Nerve Growth Factor–Evoked Masseter Muscle Sensitization and Perturbation of Jaw Motor Function in Healthy Women

Peter Svensson, DDS, PhD, Dr Odont
Professor and Chairman
Department of Clinical Oral Physiology
School of Dentistry
University of Aarhus

Clinical Consultant
Department of Oral and Maxillofacial Surgery, Aarhus University Hospital
Aarhus C, Denmark

Adjunct Professor
Orofacial Pain Laboratory
Center for Sensory-Motor Interaction
Aalborg University
Aalborg, Denmark

Eduardo Castrillon, DDS, MS, PhD
Postdoctoral Student
Department of Clinical Oral Physiology
School of Dentistry
University of Aarhus
Aarhus C, Denmark

Brian E. Cairns PhD, RPh
Associate Professor
Canada Research Chair in Neuropharmacology
Faculty of Pharmaceutical Sciences
The University of British Columbia
Vancouver, British Columbia, Canada

Correspondence to:
Professor Peter Svensson
Department of Clinical Oral Physiology
School of Dentistry
University of Aarhus
Vennelyst Boulevard 9
DK-8000 Aarhus C, Denmark
E-mail: psvensson@odont.au.dk

Aim: *To replicate and extend previous findings of nerve growth factor (NGF)-induced mechanical sensitization in healthy young men to women and test for associations between mechanical sensitization and oral motor function. Combined these data would indicate if injection of NGF into the masseter muscle is a valid model of muscle pain related to temporomandibular disorders (TMD).* ***Methods:*** *A double-blind, placebo-controlled study was conducted on 14 healthy women. Each subject received an injection of NGF (5 μg in 0.2 mL) into 1 masseter muscle and buffered isotonic saline (control, 0.2 mL) into the other. Pressure pain thresholds (PPT) and pressure pain tolerance (PPTOL) as well as self-assessed pain intensity (numeric rating scale of 1 to 10) with the jaw at rest and in relation to various motor activities (chewing, yawning, talking, swallowing, drinking, and smiling) were recorded prior to and 3 hours, 1 day, 7 days, 14 days, and 21 days postinjection. ANOVAs were used to test data.* ***Results:*** *It was found that NGF significantly reduced PPT and PPTOL 3 hours, 1 and 7 days postinjection (*$P < .001$*). Numerical rating scale (NRS) scores during chewing and yawning were significantly increased 3 hours and 1 day following NGF injection (*$P < .001$*). After 3 hours, there were significant correlations between relative changes in PPTs and NRS scores during chewing (*$r = -0.556$; $P = .037$*), between relative changes in PPTOL and NRS scores during yawning (*$r = -0.607$; $P = .020$*), and between relative changes in PPTOL and maximum unassisted jaw-opening capacity (*$r = 0.868$; $P < .001$*).* ***Conclusion:*** *This study shows that injection of NGF into the masseter muscle of women causes local signs of mechanical allodynia and hyperalgesia that persist for at least 7 days as well as pain during strenuous jaw movement. Taking the authors' previous results on NGF effects in men into consideration, these findings lend additional support to the suggestion that this model may serve as a proxy of some of the clinical features of TMD-related muscle pain.* J OROFAC PAIN 2008;22: 340–348

Key words: allodynia, jaw motor function, nerve growth factor, pain measurement, TMD pain

There is now good evidence that neurotrophic protein nerve growth factor (NGF) is a potent modulator of nociceptive transmission.[1,2] For example, increased levels of NGF have been implicated in various inflammatory pain conditions such as pancreatitis, prostatitis, and cystitis[3–5] and also appear to be involved in the pathophysiology of chronic headache conditions.[6,7]

NGF has also been mentioned as a possible therapeutic target in the treatment of osteoarthritis and pain.[8] However, relatively little is still known about NGF actions on muscle tissue despite the large number of persistent pain conditions that afflict this tissue.

Studies in animal preparations with intramuscular injections of NGF into the cervical muscles have also shown long-lasting facilitatory effects on the jaw-opening reflex[9,10] that may lead to neuroplastic changes in nociceptive synaptic transmission indicative of a process of central sensitization. The initiator of this central sensitization may involve NGF-evoked excitation of nociceptive C-fibers, which has been reported to occur upon intramuscular injection of NGF into the rat gastrocnemius muscle.[11] Recently these findings were extended by intracellular recordings of dorsal horn neurons which indicated that 8 of 15 neurons did not react to injection of NGF, 4 neurons responded with excitatory postsynaptic potentials, and 3 neurons showed both excitatory postsynaptic potentials and action potentials.[12] This pattern of neuronal response was suggested by the authors to be sufficient to induce a sensitization of central nociceptive neurons but inadequate to evoke overt painful sensations in humans after NGF injections into the masseter muscle.

Indeed, recent studies in humans have shown that direct administration of small doses (5 μg) of NGF into the masseter muscle of healthy male subjects is associated with a prolonged period of increased sensitivity to mechanical pressure stimuli and stimulation of the masseter and movement-produced pain.[13] These features in many ways mimic the clinical symptoms of myofascial temporomandibular disorders (TMD), which are characterized by pain on palpation of the jaw muscles, pain in the masticatory muscles, and pain on movement.[14] Furthermore, it has recently been found that within 1 hour of injection of human NGF into the rat masseter muscle, the mechanical threshold of Aδ fibers decreases in female rats, which may suggest that females are particularly sensitive to elevated levels of NGF.[15]

Taken together with the fact that the majority of myofascial TMD patients are women, the aim of this study was to replicate and extend previous findings of NFG-induced mechanical sensitization of the masseter muscle and to test for associations between mechanical sensitization and oral motor function.

Materials and Methods

Subjects

Fourteen healthy female volunteers with a mean age of 25.4 ± 2.3 years were recruited from among the students at the University of Aarhus. All of them were taking estrogen-containing oral contraceptives to minimize the impact of hormonal changes on pain sensitivity across the menstrual cycle. None of the subjects reported painful TMD or other orofacial pain complaints or had taken analgesics within 48 hours of the investigation. The subjects were screened for TMD in accordance with the Research Diagnostic Criteria (RDC) for TMD.[14] The local ethics committee approved the experiments, and informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki.

Study Design

The study was performed in a randomized, double-blinded, placebo-controlled manner. The sequence and side (left or right) of the single NGF and buffered isotonic saline (control) injections were both randomized. The subjects were asked to score the perceived intensity of pain on a 0- to 10-cm electronic visual analog scale (VAS) after each injection. Fifteen minutes after the first injection on 1 side, a second injection with the other substance was done on the contralateral side. One examiner prepared the NGF or saline injection while the second examiner performed all assessments at baseline before injection of NGF or saline. The tests performed were assessment of pressure pain threshold (PPT) and pressure pain tolerance (PPTOL) in the masseter muscles on both sides, maximum bite force (MBF) on both sides in the posterior area (first molars), maximum unassisted jaw opening, and a chewing test which consisted of 6 minutes of unilateral chewing on 1 piece of chewing gum.[16] Moreover, all subjects described the sensation of pain after the injections on a Danish version of the McGill Pain Questionnaire (MPQ) and drew the distribution of pain on a anatomic figure of the head.[17] After the second injection, MPQ and pain drawings were assessed again. Three hours after the first injection, the second examiner repeated all the measurements, and the subjects filled in questionnaires about pain and were asked to rate their pain intensity on a 0-to-10 numerical rating scale (NRS) during various jaw functions. The exact same mea-

surements were repeated 1, 7, 14, and 21 days after the injections. All subjects and the second examiner were blinded to administration of NGF or saline. The code was broken after data entry and analysis.

NGF Administration and Subjective Sensations

Based on previous studies[13,18,19] a single dose of 5 μg was given in 0.2 mL (~ 0.1 μg/kg) as a bolus injection to all 14 subjects. The manual injection into the masseter muscle followed published techniques.[20] As a control, 0.2 mL buffered isotonic saline was injected into the contralateral masseter muscle. Sterile solutions of recombinant human NGF (25 μg/mL) were prepared by the pharmacy at Aalborg Hospital.

The subjects used an electronic 0- to 10-cm VAS to score their perceived pain intensity of the NGF and isotonic saline injection. The VAS signal was sampled and stored in a computer every 5 seconds. The areas under the VAS curve (VAS_{auc}) and the maximum pain (VAS_{peak}) were calculated.

The area of perceived pain on the MPQ drawings was digitized and expressed in arbitrary units.[21]

Mechanical Sensitivity

A pressure algometer (Somedic, Hörby, Sweden) was used to test the sensitivity to deep stimuli applied to the masseter muscles. The PPT was defined as the amount of pressure (kPa) that the subjects first perceived to be painful.[20] The subject pushed a button to stop the pressure stimulation when the threshold was reached. Subjects were instructed to keep their teeth slightly apart to avoid contraction of the jaw-closing muscles during pressure stimulation. The PPTs were determined in duplicate with a constant application rate of 30 kPa/s and a probe diameter of 1 cm^2. The mean value was used for further statistical evaluation. PPTOLs, however, were measured just once at the end of each session to avoid sensitization that might be caused by the procedure itself. PPTOL was defined as the maximum pain that the subjects were willing/able to accept.

Assessment of Oral Motor Function

Based on the RDC/TMD questionnaire,[14] the subjects were asked how much pain on a 0-to-10 NRS was evoked with the jaw at rest and by various oral motor activities: chewing, yawning, talking, swallowing, drinking, and smiling.

The MBF was measured with the use of a 7-mm-high bite force meter as previously described.[22] The subjects were asked to bite as hard as possible on the bite force meter placed between the first molars. Verbal encouragement was given to the subjects during this task, which lasted 3 to 5 seconds. The MBF recording was repeated 3 times with 1 to 2 minutes between recordings to prevent fatigue and allow recovery. The mean of the 3 measurements was used for the further analysis. MBF was measured on both sides (NGF and control side) in randomized order.

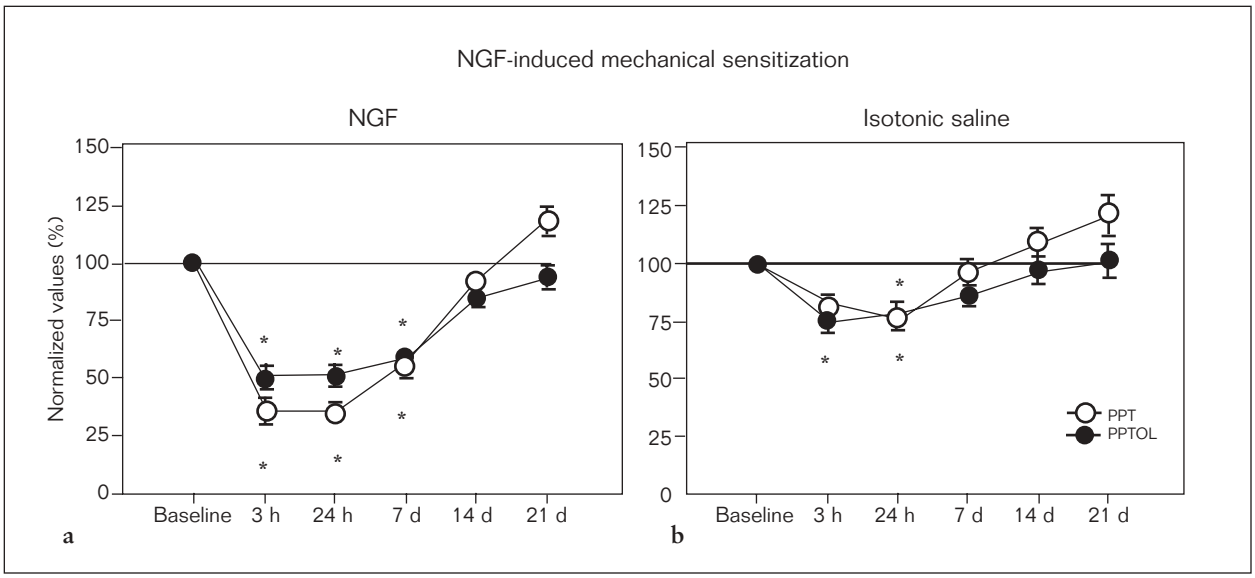
In addition, the influence of NGF injections on normal chewing was assessed using the same paradigm as Karibe et al.[16] The subjects were asked to chew 1 portion of chewing gum on their preferred chewing side (approximately 8 g; 6 pieces of Dental V-6 sugar-free chewing gum, Dandy A/S DK) for 6 minutes. The subjects reported on a 0-to-10 NRS the intensity of pain in the masseter muscles every 1 minute. After completion of the chewing task the subjects were also asked to draw the perceived pain area on a figure of the head. These pain areas were also digitized and expressed in arbitrary units.

Statistical Analyses

The results are presented as mean ± standard errors of the mean (SEM). The PPT and PPTOL were normalized to the baseline values to calculate the relative changes. Analysis of variance (ANOVA) was used to test the normalized PPT and PPTOL data with injection type (treatment) as one factor and time as the repeated factor (6 levels: baseline, 3 hours, 1 day, 7 days, 14 days, and 21 days). MBF data were analyzed with a repeated measure ANOVA (6 time levels). The NRS scores and pain drawing areas from the chewing test were analyzed with repeated measures ANOVA with time (6 levels) and trials (6 levels) as the factors. Post-hoc tests were performed with Tukey tests. Associations between relative changes in PPTs and PPTOLs and NRS scores during oral motor function were tested with Spearman correlation tests. The level of significance was set at $P < .05$.

Results

At baseline, all subjects were free of TMD complaints or signs with normal nonpainful responses to standardized palpation of the masseter and temporalis muscles and unrestricted jaw movements. Intramuscular injection of NGF and buffered iso-



Figs 1a and 1b Normalized PPT and PPTOL values in the masseter muscle at baseline and at various timepoints after administration of *(a)* NGF or *(b)* isotonic saline in women (n = 14; mean values ± SEM). * Indicates values significantly different from baseline values (Tukey: $P < .05$).

tonic saline was not associated with any spontaneous pain reports (VAS_{peak} and VAS_{auc} equal to zero), and there were no reports of systemic adverse effects.

Influence on Mechanical Sensitivity

The absolute values at baseline were 257 ± 13 kPa for PPT and 543 ± 32 kPa for PPTOL, with no significant differences between sides (paired *t* test: PPT: $P = .81$; PPTOL: $P = .94$).

A 2-way ANOVA test of the normalized PPTs showed that there was a strong main effect of time (ANOVA: F = 61.04; $P < .001$), as well as treatment (ANOVA: F = 32.19; $P < .001$), with a significant interaction between factors (ANOVA: F = 9.51; $P < .001$). For the masseter muscle injected with NGF, the PPTs were significantly different from baseline, with significantly lower PPTs after 3 hours, 1 day, and 7 days (ie, allodynia) (Tukey: $P < .05$; Fig 1a). The masseter muscle injected with isotonic saline demonstrated significantly lower PPT after 1 day compared with baseline values (Tukey: $P < .03$) (Fig 1b), but the relative PPT changes from baseline to day 1 were significantly smaller for the isotonic injections (24.0% ± 4.7%) compared with the NGF injections (66.3% ± 4.2%; paired *t* test: $P < .001$).

For the normalized PPTOLs, there was a main effect of time (ANOVA: F = 39.99; $P < .001$), as well as treatment (ANOVA: F = 13.13; $P = .001$) with a significant interaction between factors (ANOVA: F = 4.65; $P < .001$). The PPTOL in the masseter muscle injected with NGF was significantly different from baseline values, with significantly lower PPTOL after 3 hours, 1 day, and 7 days (ie, hyperalgesia; Tukey: $P < .001$; Fig 1a). The control injection of isotonic saline into the masseter muscle showed consistent decreases in PPTOL values after 3 hours and 1 day (Tukey: $P < .003$; Fig 1b), but again the relative PPTOL changes from baseline to 3 hours and 1 day were significantly smaller for the isotonic injections (24.8% ± 5.1%; 21.7% ± 4.9%) compared with the NGF injections (52.3% ± 5.3%; 49.5% ± 4.8%; paired *t* tests: $P < .004$).

Influence on Oral Motor Function

Injection of NGF caused pain in the ipsilateral masseter muscle on chewing in 1 of 14 subjects after 15 minutes, 10 of 14 subjects after 3 hours and 1 day, 6 of 14 subjects after 7 days, and 2 of 14 subjects after 14 days. The NRS scores of pain on chewing were significantly increased (ANOVA: F = 9.95; $P < .001$), with significantly higher NRS scores

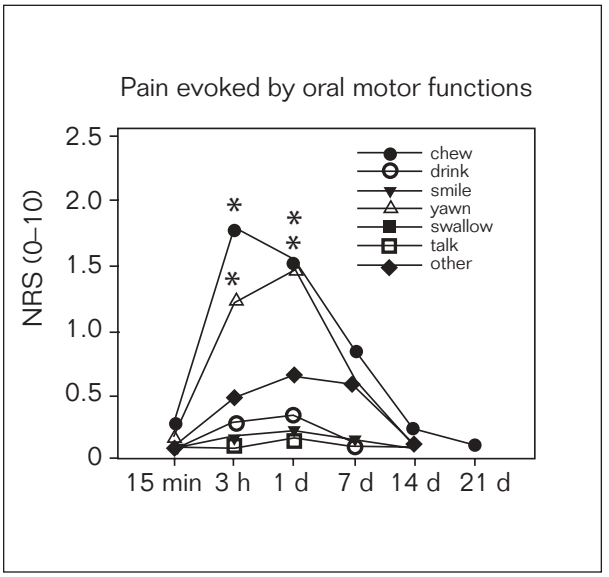


Fig 2 NRS scores of various oral motor functions at various time points following the administration of NGF in women (n = 14; mean values). * Indicates significantly different values from baseline values, which all were zero (Tukey: $P < .05$). For clarity, the SEMs have not been shown.

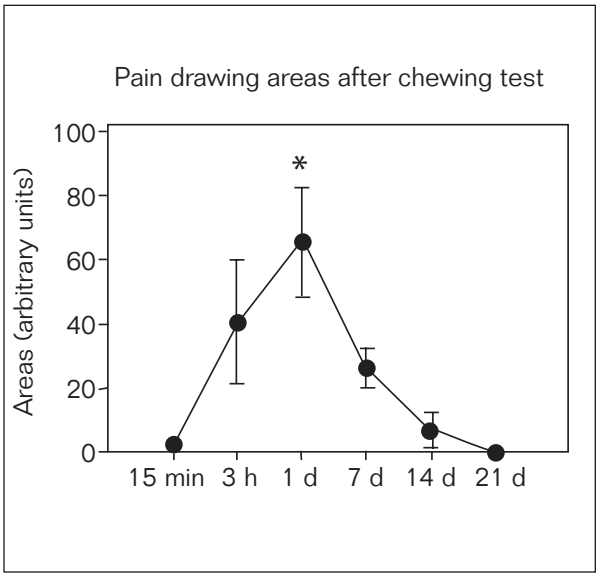


Fig 3 Pain-drawing areas in arbitrary units following a 6-minute chewing task at various timepoints after administration of NGF in women (n = 14; mean values ± SEM). * Indicates significantly different values from first values (Tukey: $P < .001$).

compared with baseline after 3 hours and 1 day (Tukey: $P < .01$; Fig 2). Also yawning was significantly influenced by the NGF injection (ANOVA: F = 6.00; $P < .001$), with significantly higher NRS scores compared with baseline after 3 hours and 1 day (Tukey: $P < .04$). No significant effects of NGF on talking, swallowing, drinking, smiling, and with the jaw at rest could be detected, although a few subjects (< 5/14) reported some disturbance in 1 or more of these oral functions (Fig 2).

The maximum unassisted jaw-opening capacity was also significantly influenced by the NGF injection (ANOVA: F = 11.344; $P < .001$), with significantly lower values after 3 hours (48.9 ± 1.9 mm) and 1 day (49.4 ± 1.7 mm) compared with baseline values (52.4 ± 5.2 mm; Tukey: $P < .001$).

The MBF at baseline was 58 ± 3.6 kg, with no differences between sides (paired *t* test: $P = .78$). Injection of NGF was associated with a significant change over time (ANOVA: F = 4.093; $P = .001$), with the lowest MBF values after 3 hours on the ipsilateral side (10% ± 5.3% decrease; $P < .02$). Also on the contralateral side, there was a time effect (ANOVA: F = 2.655; $P = .03$), but post-hoc tests could not identify at which timepoints this occurred.

Analysis of the NRS scores in response to the chewing test revealed a significant effect of time (ANOVA: F = 9.333; P < .001) and trials (ANOVA: F = 7.427; $P < .001$), with no interaction between the 2 factors. Compared to baseline values, the NRS scores after chewing on days 7, 14, and 21 were significantly increased (Tukey: $P < .001$) and the NRS scores after the 5th and 6th trials were significantly higher than after the first trial (Tukey: $P < .014$). However, the scores were generally low, ranging from 1 to 2 on the 0-to-10 NRS.

Analysis of the pain-drawing areas obtained following the chewing tests showed a significant effect of time (ANOVA: F = 6.671; $P < .001$), with significantly higher values after 1 day (65.6 ± 16.6 arbitrary units) compared with the values immediately after (15 min) the injections (2.7 ± 2.7 arbitrary units; Tukey: $P < .001$; Fig 3).

After 3 hours, but not after 24 hours, there were significant correlations between relative changes in PPTs and NRS scores during chewing ($r = –0.556$; $P = .037$), between relative changes in PPTOL and NRS scores during yawning ($r = –0.607$; $P = .020$), and between relative changes in PPTOL and maximum unassisted jaw-opening capacity ($r = .868$; $P < .001$).

Discussion

The main finding in this study was the consistent and long-lasting increase in mechanical sensitivity (allodynia and hyperalgesia) in the NGF-injected masseter muscle and pain associated with jaw functions but without spontaneous pain or any systemic side-effects in healthy young women.

Spontaneous Pain and Mechanical Sensitization

The present study has shown that NGF injections into the masseter muscle of women do not evoke painful sensations that can be recorded on the VAS or MPQ. This finding suggests that NGF in itself does not activate a sufficient number of nociceptive afferents necessary for a conscious sensation of pain and that volume effects of the injected solution play a minor role. The lack of significant spontaneous pain in relation to NGF injections is, indeed, an important point and was also found in a previous study in healthy male subjects.[13] Recent findings suggest that injection of NGF into jaw-closing muscles does not evoke significant discharge in Aδ fibers in either male or female rats but does induce a prolonged mechanical sensitization of these fibers that lasts for at least 3 hours.[15] In contrast, Hoheisel et al[11] found a robust activation of 10 of 28 C-fibers in the gastrocnemius-soleus muscle of rats but no significant changes in C-fiber discharges in response to fixed mechanical stimuli, which indicates a lack of mechanical sensitization in these fibers. These findings suggest that mechanical sensitization of muscle nociceptors may contribute to the effects of NGF when injected into the masseter muscle but that different mechanisms appear to be involved in the effects of NGF on other muscles.

A consistent finding in this study and the previous study in healthy men[13] was the pronounced sensitization of the masseter muscle in a localized area around the injection site. Smaller decreases in PPT and PPTOL values on the control side (isotonic saline injections) (Fig 1b) occurred, suggesting that the insertion of the needle and the injection itself may lead to some degree of sensitization in women; however, in the previous experiment in healthy men the isotonic saline injection was not associated with significant decreases in PPTOLs.[13]

The previous study in men examined mechanical sensitivity 1 hour after NGF injections and indicated no allodynia or hyperalgesia. In the present study in women, the first examination after NGF injections was delayed to 3 hours, and a significant decrease in both PPTs and PPTOLs was found. This effect could be mediated by a peripheral mechanism, as it has been found that NGF also sensitizes masseter muscle Aδ-fibers within 1 to 3 hours after injection into the rat masseter muscle. However, we cannot rule out the possibility that increases in mechanical sensitivity observed after 1 and 7 days are due, at least in part, to a central mechanism involving upregulation of sensory neuropeptides and neuromodulators such as calcitonin gene-related peptide, substance P, and brain-derived neurotrophic factor; receptors such as TRPV1 and P2X3, and ion channels such as TTX and TTXr (for a review see Pezet and McMahon[1]). The findings from Makowska et al[10] indicate that there is indeed a central component to the changes in nociceptive transmission following intramuscular injection of NGF. Furthermore, recent intracellular recordings of dorsal horn neurons have suggested that injection of NGF into the rat gastrocnemius-soleus muscles may produce a pattern of neuronal response that is sufficient to induce a sensitization of central nociceptive neurons but inadequate to evoke overt painful sensations.[12] Additional studies in animals will be required to separate the peripheral versus the central component of NGF-induced mechanical sensitization.

NGF as a Model of Myofascial TMD Pain?

Myofascial TMD is characterized by fluctuating levels of spontaneous pain, and it has been shown that there are substantial variations in present VAS pain scores, average VAS pain scores during a month, and the highest (worst) VAS pain scores.[23] However, it is a highly consistent finding in myofascial TMD patients that their PPTs in the masseter muscles are decreased compared to matched controls.[20,23–26] A number of studies also suggest that the mechanical sensitivity outside the trigeminal region is increased in myofascial TMD patients (eg, Maixner et al[23]). Epidemiologic data strongly suggest that women are at higher risk than men to develop a TMD problem, and in particular the use of oral contraceptives seems to be an additional risk factor.[27–29] While the mechanisms which underlie sex-related differences in the prevalence of TMD have yet to be elucidated, there is some indirect evidence that suggests estrogen levels may play a role. It recently has been shown that artificial manipulation of the estradiol levels (low versus high) in healthy women was associated with distinct differences in regional increases in baseline mu-opioid receptor availability in vivo and activation of endogenous opioid neurotransmission during pain induced by infusion

of hypertonic saline into the masseter muscle.[30] This finding suggests a role for estrogen in modulating endogenous opioid neurotransmission and psychophysical measures of experimental jaw-muscle pain and is in accordance with the clinical literature demonstrating that low levels of estrogen during the menstrual cycle are associated with small but significant increases in TMD pain.[28] In addition, the mechanical threshold of masseter nociceptors in female rats is decreased in association with a drop in serum estrogen levels.[15,31] Taken together, these findings suggest that both peripheral and central changes in masticatory muscle pain processing are associated with natural fluctuations in the level of sex hormones. To minimize the effect that natural fluctuations in sex hormone levels might exert on PPTs and PPTOLs, women in the present study were required to be taking oral contraceptives, and so it was not possible to examine the influence of serum sex hormone concentrations on NGF-evoked sensitization. Nevertheless, the literature supporting an association between TMD-related pain and menstrual cycle stage suggests that future studies be undertaken to examine the effect of NGF in normally cycling women.

Stohler,[32] in a response to a review on TMD pain,[33] was the first to consider a potential sex-related link between NGF and myofascial TMD. Levels of NGF or analysis of TrkA receptor distribution and density have not yet been performed in TMD pain patients, but the present investigation and a previous study by the present authors[13] examined the hypothesis that experimental elevations of NGF in the masseter muscle leads to a localized sensitization of the injected muscle. Numerous other models of myofascial pain exist, eg, endogenous activation of nociceptors through extensive and primarily eccentric muscle work (for a review see references 34,35) or via injections of algesic compounds such as hypertonic saline, acid solutions, serotonin, bradykinin, substance P, ATP, capsaicin, and glutamate.[21,24,36–42] For example, single injections of substance P and serotonin are associated with no or very little pain, which is comparable to the findings with NGF injections. However, none of the mentioned algesic substances have in any study been shown to lead to prolonged periods (weeks) of increased sensitivity to mechanical stimuli. Some algesic substances, such as capsaicin, acting on the TRPV1 receptor lead to very high pain ratings (VAS scores > 6)[38] but again, with relatively little and rather short-lasting (hours) changes in mechanical sensitivity. We have systematically examined the importance of peripherally administered glutamate and identified sex-related differences in the muscle pain intensity[41] and ~15% to 20% decrease in the PPTs that lasted only 30 to 60 minutes, but without sex differences. Subcutaneous administration of glutamate in the trigeminal area, however, evokes a larger area of pin-prick hyperalgesia in women than in men.[43] The magnitude of the NGF-mediated mechanical sensitization in the present study was in the range of 65% in women compared to about 40% in men[13] after 1 day and in both studies persisted for up to 7 days. Future studies will need to be designed to address whether there are indeed sex-related differences in these pain and sensitization phenomena.[44] Nonetheless, injection of NGF into the masseter muscle seems to be a relevant model of myofascial TMD pain with pronounced sensitization and influence on oral motor function. Furthermore, most TMD conditions do not show clear evidence of tissue inflammation, and there is evidence from animal studies that injection of NGF does not lead to overt inflammation as assessed by plasma extravasation in rat masseter muscles.[15] This is a marked difference to other animal models of myositis, such as injection of complete Freund adjuvant, mustard oil, or formalin (eg, Hu et al,[45] Watanabe et al,[46] and Sugiyo et al[47]).

Another important point is that NGF injections are associated with significant perturbations in normal oral motor function such as chewing and yawning, with significant correlations between measures of mechanical sensitization and NRS scores. Thus, greater levels of sensitization (expressed as relative changes in PPTs or PPTOLs) were associated with higher pain scores evoked by chewing, yawning, and reduction in maximum unassisted jaw-opening capacity. This sensory-motor interaction is also supported by studies on the jaw-opening reflex in animals receiving NGF injections into the cervical muscles.[10] Although the NRS scores following the chewing tasks were low in the present study, they consistently indicated that chewing leads to higher pain scores, in accordance with clinical reports.[48,49] Injections of hypertonic saline also lead to alterations in jaw-motor function and reflex sensitivity (eg, Svensson et al[50,51] and Wang et al[52]), but chewing is frequently associated with a decrease and reduction of pain, which also can be observed in some (6% to 30%) myofascial TMD pain patients.[49] However, the majority of myofascial TMD pain patients experience an increase in pain when chewing, and this feature was replicated in the present study.

Based on previous observations in men[13] and the current observations, it is therefore proposed that intramuscular administration of NGF is an interesting model or proxy of some aspects of TMD-like muscle pain without an inflammatory component and could be used to obtain more insight into the mechanisms of mechanical sensitization of deep nociceptive afferent fibers in the craniofacial region.

Acknowledgments

The International Association for the Study of Pain kindly awarded a travel grant to 2 authors (BEC and PS), and the Danish Dental Association funded the project in part. The skillful help of Bente Haugsted is greatly acknowledged.

References

1. Pezet S, McMahon SB. Neurotrophins: Mediators and modulators of pain. Annu Rev Neurosci 2006;29:507-538.
2. McMahon SB, Bennett DLH, Bevan S. Inflammatory mediators and modulators of pain. In: McMahon SB, Koltzenburg M (eds). Wall and Melzak's Textbook of Pain, ed 5. Philadelphia: Elsevier/Churchill Livingstone, 2006:49-72.
3. Lowe EM, Anand P, Terenghi G, et al. Increased nerve growth factor levels in the urinary bladder of women with idiopathic sensory urgency and interstitial cystitis. Br J Urol 1997;79:572-577.
4. Friess H, Zhu ZW, di Mola FF, et al. Nerve growth factor and its high-affinity receptor in chronic pancreatitis. Ann Surg 1999;230:615-624.
5. Miller LJ, Fischer KA, Goralnick SJ, et al. Nerve growth factor and chronic prostatitis/chronic pelvic pain syndrome. Urology 2002;59:603-608.
6. Saldanha G, Hongo J, Plant G, Acheson J, Levy I, Anand P. Decreased CGRP, but preserved Trk A immunoreactivity in nerve fibres in inflamed human superficial temporal arteries. J Neurol Neurosurg Psychiatry 1999;66:390-392.
7. Sarchielli P, Alberti A, Floridi A, Gallai V. Levels of nerve growth factor in cerebrospinal fluid of chronic daily headache patients. Neurology 2001;57:132-134.
8. Dray A, Read SJ. Arthritis and pain. Future targets to control osteoarthritis pain. Arthritis Res Ther 2007;9:212.
9. Ellrich J, Makowska A. Nerve growth factor and ATP excite different neck muscle nociceptors in anaesthetized mice. Cephalalgia 2007;27:1226-1235.
10. Makowska A, Panfil C, Ellrich J. Nerve growth factor injection into semispinal neck muscle evokes sustained facilitation of the jaw-opening reflex in anesthetized mice: Possible implications for tension-type headache. Exp Neurol 2005;191:301-309.
11. Hoheisel U, Unger T, Mense S. Excitatory and modulatory effects of inflammatory cytokines and neurotrophins on mechanosensitive group IV muscle afferents in the rat. Pain 2005;114:168-176.
12. Hoheisel U, Unger T, Mense S. Sensitization of rat dorsal horn neurons by NGF-induced subthreshold potentials and low-frequency activation. A study employing intracellular recordings in vivo. Brain Res 2007;1169:34-43.
13. Svensson P, Cairns BE, Wang K, Arendt-Nielsen L. Injection of nerve growth factor into human masseter muscle evokes long-lasting mechanical allodynia and hyperalgesia. Pain 2003;104:241-247.
14. Dworkin SF, LeResche L (eds). Research Diagnostic Criteria for Temporomandibular Disorders: Review, criteria, examinations and specifications, critique. J Craniomandib Disord Facial Oral Pain 1992;6:301-355.
15. Mann MK, Dong X-D, Svensson P, Cairns BE. Influence of intramuscular nerve growth factor injection on the response properties of rat masseter muscle afferent fibers. J Orofac Pain 2006;20:325-336.
16. Karibe H, Goddard G, Gear RW. Sex differences in masticatory muscle pain after chewing. J Dent Res 2003;82:112-116.
17. Melzack R. The McGill Pain Questionnaire: Major properties and scoring methods. Pain 1975;1:277-299.
18. Petty BG, Cornblath DR, Adornato BT, et al. The effect of systemically administered recombinant human nerve growth factor in healthy human subjects. Ann Neurol 1994;36:244-246.
19. Dyck PJ, Peroutka S, Rask C, et al. Intradermal recombinant human nerve growth factor induces pressure allodynia and lowered heat-pain threshold in humans. Neurology 1997;48:501-505.
20. Svensson P, Arendt-Nielsen L, Nielsen H, Larsen JK. Effect of chronic and experimental jaw muscle pain on pain-pressure thresholds and stimulus-response curves. J Orofac Pain 1995;9:347-356.
21. Svensson P, Graven-Nielsen T, Arendt-Nielsen L. Mechanical hyperesthesia of human facial skin induced by tonic painful stimulation of jaw muscles. Pain 1998;74:93-100.
22. Svensson P, Arendt-Nielsen L. Effects of 5 days of repeated submaximal clenching on masticatory muscle pain and tenderness: An experimental study. J Orofac Pain 1996;10:330-338.
23. Maixner W, Fillingim R, Sigurdsson A, Kincaid S, Silva S. Sensitivity of patients with painful temporomandibular disorders to experimentally evoked pain: Evidence for altered temporal summation of pain. Pain 1998;76:71-81.
24. Svensson P, List T, Hector G. Analysis of stimulus-evoked pain in patients with myofascial temporomandibular pain disorders. Pain 2001;92:399-409.
25. Reid KI, Gracely RH, Dubner RA. The influence of time, facial side, and location on pain-pressure thresholds in chronic myogenous temporomandibular disorder. J Orofac Pain 1994;8:258-265.
26. Sarlani E, Grace EG, Reynolds MA, Greenspan JD. Evidence for up-regulated central nociceptive processing in patients with masticatory myofascial pain. J Orofac Pain 2004;18:41-55.
27. LeResche L, Saunders K, Von Korff MR, Barlow W, Dworkin SF. Use of exogenous hormones and risk of temporomandibular disorder pain. Pain 1997;69:153-160.
28. LeResche L, Mancl L, Sherman JJ, Gandara B, Dworkin SF. Changes in temporomandibular pain and other symptoms across the menstrual cycle. Pain 2003;106:253-261.
29. Warren MP, Fried JL. Temporomandibular disorders and hormones in women. Cells Tissues Organs 2001;169:187-192.
30. Smith YR, Stohler CS, Nichols TE, Bueller JA, Koeppe RA, Zubieta JK. Pronociceptive and antinociceptive effects of estradiol through endogenous opioid neurotransmission in women. J Neurosci 2006;26:5777-5785.

31. Dong XD, Mann MK, Kumar U, et al. Sex-related differences in NMDA-evoked rat masseter muscle afferent discharge result from estrogen-mediated modulation of peripheral NMDA receptor activity. Neuroscience 2007; 146:822-832.
32. Stohler CS. Masticatory myalgias. Emphasis on the nerve growth factor-estrogen link. Pain Forum 1997;6:176-180.
33. Svensson P. Pain mechanisms in myogenous temporomandibular disorders. Pain Forum 1997;6:158-165.
34. Graven-Nielsen T, Segerdahl M, Svensson P, Arendt-Nielsen L. Methods for induction and assessment of pain in humans with clinical and pharmacological examples. In: Kruger L (ed). Methods in Pain Research. Boca Raton, FL: CRC Press, 2001:263-304.
35. Svensson P, Graven-Nielsen T. Craniofacial muscle pain: Review of mechanisms and clinical manifestations. J Orofac Pain 2001;15:117-145.
36. Svensson P, Cairns BE, Wang K, et al. Glutamate-induced sensitization of pressure pain thresholds in human masseter muscle. Pain 2003;101:221-227.
37. Ernberg M, Lundeberg T, Kopp S. Pain and allodynia/hyperalgesia induced by intramuscular injection of serotonin in patients with fibromyalgia and healthy individuals. Pain 2000;85:31-39.
38. Arima T, Svensson P, Arendt-Nielsen L. Capsaicin-induced muscle hyperalgesia in the exercised and non-exercised human masseter muscle. J Orofac Pain 2000;14:213-223.
39. Mork H, Ashina M, Bendtsen L, Olesen J, Jensen R. Experimental muscle pain and tenderness following infusion of endogenous substances in humans. Eur J Pain 2003;7:145-153.
40. Sohn MK, Graven-Nielsen T, Arendt-Nielsen L, Svensson P. Effects of experimental muscle pain on mechanical properties of single motor units in human masseter. Clin Neurophysiol 2004;115:76-84.
41. Cairns BE, Hu JW, Arendt-Nielsen L, Sessle BJ, Svensson P. Sex-related differences in human pain perception and rat afferent discharge evoked by injection of glutamate into the masseter muscle. J Neurophysiol 2001;86:782-791.
42. Cairns BE, Svensson P, Wang K, et al. Activation of peripheral NMDA receptors contributes to human pain and rat afferent fiber discharge evoked by injection of glutamate into the masseter muscle. J Neurophysiol 2003;90:2098-2105.
43. Gazerani P, Wang K, Cairns BE, Svensson P, Arendt-Nielsen L. Effects of subcutaneous administration of glutamate on pain, sensitization and vasomotor responses in healthy men and women. Pain 2006;124:338-348.
44. Greenspan JD, Craft RM, LeResche L, et al; Consensus Working Group of the Sex, Gender, and Pain SIG of the IASP. Studying sex and gender differences in pain and analgesia: a consensus report. Pain 2007;132:26-45.
45. Hu JW, Sessle BJ, Raboisson P, Dallel R, Woda A. Stimulation of craniofacial muscle afferents induces prolonged facilitatory effects in trigeminal nociceptive brainstem neurones. Pain 1992;48:53-60.
46. Watanabe M, Tanaka E, Nishi M, et al. Expression of c-Fos-like immunoreactive neurons in the supratrigeminal region in the rat following noxious stimulation of the orofacial tissues. Neurosci Lett 2002;335:99-102.
47. Sugiyo S, Takemura M, Dubner R, Ren K. Trigeminal transition zone/rostral ventromedial medulla connections and facilitation of orofacial hyperalgesia after masseter inflammation in rats. J Comp Neurol 2005;493:510-523.
48. Dao TT, Lund JP, Lavigne GJ. Pain responses to experimental chewing in myofascial pain patients. J Dent Res 1994;73:1163-1167.
49. Gavish A, Winocur E, Menashe S, Halachmi M, Eli I, Gazit E. Experimental chewing in myofascial pain patients. J Orofac Pain 2002;16:22-28.
50. Svensson P, Arendt-Nielsen L, Houe L. Sensory-motor interactions of human experimental unilateral jaw muscle pain: A quantitative analysis. Pain 1996;64:241-249.
51. Svensson P, Macaluso GM, De Laat A, Wang K. Effects of local and remote muscle pain on human jaw reflexes evoked by fast stretches at different clenching levels. Exp Brain Res 2001;139:495-502.
52. Wang K, Svensson P, Arendt-Nielsen. Effect of tonic muscle pain on short-latency jaw-stretch reflexes in humans. Pain 2000;88:189-197.