus its effects on glutamate-evoked pain are likely mediated through peripheral NMDA receptor antagonism. Moreover, a lower concentration of glutamate was not selected because glutamate-induced mechanical sensitization of the masseter muscle has only been shown to occur with glutamate concentrations of 1 mol/L.[11,25] However, since lower concentrations of glutamate (eg, 500 mmol/L) evoke a similar intensity of pain, future studies might be designed to investigate whether ketamine is more effective against lower concentrations of glutamate injections in women. Furthermore, 1 other possible biologic explanation for this apparent sex-related difference in the effect of ketamine on glutamate-evoked muscle pain could be that other peripheral glutamate-receptor subtypes, such as amino-5-methyl-4-isoxazolone-propionic acid (AMPA), kainate, or metabotropic receptors[15,26] may play a more significant role than NMDA receptors in glutamate-evoked muscle pain in women compared to men. Further investigation of non-NMDA receptor activation in glutamate-evoked muscle pain will require the development of non-NMDA receptor antagonists that are approved for human use.

The apparent sex-related difference in the ability of ketamine to antagonize glutamate-evoked masseter muscle pain also raises questions about the possible differences in the mechanisms responsible for ketamine's ability to block NMDA receptors. Several studies using other drugs such as ibuprofen[27] and pentazocine[28,29] in both animals and humans have reported some sex differences in analgesic effects, while other studies have reported no such differences.[30,31] Future studies of NMDA and non-NMDA receptor mechanisms in glutamate-evoked pain need to take account of findings that sex-related differences in analgesia may be influenced by multiple factors, such as anatomic location of the pain, types of receptors involved, pain model, type of drug, dose, mechanisms of action of the drug, psychologic factors,[30–34] and the age and health status of the subjects studied.

Glutamate-Induced Mechanical Allodynia

Baseline PPT values in the masseter muscle in this study are comparable to results from several other studies of women and men.[11,14,35,36] There were also no differences between the authors' baseline results in women and their previous PPT data for men.[9,14,35] These findings are consistent with reports of a lack of sex-related differences in PPT values for the masseter muscle in healthy men and women,[11,37] and with data on the mechanical activation threshold of masseter nociceptive afferents in rats.[25] However, the results regarding sex differences in masseter PPT are conflicting, and there are also studies showing higher PPTs in men.[34] Nevertheless, there is evidence indicating that women exhibit greater sensitivity than men to painful mechanical stimuli at other anatomic locations.[38] For example, a study of 240 human subjects found a significant difference between men and women in the mean PPT measured at the first dorsal interosseous muscle.[39]

The results of the current study also indicate that there were no major differences between the 2 groups of women (W-OC and W+OC) in terms of PPT, PPTOL, or response to glutamate-evoked pain. This finding suggests that estrogen has limited or no influence on the glutamate-related pain parameters in women, although the present study was powered in order to detect differences of about 25% between groups. A consequence of this finding is that, at least for experimental pain research purposes, significantly larger groups will be needed to detect any possible differences in glutamate-evoked masseter pain between women using OC and those not using OC.

The results of this study are also consistent with earlier findings that repeated injection of glutamate

into the masseter muscle significantly decreases the PPT at the site of the injection (Figs 4 and 5).[11,14] A glutamate-induced decrease in mechanical activation threshold of rat masseter nociceptive afferents has also been documented.[10,25] The present study also showed a decrease in PPTOL values after glutamate injection (Fig 5), and the PPT and PPTOL findings collectively indicate that glutamate injection results in both mechanical hyperalgesia and mechanical allodynia. Again, there were no significant differences between the 2 groups of women (W-OC and W+OC) in terms of the degree of mechanical sensitization produced by glutamate injections. Moreover, coinjection of 10 mmol/L ketamine did not affect the ability of glutamate to induce mechanical sensitization in the masseter muscle of female subjects, whereas 10 mmol/L ketamine could significantly prevent glutamate-induced mechanical sensitization of the masseter muscle in men[14] and glutamate-induced sensitization of masseter-nociceptive afferents in male rats.[9] Thus, different mechanisms may underlie the development of mechanical sensitization in women than in men. For example, it is possible that the active participation of other peripheral receptor processes, such as those involving TRPV1,[1] neuropeptides, or serotonin[40] may play a greater role in the development of glutamate-induced masseter muscle mechanical sensitization in women. Further studies will be needed to reveal the basis of such sex-related differences.

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