Pain Effects of Glutamate Injections Into Human Jaw or Neck Muscles

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Aims: *To document and compare the intensity, localization, and quality of pain evoked by glutamate injections into the human masseter or splenius muscles and to determine the effect of glutamate-evoked pain on the pressure pain thresholds (PPTs) in both jaw and neck muscles.* ***Methods:*** *Twenty-six healthy men were given painful injections of glutamate (1.0 mol/L) and control injections of isotonic saline (0.165 mol/L) into the masseter and splenius muscles. The subjects rated the perceived intensity of pain on a visual analog scale (VAS), drew the area of the pain on maps of the face and neck, and filled out a Danish version of the McGill Pain Questionnaire (MPQ). PPTs were used to assess the sensitivity of the masseter and splenius muscles to mechanical stimuli (n = 11).* ***Results:*** *Glutamate injection into the masseter or splenius evoked pain lasting almost 10 minutes. Peak pain intensity usually occurred within 2 minutes of the injection, and VAS scores of peak pain were significantly higher for the masseter muscle compared with the splenius muscle (paired t test, P = .003). The pain area from the masseter injections did not extend into the neck region, although in some subjects the pain from the neck region extended into the temporal region. There were no significant relationships between the area of perceived pain and the VAS pain scores (Pearson correlation, P > .297). Glutamate-evoked pain in either the masseter or splenius muscles was associated with significant decreases in masseter or splenius PPTs, respectively (2-wayANOVAs, P < .016). Isometric saline injections were almost pain-free and caused no PPT changes.* ***Conclusion:*** *The data suggest that the masseter muscle is more sensitive to glutamate injections and mechanical stimuli than the splenius muscle. The relatively limited overlap between the sensory manifestations of pain from masseter and splenius muscles may have potential implications for diagnosis and management of myofascial pain complaints in the craniofacial and neck region.*
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Key words: cervical spine pain disorders, experimental muscle pain, glutamate, temporomandibular disorders, trigeminal pain

The deep craniofacial tissues represent common sites for acute and chronic symptoms.[1,2] For example, temporomandibular disorders (TMD) typically manifest jaw muscle pain, as well as temporomandibular joint (TMJ) sounds and neuromuscular changes reflected in limited jaw motion.[3–6] The pain is often poorly localized and referred, and indeed is often associated with pain in the neck muscles as well as the jaw muscles.

Furthermore, there are also reports that pain in cervical musculoskeletal tissues may be referred to cranial structures including the jaw muscles.[7–9] Several clinical reports have shown that a substantial proportion of TMD patients have cervical spine disorders,[10–14] although a causal relationship cannot be assumed from findings based solely on prevalence data.[13]

Studies in animals have clearly demonstrated convergence of craniofacial and cervical afferents onto nociceptive neurons in the trigeminal brainstem sensory nuclear complex and central sensitization of these neurons; these findings have been implicated in the spread or referral of pain between the jaw and neck regions.[3,15–17] Furthermore, there is good evidence from both neurophysiological and biomechanical studies for a significant interplay between the human craniofacial and cervical neuromuscular systems.[18–20] However, there have been limited experimental pain studies exploring the possible association between jaw and cervical muscle pains in humans and their effects on the sensitivity of deep craniofacial and cervical tissues.

Various algesic chemicals (eg, hypertonic saline, capsaicin, acidic solutions) injected into deep tissues can be used to elicit pain in human volunteers.[5] Recently, a series of studies has used the excitatory amino acid glutamate to evoke jaw muscle pain in humans and nociceptive activity in rats.[21–24] For example, glutamate injection into the TMJ of rats evokes a nociceptive jaw-muscle reflex mediated through activation of peripheral N-methyl-D-aspartate (NMDA) and non-NMDA receptors.[25] Recently, it was shown that injection of 1.0 mol/L glutamate, but not 0.1 mol/L glutamate or isotonic saline, into the masseter muscle evokes activity in putative nociceptive afferents in the masseter muscle of rats and a reduction in the mechanical threshold of the deep afferents by about 50% for a period of at least 30 minutes after the injection.[22] Repeated injection of 0.2 mL of 1 mol/L glutamate into the human masseter muscle produces pain and has also been shown to reduce the masseter pressure pain thresholds (PPTs) by about 40% to 50% in healthy subjects.[24] However, there are no reports on the sensitivity of neck muscles to painful injections of glutamate.

The aim of the present study was therefore to document and compare the intensity, localization, and quality of pain evoked by glutamate injections into the human masseter or splenius muscles. A second aim was to determine the effect of glutamate-evoked pain on the PPTs in both jaw and neck muscles.

Materials and Methods

Subjects

The volunteers for this study were all healthy and unmedicated subjects without signs or symptoms of TMD or cervical spine disorders.[26] The study was conducted at Aalborg University. Informed consent was obtained from all subjects, and the local Ethics Committee approved the study. Parameters (intensity, localization, quality) of the pain evoked by glutamate injection into the masseter or splenius were obtained from a total of 26 men (mean age: 26.4 ± 1.2 years). PPTs in the masseter and splenius muscles were determined in addition to the pain parameters in 11 of the 26 men. Only men were studied in order to avoid bias caused by gender differences in the processing and perception of deep craniofacial nociceptive activity.[21,24,27]

Experimental Design

Subjects were given standardized instructions and were unaware of which solution was about to be injected (single blind). To avoid sequence effects, injections of glutamate and isotonic saline into the masseter and splenius muscles were performed in a randomized and balanced fashion during 2 sessions separated by 1 week. In each session, the subject received 1 injection of glutamate and 1 injection of isotonic saline; 1 solution was injected into the masseter and the other into the splenius. The first injection (glutamate or isotonic saline) was made into either the right masseter or splenius muscle. Thirty to 40 minutes later, a second injection (isotonic saline or glutamate) was made into the splenius or masseter muscle. The site of the first injection was chosen at random. During the second session, the sequence of substance injection was reversed, eg, if a subject in the first session received a first injection of glutamate into the masseter muscle and a second injection of isotonic saline into the splenius muscle, then he would in the second session first receive an injection of isotonic saline into the masseter muscle and a second injection of glutamate into the splenius muscle.

Intramuscular Injection Technique

All injections were given manually over a 10-second period with a 27-gauge hypodermic needle and a disposable syringe. The masseter injection site was the deep masseter muscle midway between its upper and lower border and 1 cm posterior to its anterior border.[28] The needle was

inserted until bony contact was made and then retracted about 2 mm before aspiration and injection of the solution. The splenius injection site was in the middle of the muscle between the mastoid process and the external occipital protuberance and was identified following careful manual palpation of the muscle during head movements and voluntary contractions. The pharmacy at Aalborg Hospital prepared the sterile solutions of glutamate (1.0 mol/L) and isotonic saline (0.165 mol/L) and adjusted the pH to 6.8 to 7.0. Pilot studies had revealed that injection of 0.2 mL glutamate into the splenius muscle was associated with low pain scores, and it was therefore decided to increase the volume and use 0.4 mL glutamate (and isotonic saline) for the splenius muscle injections and 0.2 mL glutamate (and isotonic saline) for the masseter muscle injections.

Pain Characteristics

All subjects were instructed to rate continuously the pain intensity evoked by the injection of glutamate or isotonic saline on an electronic 10-cm visual analog scale (VAS) for 15 minutes; each subject sat upright with his jaw at rest over this period. The lower endpoint of the VAS (0) was labeled "no pain at all," and the upper endpoint (10) was labeled "most pain imaginable." The VAS signals were sampled every 1 second and stored on a personal computer. The maximum pain was measured as the peak VAS score. The area under the VAS curve (VAS_{AUC}) was used to obtain a measure of the overall amount of pain, and the onset and offset of pain was determined from the VAS profiles. Furthermore, the time from start of injection until the time at which VAS scores peaked was determined.

Fifteen minutes after each injection, the subjects described the quality of their overall pain experience on a validated Danish version of the McGill Pain Questionnaire (MPQ).[29] The pain rating index (PRI) of the sensory, affective, evaluative, and miscellaneous dimension of pain was calculated according to Melzack,[30] and the words chosen by at least 30% of the subjects were noted. The subjects also drew the distribution of pain on a map showing the lateral projection of the face and posterior view of the neck. In addition, all subjects were asked about referral of pain to the teeth. The pain maps were digitized (ACECAD, model D9000+ digitizer, Taiwan) to calculate the area of perceived pain expressed in arbitrary units (au).

PPTs

A pressure algometer (Somedic) was used to test the sensitivity to deep stimuli applied to the masseter and splenius muscle in 11 subjects.[28] The PPT was defined as the amount of pressure (kPa) that the subjects first perceived to be painful. The subject pushed a button to stop the pressure stimulation when the threshold was reached. The PPT was determined at the injection site with a constant application rate of 30 kPa/s and a probe diameter of 1 cm. The probe was held perpendicular to the skin, and the subjects were asked to keep their head still and jaw at rest and not to clench their teeth, because contraction of the jaw-closing muscles may influence the determination of PPTs.[31] The PPTs were determined at baseline before each injection and at 1, 5, 10, 20, and 30 minutes after the injection, in accordance with the methods detailed in the authors' recent study.[24]

Statistical Analyses

The mean (± SEM) VAS pain parameters, perceived pain area, and MPQ data are reported for the entire group of subjects (n = 26). These parameters for the injection into the masseter and splenius muscles were compared with the use of paired *t* tests, repeated-measures analysis of variance (ANOVA), and Tukey post hoc tests. The PPTs (n = 11) were normalized with respect to baseline values and described with 2-way ANOVA models with time (6 levels: baseline, 1, 5, 10, 20, and 30 minutes) and solution (2 levels: glutamate and isotonic saline) as the repeated factors, followed by Tukey post hoc tests. Additional ANOVA tests were used to examine potential sequence effects (4 baseline measurements for each muscle) and differences between masseter and splenius PPTs at baseline. Pearson product-moment correlation was used to test the association between VAS pain scores and area of perceived pain. Furthermore, the association between relative changes in PPTs and VAS pain scores was examined with Pearson tests. For all tests, the significance level was set at $P < .05$.

Results

Pain Characteristics

Injections of glutamate into the masseter and splenius muscles were associated with a painful sensation in all subjects, whereas injections of isotonic

Table 1 Perceived Pain Intensity Following Injection of Glutamate or Isotonic Saline into Masseter (0.2 mL) or Splenius (0.4 mL) Muscles

	Pain onset	Pain offset	Time to VAS peak	VAS peak	VAS_{AUC}
	(s)	(s)	(s)	(cm)	(cm × s)
Glutamate					
Masseter	7 ± 1	527 ± 39	74 ± 9	6.0 ± 0.3*	1727 ± 131
Splenius	6 ± 2	578 ± 38	81 ± 14	4.8 ± 0.4	1530 ± 160
Isotonic saline					
Masseter	12 ± 5	204 ± 49	30 ± 11	1.4 ± 0.2	170 ± 33
Splenius	18 ± 3	102 ± 41	27 ± 14	1.7 ± 0.3	228 ± 58

Mean values and SEM (n = 26) of VAS pain parameters. For injections with isotonic saline, only the data from subjects who reported pain were included in this analysis (10 subjects with respect to the masseter; 9 with respect to the splenius). *Indicates significantly higher compared to splenius (paired *t* test, $P = .003$).

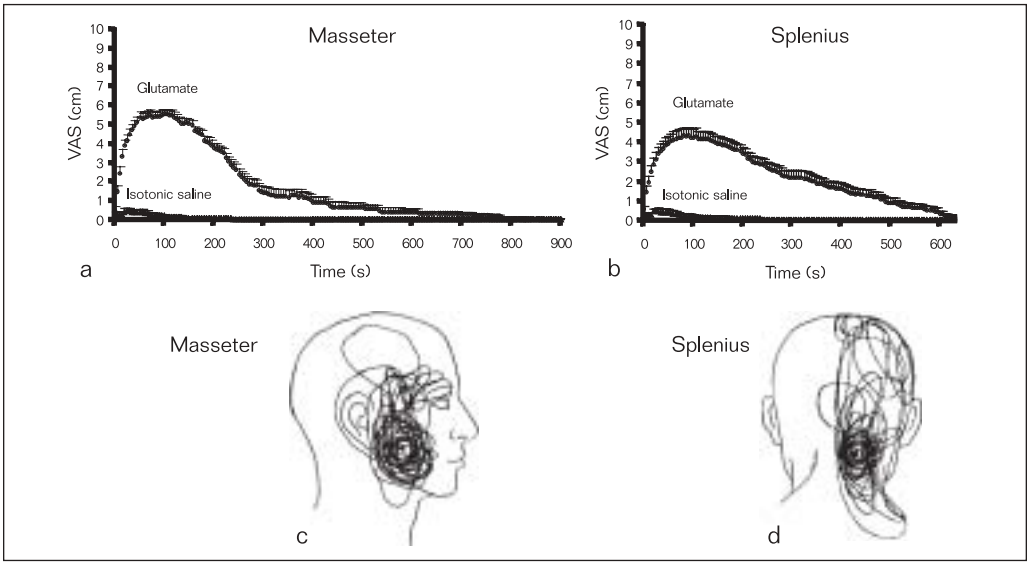

Fig 1 Pain evoked by injection of glutamate or isotonic saline into the masseter or splenius muscles scored continuously on a 0-to-10 VAS *(a and b)*. Mean VAS scores ± SEM are shown for the 26 subjects. Note that pain in the masseter muscle was more intense. The subjects drew the areas of perceived pain on 2 different aspects of the face *(c and d)*. Note here the widespread nature of muscle pain and the lack of referral of masseter pain into the neck region.

saline often were almost pain-free. Only 9 subjects scored any pain on the VAS following the isotonic saline injections into the masseter; only 10 registered any pain after injections into the splenius (Fig 1). The VAS pain parameters are shown in Table 1. In brief, the glutamate-evoked pain started shortly after completion of the injection (mean onset time: 6 to 7 seconds) and usually lasted less than 10 minutes (mean offset time: 527 to 578 seconds), with the peak intensity occurring after 1 to 2 minutes (mean VAS peak time: 74 to 81 seconds). The peak VAS pain score was significantly higher for glutamate injection into the masseter muscles compared with injection into the splenius muscles (paired *t* test, $P = .003$) (Table 1 and Fig 1).

Glutamate-evoked pain in the masseter spread to a large area around the injection site; in some subjects, pain was referred toward the ipsilateral upper head and temporal region (11/26), upper or lower molar teeth (9/26,) or the TMJ (4/26), but never to the neck region (Fig 1c). The glutamate-evoked pain in the splenius spread or was referred to the ipsilateral neck and occipital region (Fig 1d), and in some subjects, toward the ipsilateral upper head and temporal region (12/26), shoulder (5/26), or very rarely, to the teeth or masseter region (1/26) (not shown in Fig 1d). There was no significant difference between the area of perceived pain in the masseter and splenius muscles (2.2 ± 0.7 au versus 3.4 ± 0.7 au; paired *t* test: $P = .074$).

Table 2 Use of Words Chosen from MPQ to Describe the Quality of Injection of Glutamate or Isotonic Saline into Masseter (0.2 mL) and Splenius Muscles (0.4 mL)

	Hurting	Taut	Pressing	Intense	Tight	Tiring	Boring
Glutamate							
Masseter	10/26	10/26	9/26	10/26	5/26	4/26	7/26
Splenius	9/26	12/26	11/26	5/26	12/26	8/26	8/26
Isotonic saline							
Masseter	–	2/26	2/26	–	–	–	–
Splenius	–	3/26	–	–	2/26	–	–

Words chosen by > 30% of the subjects (≥ 8 subjects) are shown in bold.

Table 3 Analysis of MPQ Data. PRIs of Sensory (S), Affective (A), Evaluative (E), and Miscellaneous (M) Aspects of Pain

	PRI(S)	PRI(A)	PRI(E)	PRI(M)
Glutamate				
Masseter	3.7 ± 0.4*	0.6 ± 0.2	0.7 ± 0.1	1.1 ± 0.2
Splenius	3.1 ± 0.3*	0.7 ± 0.2	0.5 ± 0.1	1.3 ± 0.2
Isotonic saline				
Masseter	0.6 ± 0.3	0	0	0
Splenius	0.7 ± 0.3	0	0	0.2 ± 0.1

Mean values ± SEM in 26 subjects. *Indicates significantly higher compared to PRI(A), (E), or (M) (ANOVAs, $P < .001$; Tukey, $P < .001$).

The 3 words most commonly used to describe the quality of the glutamate-evoked muscle pain were "hurting," "taut," and "intense" for the masseter muscle and "taut," "pressing," and "tight" for the splenius muscle (Table 2).

Quantitative analysis of the MPQ data did not indicate any significant differences in the PRI values for glutamate injections into the masseter and splenius muscles (ANOVAs, $P > .073$) (Table 3). The PRI(S) was significantly higher than the 3 other PRI values (ANOVA, $P < .001$; Tukey, $P < .001$).

Correlation Between Pain Intensity and Pain Area

Pearson correlation coefficients (r) were used to describe the associations between the VAS peak and VAS_{AUC} pain scores versus the pain areas in arbitrary units for the injections into the masseter and splenius muscles. No significant relationships were found for the masseter muscle ($r < .213$, $P > .297$) or splenius muscle ($r < .158$, $P > .442$).

Pressure Pain Thresholds

Overall, the analysis of the 4 baseline PPTs indicated significantly higher values in the splenius muscle (389 ± 21 kPa) compared to the masseter muscle (289 ± 14 kPa) (2-way ANOVAs, $P < .001$). There were no sequence effects for the repeated measurement of PPTs at baseline in the masseter or splenius muscles (2-way ANOVAs, $P = .547$).

Injections into the masseter muscle were associated with a significant effect of solution (2-way ANOVA, $P = .011$) and significant interaction between solution and time for the normalized masseter PPTs (2-way ANOVA, $P = .005$). Overall, the PPTs in the masseter were smaller following injection of glutamate than injection of isotonic saline (Tukey, $P = .011$). Masseter PPTs at 5, 10, and 20 minutes after the glutamate injection were significantly lower than the baseline values (Tukey, $P < .05$) (Fig 2a), and direct comparisons revealed significantly lower values following glutamate injections compared with isotonic saline injections at 5, 10, 20, and 30 minutes (Tukey, $P < .05$) (Fig 2a). Injections into the masseter muscle were not associated with any significant effect of time (2-way ANOVA, $P = .667$), solution (2-way ANOVA, $P = .202$) or interaction between the factors for the normalized splenius PPTs (2-way ANOVA, $P = .053$) (Fig 2b).

Injections into the splenius muscle did not produce any significant time effects (2-way ANOVA, $P = .871$), solution effects (2-way ANOVA, $P =$

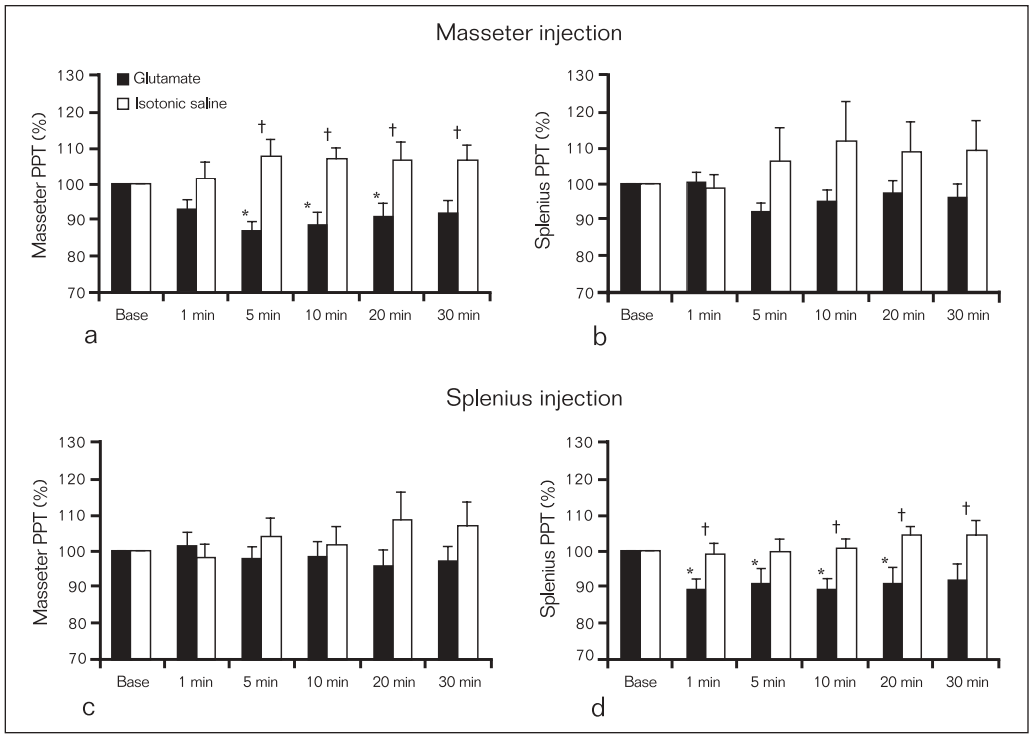

Fig 2 Normalized PPTs in the masseter and splenius muscles following injection of isotonic saline or glutamate into the masseter *(a and b)* and splenius *(c and d)* muscles. PPTs were measured at baseline and 1, 5, 10, 20, and 30 minutes after the injections. Mean values ± SEM are shown for 11 subjects. *Indicates values significantly different from baseline values (Tukey, $P < .05$). †Indicates significant difference between glutamate and isotonic saline (Tukey, $P < .05$).

.456), or interaction between the factors for the normalized masseter PPTs (2-way ANOVA, $P = .137$) (Fig 2c). Injections into the splenius muscle demonstrated a significant effect of time (2-way ANOVA, $P = .011$) and solution (2-way ANOVA, $P = .016$) and a significant interaction between the factors for the normalized splenius PPTs (2-way ANOVA, $P = .038$). Overall, the splenius PPTs were lower following the glutamate injection compared with the isotonic saline injection (Tukey, $P = .016$). For glutamate injections, the splenius PPTs were significantly lower at 1, 5, 10, and 20 minutes compared with baseline values (Tukey, $P < .05$), and direct comparisons showed significantly lower values at 1, 10, 20, and 30 minutes following the glutamate injection compared with the isotonic saline injection (Tukey, $P < .05$) (Fig 2d).

Correlation Between PPTs and Pain Intensity

The relative decrease in PPTs 5 minutes after injections of glutamate into the masseter and splenius muscle could not be correlated with the VAS peak pain or VAS_{AUC} scores (Pearson $r < .547$, $P > .082$).

Discussion

The present study has demonstrated that the masseter muscle appears to be more sensitive than the splenius muscle to intramuscular injections of glutamate and to pressure stimuli. There was no spread or referral of pain from the masseter muscle to the neck region, whereas pain in the splenius muscle often spread as far as the temporal region. Glutamate-evoked pain in either masseter or splenius muscles was associated with significant decreases in PPTs for up to 30 minutes in the masseter or splenius, respectively.

Methodological Considerations

The present study was designed as a single-blind study with a randomized sequence of injections into the masseter and splenius muscles and the use of isotonic saline injections as a control. In accordance with previous studies,[21,32] the isotonic saline injections were almost entirely pain-free, indicating that tissue trauma due to the needle insertion and volume effects do not play any significant role in acti-

vation or sensitization of deep craniofacial or cervical afferent fibers. Furthermore, it seems highly unlikely that expectancy effects related to the experimental design can explain the very large differences in VAS pain scores evoked by the isotonic saline and glutamate injections (Fig 1, Table 1).

Only intramuscular injections of 1.0 mol/L glutamate were used in the present study because the authors have previously documented that this concentration of glutamate reliably evokes both muscle pain and mechanical sensitization in humans.[21,23,24] In contrast, intramuscular injection of hypertonic saline, while painful, appears to have little effect on PPTs during or after pain.[28,33,34] Further, unlike hypertonic saline, the mechanisms whereby injection of 1.0 mol/L glutamate evokes muscle pain and induces mechanical sensitization are reasonably well understood. The authors have demonstrated that glutamate evokes masseter muscle pain in human subjects, in part, through activation of *peripheral* NMDA receptors.[23] Activation of peripheral NMDA and non-NMDA receptors by intramuscular injection of glutamate in rats excites predominantly slowly-conducting (< 10 m/s) masseter afferent fibers thought to mediate nociceptive function.[23] Activation of peripheral NMDA and non-NMDA receptors is also responsible for glutamate-induced mechanical sensitization of masseter muscle afferent fibers.[22]

It needs to be acknowledged, however, that only 1 pain-producing substance was employed, and only 1 neck muscle and 1 jaw muscle were tested. Injection of other algogenic substances into other neck or jaw muscles could have different pain characteristics and different patterns of spread or referral of pain. However, a systematic study of the effects of injections of hypertonic saline into the splenius muscle, sternocleidomastoid muscle, trapezius muscle, masseter muscle and anterior and posterior parts of the temporalis muscle did not indicate a substantial spread of jaw muscle pain to the neck region (Schmidt-Hansen et al, unpublished data, 2003) whereas, in accordance with the present findings, a frequent spread or referral of pain in the splenius muscle to the temporal region was observed. The splenius muscle was chosen for the present study because it is a frequent site of neck pain and also has been implicated in tension-type headaches and cervical spine disorders.[8,35] The masseter muscle was chosen since it is commonly involved in painful TMD conditions.[4–7] The authors' recent research[21–24] has focused on this muscle in particular. Finally, it should be mentioned that injections into the splenius muscle may be technically more difficult than injections into the masseter muscle because of the layered arrangement of the cervical muscles. Electromyographic recordings could offer an advantage for the identification of the correct position of the needle; however, in this study the researchers relied on a standard manual palpation of the muscle during head movements and voluntary contractions to ensure the injection was correctly placed in the muscle.[8]

Pain Characteristics of Jaw and Neck Muscle Pain

Injections of 1.0 mol/L glutamate into the masseter and splenius muscles evoked moderate to strong pain in all subjects. Although the splenius muscle was injected with 0.4 mL glutamate, this dose was associated with significantly lower levels of pain than the 0.2 mL of glutamate injected into the masseter muscle. Theoretically, differences in size and thickness of the masseter and splenius muscles could contribute to this finding. However, the suggestion that deep craniofacial tissues are more sensitive to injections of glutamate than cervical tissues was further supported by the findings of lower PPTs in the masseter muscle. The quality of glutamate-evoked pain was comparable between the masseter and splenius muscles, although the word "intense" was more often associated with masseter pain, in accordance with the higher VAS pain scores in the masseter. However, splenius pain was linked to more words, eg, "tight" and "tiring," but no significant differences in the PRI could be detected between the 2 muscles (Tables 2 and 3). Both masseter and splenius pain were perceived by the subjects in a large area around the site of injection (spread of pain) and were reported by the subjects to refer to more distant areas (Figs 1c and 1d). However, in this respect, masseter muscle pain differed from splenius muscle pain, since glutamate-evoked pain in the masseter muscle was never referred to the neck region, whereas splenius pain was often (12/26) referred to the temporal region and rarely (1/26) to the masseter region or teeth. The spread of glutamate-evoked muscle pain is in general accordance with other experimental studies on the pain spread or referral following glutamate or hypertonic saline injection into the masseter muscle.[21,24,28,32] In addition to the clinical observations of spread and referral patterns from the jaw and neck muscles,[8,9] Campbell and Parsons[36] reported that injection of hypertonic saline into the occipital and C1 regions caused pain in the frontal and parieto-frontal regions in the majority (62% to 85%) of 20 subjects tested. Injections of hypertonic saline into the paravertebral muscles of 5 subjects at the C1 level were later shown to produce consis-

tent pain in the occipital and posterior neck regions, but only a single subject reported spread or referral of pain to the forehead.[37] The present findings with glutamate-evoked spread or referral of pain from the splenius muscle and the studies mentioned demonstrate that painful stimulation of deep cervical regions has the propensity to spread or refer to the temporal region and forehead.

There is a theoretical basis for the spread or referral of pain from the splenius muscle to the jaw muscles. The trigeminal brainstem nociceptive neurons that respond to masticatory muscle stimulation (temporalis, masseter) have convergent inputs from facial cutaneous and other deep orofacial structures (tongue, teeth, oral mucosa, other masticatory muscles) as well as the neck.[15] Hu et al[38] found that injection of a noxious inflammatory substance into the masseter muscle induced a central sensitization reflected in a 30-minute expansion of receptive fields as well as increased responses to peripheral afferent inputs in about 40% of brainstem nociceptive neurons tested. This suggests that painful stimulation of the masseter muscle could induce central sensitization of trigeminal nociceptive neurons, including those which receive neck muscle afferent input. However, spread or referral of pain may not be a simple "bidirectional" phenomenon. The glutamate-evoked pain in the splenius muscle was associated with a frequent spread or referral to the temporal region, but very rarely to the masseter region. Moreover, spread or referral of pain from the masseter muscle to the neck region was not observed. These findings may have clinical implications for the understanding of the sensory manifestations of painful TMD and cervical spine disorders and their potential inter-relationship. Nonetheless, the present findings, in agreement with other studies,[13] do not infer a direct causal relationship between TMD pain and cervical spine disorders, which may be important to consider from a diagnostic and therapeutic point of view.

PPTs

PPTs are often used in studies on musculoskeletal pain conditions as a quantitative measure of muscle sensitivity (ie, mechanical allodynia and hyperalgesia). Relatively few studies have systematically examined differences in PPTs between craniofacial and cervical muscles.[39–41] The present PPT results indicate that the masseter muscle is more sensitive than the splenius muscle to pressure stimulation.

Various inflammatory and algogenic substances may act peripherally within muscle tissues to sensitize afferent fibers and cause a decrease in PPTs. For example, injection of serotonin (5-HT) into the human masseter causes a significant reduction in PPTs, and injections of a combination of 5-HT and bradykinin effectively produce allodynia to mechanical pressure stimulation of the tibialis anterior muscle.[42–45] Recently, it has also been shown that nerve growth factor decreases PPTs in the masseter muscle for at least 7 days.[46] In addition, peripheral glutamate receptors have been implicated in the development of allodynia after cutaneous and deep tissue injury,[22,47–53] and activation of peripheral NMDA and non-NMDA receptors by intramuscular injection of glutamate has consistently been shown to activate nociceptive masseter afferent fibers.[23] In support of a peripheral site of action, peripheral administration of glutamate receptor antagonists can attenuate the development of allodynia.[22] In the authors' recent study in humans, a significant decrease in PPTs in the masseter muscle was observed following repeated glutamate injections.[24] In that study, there were no significant changes in PPTs on the contralateral (control) side, which was taken as further indication of a peripheral mechanism of action for the injected glutamate. The present study has extended previous research by showing that a neck muscle can also be sensitized by injection of glutamate. The clinical implication of these findings is that peripheral administration of drugs interfering with glutamatergic nociceptive transmission may be effective in the management of musculoskeletal pain conditions, although further studies are required to test this hypothesis.[54]

In conclusion, the present data suggest that the masseter muscle is more sensitive to painful glutamate injections and mechanical stimuli than the splenius muscle. Although neurophysiological and biomechanical data have demonstrated significant interplay between the trigeminal and cervical neuromuscular systems,[18–20] the present study of glutamate-evoked pain suggests there is a relatively limited overlap in the sensory manifestations of pain between the masseter and splenius muscles. This may be important to consider for the diagnosis and management of painful TMD and cervical spine disorders.

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