The Effect of Glutamate-Evoked Masseter Muscle Pain on the Human Jaw-Stretch Reflex Differs in Men and Women

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Aims: *To compare jaw-stretch reflex responses in male and female subjects and to determine whether injection of glutamate into the masseter muscle facilitates these responses in both sexes.* ***Methods:*** *Jaw-stretch reflex responses were evoked with a muscle stretcher, and pain intensity was scored by 11 men and 13 women before and after the injection of glutamate (1.0 mol/L, 0.2 ml) into the masseter muscle. The subjects rated glutumate-evoked pain intensity on a visual analog scale.* ***Results:*** *Baseline jaw-stretch reflex responses were larger and glutamate injections into the masseter muscle were significantly more painful in women than in men, however, glutamate significantly facilitated jaw-stretch reflex responses in men but not in women.* ***Conclusion:*** *These results suggest that there is a significant sex-related difference in human jaw-stretch reflex responses and their modulation by painful stimuli. Since one possible function of facilitated jaw-stretch reflex responses during jaw muscle pain may be to reduce jaw mobility and thus protect against further exacerbation of an existing injury, the finding of a sex-related difference in modulation of jaw-stretch reflex responses may prove to be important in clarifying why the prevalence of temporomandibular disorders is greater in women than in men.* J OROFAC PAIN 2003;17:317–325.

Key words: glutamate, jaw-stretch reflex, masticatory muscle, sex differences, trigeminal pain mechanisms

There is evidence to suggest that jaw-stretch reflex responses are altered during chronic orofacial pain conditions such as temporomandibular disorders (TMD).[1–4] Although the clinical relevance of this finding remains controversial, it has been suggested that the facilitation of jaw-stretch reflex responses by muscle pain reduces jaw mobility and thus serves a protective function.[5,6] Recent studies have shown that experimental masseter muscle pain induced by injection of hypertonic saline into the masseter muscle enhances the amplitude of the human jaw-stretch reflex response.[7–10] It is unclear how hypertonic saline injections evoke muscle pain, although 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.[11,12] The mechanistic basis for facilitation of human jaw-stretch reflex responses after injection of hypertonic saline into the masseter muscle is also not well understood. It has been proposed, based on results obtained in animal studies, that hypertonic saline injections facilitate human jaw-stretch reflex responses by increasing the excitability of spindle afferent fibers through an effect on gamma-motoneuron tone, as well as by altering the response properties of trigeminal brainstem interneurons,[7,8,13–15] rather than by directly altering the excitability of spindle and/or other stretch-sensitive masseter muscle afferent fibers.[15]

In contrast to hypertonic saline, the mechanism whereby injection of the excitatory amino acid glutamate into the human masseter muscle results in reports of localized and referred muscle pain is better characterized.[16,17] Animal models have revealed that glutamate exerts its effects on deep craniofacial tissues in part through activation of peripheral N-methyl-d-aspartate (NMDA) and non-NMDA receptors associated with craniofacial group III and IV afferent fibers.[18,19] However, unlike hypertonic saline, injection of glutamate into the human masseter muscle has also been demonstrated to induce a prolonged period (> 30 minutes) of mechanical sensitization.[17,20] Animal studies have shown that injection of glutamate into the masseter muscle excites and decreases the mechanical threshold of afferent fibers over a broad conduction velocity range (2.5 to 45 m/s); it can both activate and sensitize group II and III afferent fibers.[19] This glutamate-induced mechanical sensitization is also mediated through activation of peripheral excitatory amino acid receptors.[19] Thus, glutamate injection into the human masseter muscle produces both localized muscle pain and prolonged mechanical sensitivity. Similar symptoms of muscle pain and localized mechanical sensitivity are also reported by patients suffering from TMD.[21–23]

The effect of injection of glutamate into the masseter muscle on human jaw-stretch reflex responses has not yet been examined. As both glutamate and hypertonic saline activate muscle nociceptors and cause pain in human subjects, glutamate injections into the masseter muscle would be predicted to enhance human jaw-stretch reflex responses. However, since glutamate-evoked pain in human subjects and glutamate-evoked afferent fiber activity in animals are significantly greater in females than in males,[16,18,19] it may be that the increased glutamate-evoked pain in women would be associated with a difference in jaw-stretch reflex responses in women and men. Therefore, the effect of glutamate injection into the masseter muscle on jaw-stretch reflex responses in male and female subjects was examined in the present study. Some of these data have been briefly reported in abstract form.[24]

Materials and Methods

Subjects

Eleven healthy men (mean age ± SD: 24.9 ± 2.4 years) and 13 healthy women (mean age ± SD: 27.9 ± 6.2 years) without signs or symptoms of TMD[25] volunteered to participate in this study, which was undertaken at the Orofacial Pain Laboratory, Center for Sensory–Motor Interaction, Aalborg University, Denmark. The study was approved by the local ethics committee (Counties of Nordjylland and Viborg, Denmark) and conducted in accordance with the Helsinki Declaration. Informed consent was obtained from all subjects.

EMG Recordings

Surface recordings of electromyographic (EMG) activity from the left and right masseter and anterior temporalis muscles were made with 4 pairs of bipolar disposable electrodes (720-01-k, Neuroline, Medicotest); the electrodes were placed 10 mm apart over a muscle along its long axis. The skin over the recording positions was cleaned with alcohol prior to electrode placement. A ground electrode soaked with saline was attached to the right wrist. The EMG signals were amplified 2,000 to 5,000 times (Counterpoint MK2), filtered with a band-pass of 20 Hz to 1 kHz, sampled at 4 kHz, and stored for off-line analysis.

Jaw-stretch Reflex Responses

Jaw-stretch reflex responses were evoked bilaterally in the masseter and temporalis muscles with a muscle stretcher based on a previously published design.[8,9,26] A stainless-steel bite-bar was mounted on a frame attached to the floor. A powerful electromagnetic vibrator (Ling Dynamic Systems, model 406) imposed servo-controlled displacements of the bite-bar. A 200-N load cell (Kistler 5039 A312) was placed in series with the moveable probe of the vibrator to measure forces on the lower bite-bar. The displacement of the vibrator probe was measured with a linear potentiometer (Sakae Type 20 FLP 30A-5K) mounted in parallel with the probe. Acceleration in the vertical plane was measured by an accelerometer (Delta Tron Accelerometer Type 4399) mounted on the bite-bar. The initial gape of the bite-bar was 4.0 mm in all experiments, and the short-latency component of the jaw-stretch reflex response was evoked by a displacement of 1 mm with a ramp time of 10 milliseconds.

Similar conditions applied to the women and men, and exactly the same instructions were given to them. To activate the muscle stretcher, subjects were required to contract their left masseter muscle to a steady level of EMG activity which corresponded to 15% of their mean maximal voluntary contraction (MMVC). The 15% MMVC clenching level was used to avoid muscle fatigue during repeated jaw-stretch reflex recordings.[9] They were first asked to perform 3 3-second maximal clenches with their incisor teeth on the bar to obtain their MMVC. The MMVC was defined as the average of the 3 EMG values and was used as a reference. To help them achieve 15% MMVC, they were shown a screen display of 200-millisecond intervals of the root-mean-square (RMS) value of their EMG activity. The screen also displayed an EMG activity window between 13.5% and 16.5% of the MMVC. The display of their EMG activity level changed from green to red when it crossed above the upper and below the lower limits of this window.[27] The program automatically triggered the jaw-muscle stretcher when the EMG activity remained within this window for more than 400 milliseconds. In total, 300 milliseconds of peri-stimulus EMG activity was recorded (100 milliseconds prestimulus and 200 milliseconds poststimulus).

Experimental Protocol

Prior to injection of glutamate into the left masseter muscle, subjects received training with the muscle stretcher. The subjects were allowed to practice activating the muscle stretcher by clenching the bar until they indicated they were comfortable with the procedure. Before the glutamate injection, a baseline of 30 jaw-stretch reflex responses was obtained over a 5-minute period (once every 10 seconds). Sterile glutamate (1.0 mol/L, 0.2 ml) was then injected into the posterior part of the left masseter muscle about 2 cm posterior to the surface EMG electrodes, and 60 jaw-stretch reflex responses were obtained over a 10-minute period (once every 10 seconds). The subjects continuously scored the pain intensity on a 10-cm electronic visual analog scale (VAS) with the lower extreme marked “no pain” and the upper extreme marked “most pain imaginable.” VAS pain ratings were sampled every 10 seconds and stored electronically for later analysis.

Data Analysis

The amplitude of reflex EMG responses evoked by each fast stretch was analyzed off-line by a computer program developed in the laboratory. The peak-to-peak amplitude of each jaw-stretch reflex response was measured from the nonrectified EMG signal.[28] The EMG activity was rectified and the average wave amplitude during the 100-millisecond prestimulus period was calculated. The amplitude of the stretch reflex has been shown to be critically dependent on the amplitude of EMG activity during the prestimulus period.[9] To control for this effect in the present study, the peak-to-peak amplitude was then normalized to the mean prestimulus level of EMG activity to yield relative jaw-stretch reflex responses.[9] Median and maximum relative jaw-stretch reflex responses were calculated from all the relative jaw-stretch reflex responses (n = 30) collected during the initial 5-minute period prior to each injection, and from each of 2 consecutive 5-minute periods (n = 30 per period) immediately following injection.

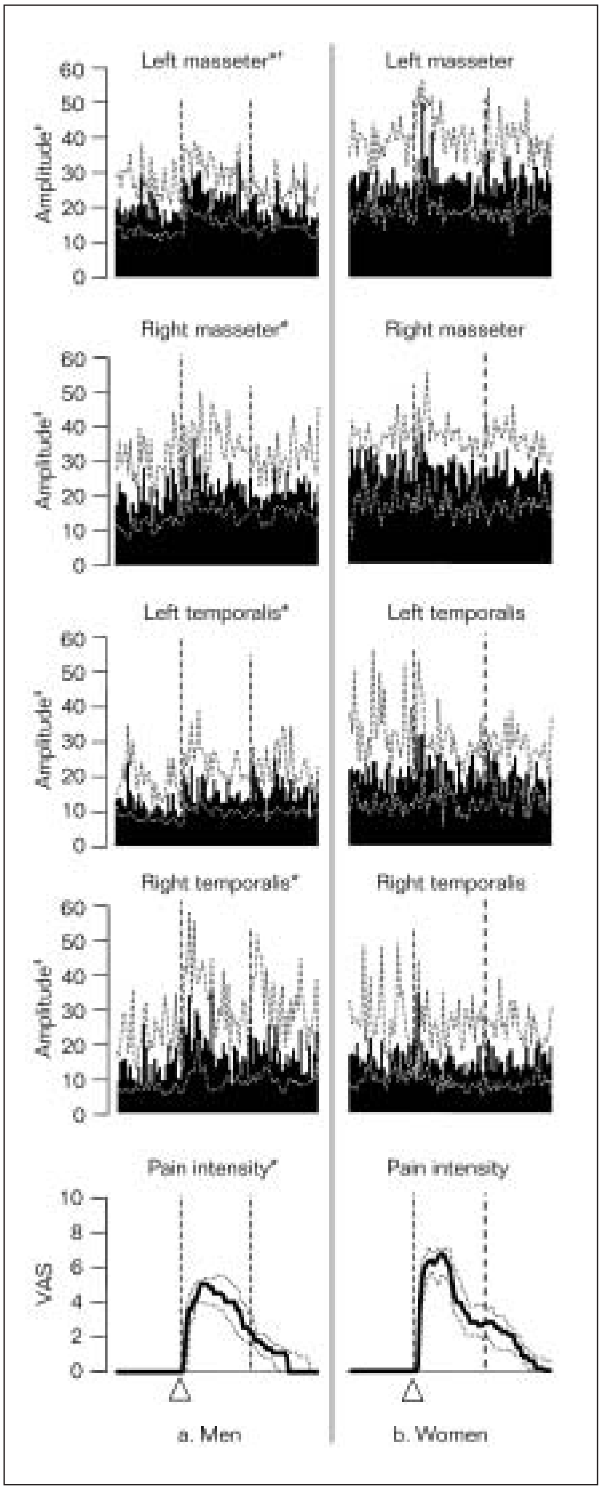
Statistical Analyses

Spearman correlation on ranks was used to determine if significant relationships existed between the VAS pain score and the relative jaw-stretch reflex responses for each muscle for the initial 5-minute period after the injection. Repeated measures analysis of variance (ANOVA) on ranks was used to compare the median and maximum relative jaw-stretch reflex responses calculated before and after injection for each muscle. A post-hoc Dunnett test was applied when the ANOVA indicated significant differences. Comparisons of VAS pain parameters, as well as median and maximum relative jaw-stretch reflex responses between men and women, were made with a Mann-Whitney rank sum test. The level of significance was set at $P < .05$.

Results

Male Subjects

Injection of glutamate into the left masseter muscle evoked pain in all 11 men and significantly enhanced the median relative jaw-stretch reflex responses bilaterally in both the masseter and temporalis muscles (Fig 1a). The maximum relative jaw-stretch reflex response in the left masseter muscle was also significantly increased (Fig 1a). There was a significant positive correlation between the intensity of pain and the relative jaw-stretch reflex responses in the left masseter muscle ($r = 0.557$, $P < .05$ Spearman correlation).



Figs 1a and 1b The effect of glutamate injections on the relative jaw-stretch reflex responses recorded from 11 male and 13 female subjects is shown. The histograms illustrate the median relative jaw-stretch reflex response, and the line graph illustrates the median VAS pain score. Dotted lines indicate the interquartile range. Jaw-stretch reflex responses and VAS pain scores were obtained every 10 seconds. The dashed vertical lines divide the experiment into three 5-minute periods—1 preinjection and 2 postinjection. Injection of glutamate (white triangles) significantly increased jaw-stretch reflex responses in all 4 muscles, but had no significant effect on jaw-stretch reflex responses in women. *$P < .05$, repeated measures ANOVA on ranks, Dunnett test for the overall median relative stretch reflex response compared to preinjection baseline. †$P < .05$, repeated measures ANOVA on ranks, Dunnett test for the maximum relative stretch reflex response, compared to preinjection baseline. ‡Relative peak-to-peak.

Female Subjects

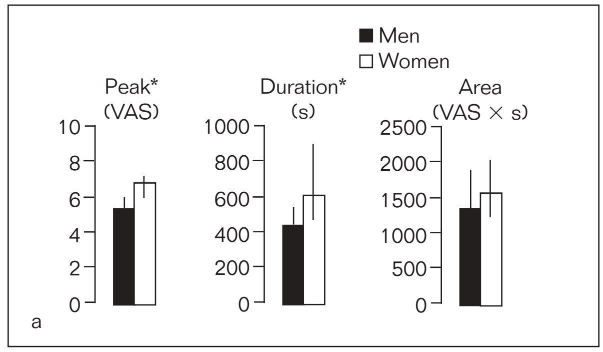
Injection of glutamate into the left masseter muscle evoked pain in all 13 women but did not significantly increase either the median or maximum relative jaw-stretch reflex responses in any muscle (Fig 1b). There was also no significant correlation between the intensity of pain and the relative jaw-stretch reflex responses in any of the 4 muscles after injection of glutamate.

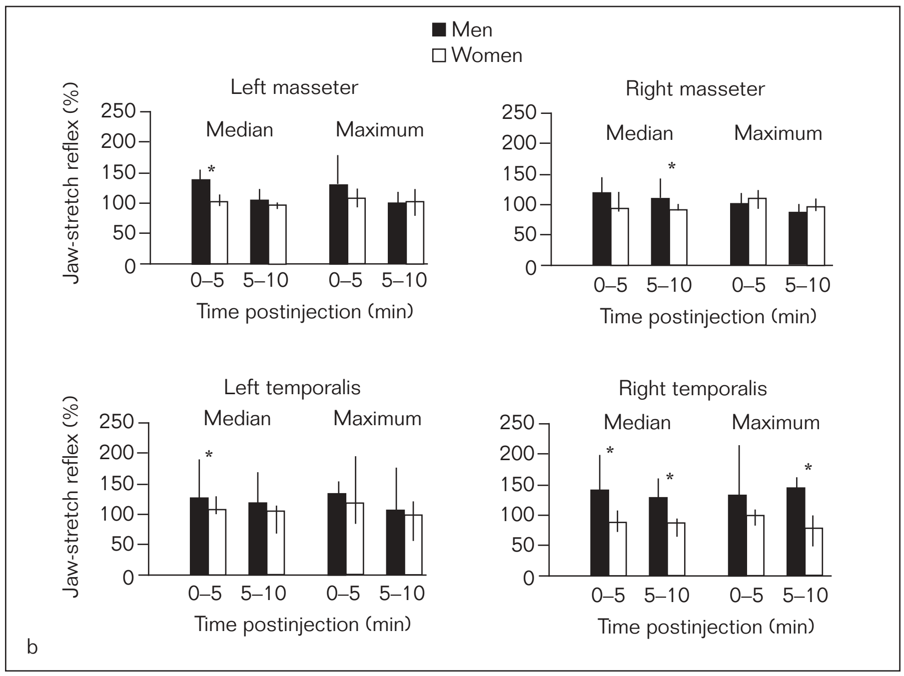
Comparison of Men and Women

The peak and duration of pain evoked by glutamate injection were significantly greater in women than in men (Fig 2a). The preinjection (baseline) median relative jaw-stretch reflex responses in all 4 muscles were larger in women than in men, but were significantly larger only in the left masseter muscle ($P < .05$, Mann-Whitney rank sum test; Fig 1). This difference in baseline complicated the direct comparison of the relative jaw-stretch reflex response parameters after injection of glutamate. Therefore, in men and women, the relative jaw-stretch reflex responses after injection of glutamate were normalized to the preinjection baseline relative jaw-stretch reflex responses for all 4 muscles. Analysis of the data in this manner revealed that after injection of glutamate into the masseter muscle, median and maximum normalized jaw-stretch reflex responses were significantly greater in male subjects than in female subjects (Fig 2b).

Discussion

Based on our recent findings, it was expected that glutamate injections into the masseter muscle would evoke greater pain ratings in female subjects than in male subjects.[16,17] It was also anticipated that this would be associated with a greater enhancement of jaw-stretch reflex responses in women than men. Glutamate injections into the masseter muscle were indeed significantly more





Figs 2a and 2b The upper bar graphs indicate pain intensity parameters *(a)* and the lower bar graphs *(b)* indicate values for the median and maximum relative jaw-stretch reflex responses from 2 consecutive 5-minute periods immediately following the injection of glutamate normalized to the pre-injection baseline relative jaw-stretch reflex responses in men (black bar, n = 11) and women (white bar, n=13). Error bars indicate the interquartile range. Note that the majority of the normalized median and maximum values were greater in men than in women. $^*P < .05$, Mann-Whitney rank sum test.

painful in women than men, which is consistent with our earlier findings.[16,17] The preinjection baseline jaw-stretch reflex responses were larger in women than in men. However, contrary to expectations, glutamate was found to significantly facilitate jaw-stretch reflex responses in men but not in women.

Mechanism of Glutamate-induced Jaw-stretch Reflex Enhancement

Jaw-stretch reflex responses appear to be principally mediated through a monosynaptic pathway that includes the jaw-closing (masseter and temporalis) muscle spindle afferent fibers and trigeminal (masseter, temporalis) motoneurons.[29–32] However, a disynaptic pathway from the jaw spindle afferent fibers to the masseter motoneuron pool via the peritrigeminal area, a region that surrounds the trigeminal motor nucleus and the trigeminal subnucleus oralis, also likely contributes to the generation of jaw-stretch reflex responses.[33–36] Thus, the amplitude of jaw-stretch reflex responses after a jaw displacement of fixed amplitude and velocity depends on the magnitude of the spindle afferent volley and the excitability of jaw muscle alpha motoneurons,[37–39] but may also be modulated to some extent by the excitability of premotor interneurons intercalated disynaptically in the jaw-stretch reflex pathway.

Although injection of both hypertonic saline[7–10] and, in the present study, glutamate into the masseter muscle increases the amplitude of human jaw-stretch reflex responses in men, the mechanism that underlies this effect has yet to be satisfactorily elucidated. It has been suggested that increased alpha motoneuron excitability is not likely to be responsible for enhancement of jaw-stretch reflex responses, since experimental pain in humans does not appear to alter the magnitude of the H-reflex, which is thought to reflect motoneuron excitability because it is a monosynaptic reflex evoked by electrical stimulation.[8,27,40–42] Although it is possible that the muscle spindle afferent volley is increased by injection of algogenic substances into the masseter muscle, recent results indicate that hypertonic saline does not exert a consistent excitatory effect on masseter spindle afferent fibers[15] and that glutamate-induced depolarization of spindle afferents is only transient.[43] In contrast, animal studies have revealed that intramuscular injection of hypertonic saline or glutamate evokes large responses in thinly myelinated and unmyelinated trigeminal afferent fibers.[16,18,19,43–45] Injection of hypertonic saline into the masseter muscle also increases the activity of a subpopulation of putative premotor interneurons in the trigeminal subnucleus oralis and the peritrigeminal area during fictive mastication in the rabbit.[46] Further, prolonged noxious stimulation of deep craniofacial tissues after injection of glutamate or the algesic compound mustard oil increases the excitability of trigeminal nociceptive neurons[47–49] in subnucleus caudalis. This is also the site of interneurons involved in the reflex increases in jaw muscle EMG activity that can be induced by mustard oil or glutamate injection into deep craniofacial tissues.[50,51] In addition, injection of glutamate into the masseter muscle also induces a prolonged mechanical sensitization of masseter muscle afferent fibers that is mediated through activation of peripheral NMDA and non-NMDA receptors.[19] These findings collectively suggest that facilitation of the human jaw-stretch reflex responses after injection of glutamate into the masseter muscle may be mediated by both increased excitability of premotor interneurons as well as increased stretch-induced afferent barrage due to the recruitment of sensitized group II, III, and IV masseter muscle afferent fibers.

Comparison of Men and Women

In the current study we found that the preinjection baseline jaw-stretch reflex responses in all 4 muscles were almost 50% larger in women than in men. This finding is consistent with some previous studies, which have reported that the peak-to-peak amplitude of masseter and temporalis stretch reflexes is significantly larger in women than in men.[52–54] A similar sex-related difference has been reported for the auditory startle reflex.[55] Several methodologic factors such as sex-related differences in skin thickness, prestretch EMG activity, jaw displacement distance, and biting position might have influenced the magnitude of the peak-to-peak amplitude of jaw-stretch reflex responses.[9] It has been suggested that a decreased resistance between the skin and the EMG electrodes in women due to their thinner skin might explain why larger peak-to-peak amplitudes were recorded in women.[53,54] However, in the present study, the peak-to-peak amplitude of jaw-stretch reflex responses was normalized to the prestretch EMG activity to correct for the influence of prestretch EMG activity.[9] This manipulation should also have eliminated any potential sex-related difference due to skin thickness or absolute MMVC levels. All male and female subjects received the same instructions and used the same biting position, although the 1 mm displacement of the jaw may have resulted in a greater angle of jaw opening in women than in men. However, a 50% increase in jaw displacement in men (from 1 to 1.5 mm) has been shown to increase jaw-stretch reflex responses by only about 10% to 20%[9] and is also unlikely to explain why baseline jaw-stretch reflex responses were almost 50% larger in women than in men. Thus, it is possible that there are sex-related differences that explain the present findings, such as differences in the number or sensitivity of spindle afferent fibers, the excitability of premotor interneurons, of masseter and temporalis alpha motoneurons, and/or the descending influences that can modulate the excitability of premotor interneurons and motoneurons.

Since the baseline jaw-stretch reflex responses were so different in men and women, we normalized the jaw-stretch reflex responses after injection to the baseline jaw-stretch reflex responses to permit direct comparison of the effect of glutamate injection in men and women. This analysis indicates that overall, glutamate increased jaw-stretch reflex responses by about 30% in men, but had no effect on jaw-stretch reflex responses in women (see Fig 2b). It is conceivable that these findings reflect a sex-related difference in the consequences of painful masseter muscle stimulation. However, there remains a possibility that the failure of glutamate to increase jaw-stretch reflex responses in women might have been due to the experimental conditions. In particular, failure to observe significant increases in jaw-stretch reflex responses after glutamate injection in women could have been due to a ceiling effect; a result of baseline jaw-stretch reflex responses in women that were closer to the maximal jaw-stretch reflex responses. If a ceiling effect underlies the failure of glutamate injection to increase jaw-stretch reflex responses in women, then it would be predicted that reduction of baseline jaw-stretch reflex responses in women by about 50% (ie, to the same level as baseline jaw-stretch reflex responses in men in the present study) would allow glutamate injections to increase jaw-stretch reflex responses in women. Future studies that examine the influence of methodologic factors on the magnitude of jaw-stretch reflex responses in women, analogous to those that have been carried out in men,[9] are required in order to test this prediction.

We have recently found that glutamate injection into deep craniofacial tissues reflexly evokes increases in background jaw muscle EMG activity in rats that are greater in females than in males.[56] Together with our recent[16,17] and present data that show that in humans glutamate injection into the jaw muscle evokes more pain in women than in men, but induces a greater enhancement of jaw stretch reflex responses in men than in women, these findings collectively suggest that the glutamate effects and the sex differences are being expressed differentially in the neural circuits and processes that underlie pain perception, background EMG activity, and stretch reflex responses.

Clinical Significance

Temporomandibular disorders, which are more prevalent in women than in men,[21] are characterized by masticatory muscle pain and tenderness, limited jaw motion, and joint sounds.[23,57,58] Injection of glutamate into the masseter muscle also causes prolonged muscle sensitization and greater pain responses in women than men,[16,17] and thus can mimic in an experimental pain model some of the key characteristics of TMD-related pain. There is some evidence to suggest that jaw-stretch reflexes are altered during chronic craniofacial pain, such as that which occurs in TMD.[1–4] In this study, glutamate injection into the masseter muscle facilitated jaw-stretch reflex responses significantly more in men than women even though baseline jaw-stretch reflex responses were significantly larger in women and even though women expressed greater muscle pain. One possible function of facilitation of jaw-stretch reflex responses during jaw muscle pain may be to reduce jaw mobility and thus protect against further exacerbation of an existing injury.[5,6] The finding that jaw-stretch reflex responses are not facilitated in women during experimental jaw muscle pain may mean that women have less protection against exacerbation of an existing jaw muscle injury than men. This sex-related difference in the effect of muscle pain on jaw-stretch reflex responses may prove to be important in clarifying why the prevalence of TMD is greater in women than in men.[21]

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