Local and systemic inflammatory mediators and their relation to pressure-pain threshold and pain of the temporomandibular joint

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Cover photo: Assessment of temporomandibular joint pressure-pain threshold with an electronic algometer over the lateral pole of the joint.

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To Madelaine, Sophie, Sonja, Per Olof and Håkan

Lider en kroppsdel, så lider också alla de andra.
Blir en del hedrad, så glädjer sig också alla de andra.

1 Kor 12:26

If one member* suffers, all suffers together with it;
if one member is honoured, all rejoice together with it.

1 Cor 12:26

(*of the body)
Abstract

The first aim of this thesis was to investigate the validity of temporomandibular joint (TMJ) pressure-pain threshold for assessment of pain conditions of inflammatory nature in the TMJ. Pressure-pain threshold was recorded over the palpable lateral pole of the TMJ condyle with a hand-held electronic pressure algometer (Somedic Production AB, Sollentuna, Sweden). TMJ pressure-pain threshold is systemically modulated and not modulated by the investigated inflammatory mediators (5-HT, TNFα, IL-1sRII) in the TMJ synovial fluid. TMJ pressure-pain threshold is therefore not valid as a tool for assessment of intra-articular pain conditions of inflammatory nature in the TMJ.

The second aim was to investigate differences in pressure-pain threshold between genders in healthy individuals and rheumatoid arthritis (RA) patients as well as between healthy females and female RA patients with clinical signs of TMJ involvement. TMJ pressure-pain threshold in female RA patients is lower than in male RA patients as well as in healthy females. No difference between healthy females and males was observed.

The third aim was to investigate the influence of synovial fluid and blood levels of 5-HT and TNFα on the effects by intra-articular injections of glucocorticoid regarding pressure-pain threshold and on pain of the TMJ in patients with chronic inflammatory TMJ disorders. The glucocorticoid was injected into the upper joint compartment of the TMJ. TMJ synovial fluid samples were obtained before and after treatment. Patients with detectable pretreatment levels of synovial fluid 5-HT experienced a larger reduction of TMJ pain intensity at rest and on maximum mouth opening after treatment with intra-articular glucocorticoid. Patients with detectable pretreatment levels of synovial fluid TNFα experience a larger reduction of TMJ pain on maximum mouth opening than those without. Pretreatment presence of TNFα or 5-HT in the synovial fluid predicts reduction of TMJ pain by intra-
arthicular injection of glucocorticoid in patients with chronic inflammatory TMJ disorders.

The fourth and final aim was to investigate the relative importance of local and systemic inflammatory mediators in the modulation of pressure-pain threshold and other pain entities in patients with seropositive or seronegative RA involving the TMJ and whether these pain entities are related to each other. TMJ synovial fluid samples were obtained from all patients. TMJ pressure-pain threshold as well as other TMJ pain entities were recorded. The results indicate that TMJ pressure-pain threshold is modulated by systemic rather than local inflammatory mediators and is unrelated or weakly related to other TMJ pain entities in RA patients. TMJ pain intensity at rest is mainly locally modulated but with an additional systemic influence. TMJ movement pain is mainly modulated by systemic mediators in the seropositive patients and mainly by local mediators in the seronegative patients. Tenderness and pain reflex to palpation are modulated mainly by systemic mediators in both groups, where TNFα dominate in seropositive and 5-HT in seronegative patients.
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List of publications
This thesis is based on the following five papers, which will be referred to in the text by their Roman numerals:

I. Fredriksson L, Alstergren P and Kopp S.  
Absolute and relative facial pressure-pain thresholds in healthy individuals.  

II. Fredriksson L, Alstergren P and Kopp S.  
Pressure-pain thresholds in the craniofacial region of female patients with rheumatoid arthritis.  

III. Fredriksson L, Alstergren P and Kopp S.  
Serotonergic mechanisms influence the response to glucocorticoid treatment in TMJ arthritis.  
Mediators of Inflammation. 2005;31:194-201.

IV. Fredriksson L, Alstergren P and Kopp S.  
Tumor necrosis factor in temporomandibular joint synovial fluid predicts treatment effect on pain of intra-articular glucocorticoid treatment.  
Mediators of Inflammation. 2006; Accepted.

V. Alstergren P, Fredriksson L, and Kopp S.  
Temporomandibular joint pressure-pain threshold is modulated by systemic inflammatory mechanisms and only weakly related to other temporomandibular joint pain entities in rheumatoid arthritis.  
Pain 2006; Submitted.

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**Abbreviations**

<table>
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<th>Abbreviation</th>
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<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine, serotonin</td>
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<td>IL-1β</td>
<td>Interleukin-1 beta</td>
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<td>IL-6</td>
<td>Interleukin-6</td>
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<td>IL-8</td>
<td>Interleukin-8</td>
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<tr>
<td>IL-1sRII</td>
<td>Soluble Interleukin-1 receptor type II</td>
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<td>RA</td>
<td>Rheumatoid arthritis</td>
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<td>TMJ</td>
<td>Temporomandibular joint</td>
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<td>TNFα</td>
<td>Tumor necrosis factor alpha</td>
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Definitions

Chronic systemic inflammatory joint disease involving the temporomandibular joint
A chronic systemic or generalized inflammatory disease that may cause polyarthritic conditions involving the TMJ according to the diagnostic classification criteria by the American Academy of Orofacial Pain (Okeson 1996) with the modification that pain with mandibular function or point tenderness on TMJ palpation was mandatory.

Local modulation of temporomandibular joint pain
Actions of mediators in the TMJ synovial tissues on nociceptors that influences TMJ pain.

Systemic modulation of temporomandibular joint pain
Actions of mediators in the blood, i.e. circulating with origin distant from the site or sites of inflammation, on nociceptive mechanisms in the TMJ synovial tissues or central pain mechanisms that influence TMJ pain.

Local inflammatory activity in the temporomandibular joint
Inflammation in the TMJ as assessed by presence and levels of mediators of inflammation and pain in the TMJ synovial fluid.

Systemic inflammatory activity
General inflammation as estimated by presence and levels of inflammatory markers and mediators of inflammation and pain in the blood.

Intra-articular pain conditions of inflammatory nature in the temporomandibular joint
Pain due to inflammation in the TMJ synovial tissues in patients with chronic systemic inflammatory joint diseases involving the TMJ.

Pressure-pain threshold
The minimum pressure needed to evoke a painful sensation recognizable by the subject.
Introduction

Chronic inflammatory joint diseases like rheumatoid arthritis (RA) often affect the temporomandibular joint (TMJ) since about 30 - 50% of the patients experience TMJ pain or impaired function (Syrjänen 1985, Tegelberg and Kopp 1987). The pathophysiologic mechanisms behind TMJ inflammation and pain are unclear but local and systemic inflammatory mediators are involved (Alstergren and Kopp 1997, Kopp and Alstergren 2002). TMJ synovial fluid analysis provides a possibility to study mediators of inflammation and pain and thereby increase knowledge about the local pathology as well as diagnostic and prognostic factors. Intra-articular injection of glucocorticoid in the TMJ are used to suppress inflammation and pain by the strong anti-inflammatory effects of glucocorticoid. TMJ pain can be divided into different pain entities such as TMJ pain at rest, external and internal mechanical stimulation of TMJ pain and TMJ pressure-pain threshold. These different pain entities may, in turn, be differently modulated by local and systemic inflammatory mediators (Alstergren and Kopp 1997, Nordahl et al. 2000, Kopp and Alstergren 2002).

Tumor necrosis factor α

The proinflammatory cytokine tumor necrosis factor α (TNFα) is produced by a number of cell types including activated monocytes/macrophages, T-cells and fibroblasts at the site of inflammation (Vilcek and Lee 1991). TNFα has direct modulatory effects on pain, e.g. sensitization of peripheral nerves. Besides its direct effects, TNFα also induces production of other mediators of inflammation and pain e.g. IL-1β, IL-6, IL-8 and prostaglandins in the inflamed synovial tissues (Haworth et al. 1991, Cunha et al. 1992, Maini et al. 1993, Watkins et al 1994, van de Loo et al. 1995). Elevated plasma levels of TNFα are considered as a part of the systemic inflammatory response (Beyaert and Fiers 1998) and TNFα is elevated in plasma and synovial fluid of patients with chronic inflammatory disorders like RA (Saxne et al. 1988, Campell et al. 1990, Chu et al. 1991, Neidel et al. 1995, Nordahl et al. 2000,
Voog et al. 2003). In healthy individuals TNFα is detectable in plasma (Alstergren and Kopp 2006) but not in TMJ synovial fluid (Kaneyama et al. 2005).

**Serotonin**

Peripherally, serotonin (5-HT) is produced in enterochromaffin cells in the gastrointestinal mucosa and is immediately uptaken and stored by platelets (Gyermek 1996). In peripheral joints like the TMJ, 5-HT is released by activated platelets in the synovial membrane or fluid upon inflammation (Zeller et al. 1983, Endresen 1989, Sommer 2006). There are seven 5-HT receptor classes identified so far. Of these receptors the 5-HT₃ receptor, which is peripherally only located on neurons, is associated with inflammatory pain in humans (Richardson and Engel 1986, Gyermek 1996) and 5-HT₃ receptor antagonists reduce inflammatory pain (Giordano and Rogers 1989). For example, intra-articular injection of the 5-HT₃ receptor antagonist granisetron in the TMJ transiently reduces resting as well as movement pain intensity in patients with RA (Voog et al. 2000). There are reason to believe that 5-HT in the TMJ may result in sensitization or activation of nociceptive afferents (Zeitz et al. 2002) or act more indirectly by activating other cells like T-lymphocytes or monocytes/macrophages (Farber et al. 2002, Fiebich et al. 2002), which in turn release mediators of inflammation and pain like TNFα or IL-1β. 5-HT can not be detected in synovial fluid from healthy TMJs but it can be found in the arthritic TMJ (Alstergren and Kopp 1997, Alstergren et al 1999, Nishimura et al 2002). Kopp and Alstergren (2002) has previously shown that a high level of 5-HT in TMJ synovial fluid is associated with TMJ pain and decreased mobility in patients with systemic inflammatory disorders.

**Interleukin-1 soluble receptor type II**

The soluble interleukin-1 receptor type II (IL-1sRII) has anti-inflammatory effects since it binds to and inactivates the potent proinflammatory cytokine IL-1β as part of the endogenous control of IL-1β. IL-1sRII can be found in blood and in the synovial fluid of
patients with polyarthritis (Arend 1993, Dinarello 1994, Dinarello 1998, Alstergren et al. 2003). Seropositive RA patients have lower plasma concentrations of IL-1sRII than the other patients, which indicates an insufficient systemic control of the effects of IL-1β (Alstergren et al. 2003). High plasma concentrations of IL-1sRII has previously been associated with local TMJ pain, general body pain and reduced pressure pain threshold (Alstergren et al. 2003).

**Pressure-pain threshold**

There are only two studies that have investigated the relation between TMJ pressure-pain threshold and mediators of inflammation and pain in the TMJ synovial fluid or blood in patients with chronic inflammatory TMJ disorders. In seropositive RA patients, low TMJ pressure-pain threshold has been related to high serum level of 5-HT (Alstergren and Kopp 1999). In addition, high plasma level of 5-HT was similarly associated with low TMJ pressure-pain threshold in healthy individuals (Kopp and Alstergren 2002). These results suggest that TMJ pressure-pain threshold is modulated by systemic mechanisms, even in healthy individuals. This has to be elucidated further.

No studies have so far tried to investigate the validity of TMJ pressure-pain threshold for assessment of intra-articular inflammatory conditions. For example, based on the presence of mediators of pain and inflammation in the TMJ synovial fluid. Chung et al. (1993) compared TMJ pressure-pain threshold between patients with capsulitis and healthy subjects. The patients with capsulitis had lower pressure-pain threshold and therefore it was concluded that pressure-pain threshold can be useful for evaluation of TMJ capsulitis. However, all previous studies have in common that the TMJ pressure-pain threshold was related to tenderness on digital palpations of the TMJ. On the contrary, Laursen et al. (2005) investigated the pressure-pain threshold in four groups of pain patients with fibromyalgia/whiplash, endometriosis, low back pain and rheumatoid arthritis. All groups showed lower pressure-pain threshold for all eight sites investigated.
compared to the healthy controls, indicating a generalized reduction of pressure-pain threshold.

Several studies on healthy subjects have shown that males have higher pressure-pain threshold than females (Dundee and Moore 1960, Merskey and Spear 1964, Fisher 1987, Brennum et al. 1989, Chung et al. 1993, Riley et al. 1998, Chesterton et al. 2002, Soetanto et al. 2006). The nature of the mechanism behind the reported lower pressure-pain threshold in females is yet unknown but it has been suggested that the gender difference can be a function of sex steroids and gender role (Otto and Dougher 1985, Riley et al. 1998, Isselée et al. 2001, Myers et al. 2001, LeResche 2003). There are also studies that indicate that there are no differences between genders regarding pressure-pain threshold (Antonaci 1992, Isselée et al. 1997 and 1998). It is therefore of interest to further investigate this matter in healthy individuals and in patients with inflammatory TMJ disorders.

**Temporomandibular joint tenderness and palpebral pain reflex to digital palpation**

TMJ tenderness and palpebral pain reflex to digital palpation of the lateral and posterior aspects are often used clinically to identify inflammatory conditions in the TMJ. TMJ tenderness to palpation seems to be locally modulated since intra-articular treatment with glucocorticoid reduces both lateral and posterior TMJ tenderness in RA patients (Kopp et al. 1991). In addition, TMJ tenderness to palpation has also been positively related to inflammatory proteins such as IL-1β and TNFα in the TMJ synovial fluid (Alstergren et al. 1998, Nordahl et al. 2000).

**Temporomandibular joint pain intensity at rest**

TMJ pain intensity at rest in patients with systemic inflammatory disorders is reduced after intra-articular injection of the 5-HT3 receptor antagonist granisetron and after injection of glucocorticoid in the TMJ in patients with RA, which indicate that this pain entity is locally modulated by 5-HT in the synovial fluid (Kopp et al. 1991, Voog et al.)
However, a possible systemic modulation of this pain entity is not known so far.

**Temporomandibular joint pain on maximum mouth opening**

TMJ pain on maximum mouth opening has previously been related to TNFα, i.e., patients with pain on mouth opening had higher levels of TNFα in TMJ synovial fluid than patients without (Nordahl et al. 2000). In addition, Voog et al. (2000) showed that intra-articular injection of the 5-HT3 receptor antagonist granisetron in the TMJ reduces TMJ pain on maximum mouth opening in patients with RA, which indicate that this pain entity is locally modulated by 5-HT as well (Alstergren and Kopp 1997). However, Kopp and Alstergren (2002) found a relation between the serum level of 5-HT and TMJ pain on maximum mouth opening and suggested that 5-HT from the blood is also involved in the modulation of this pain entity.

**Glucocorticoid effects**

The exact mechanism of the anti-inflammatory action of glucocorticoids is not fully understood but the lipidophilic glucocorticoids pass through the cellular membrane and bind to glucocorticoid receptors in the cytoplasm of the target cells. Based on the fact that the glucocorticoid receptors are ubiquitously expressed, it is reasonable to assume that glucocorticoids have an effect on nearly all cells in the body (Bladh 2005). The glucocorticoid receptor interact with the transcription factor in the cell nucleus, which increases transcription of anti-inflammatory proteins like IL-1sRII, decreases the transcription of proinflammatory proteins like IL-1β and TNFα and reduces prostaglandin production by inhibition of the phospholipase A2 enzyme (Boumpas et al. 1993, Chrousos 1995, Barnes 1998). Glucocorticoids administered systemically or locally suppress inflammation and pain in patients with systemic inflammatory disorders (Barnes 1998). Intra-articular treatment of the TMJ with glucocorticoid reduces TMJ pain in patients without systemic disorders and in RA patients with signs of TMJ inflammation (Kopp et al. 1985, 1987, 1991).
However, no study have investigated the predictive value of TNFα or 5-HT in the TMJ synovial fluid or blood on the treatment response for different pain entities.
Aims

The aims of this thesis were to investigate:

- the validity of TMJ pressure-pain threshold for assessment of pain conditions of inflammatory nature in the TMJ

- differences in pressure-pain threshold between genders in healthy individuals and RA patients as well as between healthy females and female RA patients with TMJ involvement

- the influence of synovial fluid and blood levels of 5-HT and TNFα on the effects by intra-articular injections of glucocorticoid on pressure-pain threshold and on pain of the TMJ in patients with chronic inflammatory TMJ disorders

- the relative importance of local and systemic inflammatory mediators (5-HT, TNFα, IL-1sRII) in the modulation of pressure-pain threshold and other pain entities in patients with seropositive or seronegative RA involving the TMJ and whether these pain entities are related to each other
Material and methods

All studies were approved by the local Ethical Committee at Huddinge Hospital, Huddinge, Sweden (176/91, 142/02, 364/02, 365/02).

Patients

Table 1 summarizes number of patients, gender and age distribution, duration of general and as well as TMJ symptoms and diagnosis of the patients included in studies II-V. All patients were referred to the Department of Clinical Oral Physiology from rheumatologists because of TMJ symptoms due to polyarthritic conditions. The patients had not been subjected to any treatment of the TMJ within the last 3 months.

Inclusion criteria for studies II-V were the diagnosis of RA according to the diagnostic classification of the American College of Rheumatology (Arnett et al. 1988) or other polyarthritic diseases involving the TMJ according to a modification of the criteria by the American Association of Orofacial Pain (Okeson 1996). In addition, in study II, further inclusion criteria were TMJ pain for more than six months and tenderness to digital palpation of the TMJ.

Inclusion criteria for study IV was a confirmed intra-articular inflammation.

Data from all patients in studies II-V are from the Department of Clinical Oral Physiology research database were collected between 1991 and 2005.

Determination of intra-articular inflammation

To determine intra-articular inflammation at least one of the proinflammatory mediators TNFα, IL-1β or 5-HT or a pathological level of the anti-inflammatory mediator IL-1 receptor antagonist had to be present in the synovial fluid directly before or after treatment. In study III and IV all but one patient had confirmed intra-articular inflammation as indicated by the presence of inflammatory mediators in the TMJ synovial fluid. The patient without determined TMJ inflammation had a previous presence of high TMJ synovial fluid levels of IL-1β and 5-HT.
Healthy individuals

Table 1 summarizes number of healthy individuals and their gender and age distribution.

Exclusion criteria for study I and II were current or prior general or local joint/muscle symptoms and current headache.

Pressure-pain threshold assessment

In all studies pressure-pain threshold were recorded from the palpable lateral pole of the TMJ (Fig. 1). The pressure-pain threshold of glabella on the frontal bone was assessed in all studies except in study IV.

Table 1. Age (years), number of subjects (n), distribution of female and male subjects (F/M), duration of general and local disease (years) and diagnosis. TMJ = temporomandibular joint, IQR = intraquartile range, na = not applicable.

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<th>Study</th>
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Diagnoses

Rheumatoid arthritis, RF-positive 17 3 5 60
Rheumatoid arthritis, RF-negative 25 5 5 74
Ankylosing spondylitis 3 3
Osteoarthritis 1 1
Psoriatic arthropathy 1 3
Chronic unspecific polyarthritis 2 2
Systemic lupus erythematosus 1
Sjögren’s syndrome 1
Common variable immunodeficiency 1
Marfan’s syndrome 2 2
The pressure-pain threshold was assessed by a hand-held electronic pressure algometer (Somedic Production AB, Sollentuna, Sweden) consisting of a pressure transducer probe connected to a pistol-grip with a display unit. The tip of the pressure transducer has a flat, circular rubber tip with an area of 1.0 cm². A linearly increasing pressure rate (50 kPa/s²) was applied until the subject responded to the first pain sensation by pressing a button on a device connected to the probe that froze the current pressure-pain threshold level on the display.

In study I, the pressure-pain threshold was consecutively measured five times over the TMJ and glabella. In study II the pressure-pain threshold was measured consecutively three times at the TMJ and glabella. The mean of the second and third measurement was used in the statistical analysis. In study III-V, the pressure-pain threshold was measured a single time.
In study II, the more painful TMJ was determined by assessing the number of painful mandibular movements on each side. If the number of painful movements was equal the pain intensity at rest was used to separate the more painful side from the less painful side.

Other pain entities (studies II-V)
A 100-mm visual analogue scale (score 0 - 100) with end-points marked with "No pain" and "Worst pain ever experienced" was used to assess the current degree of TMJ pain intensity at rest and on maximum mouth opening.

Absence or presence of tenderness and palpebral pain reflex to digital palpation of the lateral and posterior (through the acustic meatus) aspects of the TMJ were assessed with the mandible in rest position.

TMJ pain upon mandibular movements (maximal voluntary mouth opening, ipsilateral and contralateral laterotrusion as well as protrusion) was scored with one unit for each movement causing TMJ pain on each side (score 0 - 4).

Synovial fluid sampling (studies III-V)
TMJ anesthesia was achieved by blocking the auriculotemporal nerve with 2.0 mL 2% lidocain (Xylocain®, Astra-Zeneca, Södertälje, Sweden). The TMJ was punctured with a standard disposable needle (diameter = 0.65 mm) inserted into the posterior part of the upper joint compartment. TMJ synovial fluid samples were obtained by washing the joint cavity with saline using a push and pull technique (Alstergren et al. 1999). The washing solution, consisting of 78% saline (NaCl 9 mg/mL, Pharmacia Upjohn, Uppsala, Sweden) and 22% hydroxocobalamin (Behepan® 1 mg/mL; Pharmacia Upjohn, Uppsala, Sweden), was slowly injected into the posterior part of the upper joint cavity approximately 1 mL at a time and then aspirated. The total volume of the injected washing solution was 4 mL. The hydroxocobalamin was included in order to determine the amount of synovial fluid in the aspirate by comparing the spectrophotometric absorbance of the aspirate with that
of the washing solution. The synovial fluid volume recovered was then calculated. Only samples that fulfilled previously established sample quality criteria were included in the studies (Alstergren et al. 1999).

**Blood sampling (studies III-V)**

Venous blood was collected in a sodium citrate tube (0.105 mol/L) to determine the erythrocyte sedimentation rate and in an EDTA tube that was immediately cooled and centrifuged (1500 g for 10 min at +4°C) frozen (-70°C) and later examined for IL-1sRII, platelet-rich 5-HT and TNFα in plasma. One EDTA tube that was immediately cooled and centrifuged (1500 g for 30 min at +4°C) and then frozen (-70°C), and later examined for 5-HT in plasma. In addition, venous blood was collected without additives for analysis of serum concentrations of 5-HT, thrombocyte particle count, rheumatoid factor and C-reactive protein. The tube for determination of serum concentration of 5-HT was left at room temperature for 60 min for coagulation and thereafter centrifuged (1500 g for 30 min at +4°C). The serum was then removed and frozen (-70°C) until analysis. Rheumatoid factor titers below 15 IE/mL and C-reactive protein levels below 10 mg/L were considered as zero values according to the standard protocol of the Department of Clinical Chemistry at Karolinska University Hospital, Huddinge, Sweden.

**Intra-articular glucocorticoid treatment (studies III-IV)**

The glucocorticoid methylprednisolone (40 mg/mL) with lidocaine (10 mg/mL) added (Depo-Medrol cum lidocain; Pfizer AB, Täby, Sweden) was injected in a volume of 0.7 mL into the upper joint compartment of the TMJ. Patients with bilateral TMJ pain, who were subjected to bilateral treatment, data from the more painful TMJ was used in the statistical analysis. The more painful TMJ was that with the highest pain intensity at rest before treatment.
Analysis of mediators (studies III-V)
The concentrations of all investigated mediators and receptors were determined by commercially available enzyme-linked immunoassays with highly specific antibodies to detect the mediators (5-HT: Kit nr 0642, Immunotech International, Germany; TNFα: TNFα EASIA™, Medgenix, B 6220 Fleurus, Belgium. IL-1sRII: Quantikine Immunoassays, R&D Systems, Minneapolis, USA). The 25th/75th percentiles plasma level of TNFα, 5-HT and IL-1sRII in healthy individuals are 8/12 pg/mL, 10/30 nmol/L and 11442/14790 pg/mL as assayed in our laboratory. The corresponding 25th/75th percentiles of serum 5-HT in healthy individuals are 584/1186. TNFα and 5-HT is undetectable in TMJ synovial fluid in healthy individuals.

Statistical Analyses
Parametric methods were applied in study I. For descriptive statistics, mean and standard deviation were used. Kolmogorov-Smirnov´s test was used to test and confirm the normality of the variables. One-way ANOVA with Bonferroni´s compensation for multiple comparisons as post-hoc test were used to test the significance of the change in TMJ pressure-pain threshold during five consecutive recordings. The significance of the differences between genders was tested with Student's independent t-test

Non-parametric methods were applied for all statistical analyses in study II-V. For descriptive statistics, median and interquartile range (75th percentile - 25th percentile; IQR) were used. Mann-Whitney U-test was used to test the significance of differences between the two groups and the Wilcoxon test was used to test the significance of differences at repeated measures. The Friedman analysis of variance on ranks was used to test the significance of the change in TMJ pressure-pain threshold during three consecutive recordings. A pair-wise multiple comparison procedure according to Dunnet was used as a post-hoc test to test the significance of the change in TMJ pressure-pain threshold during the three consecutive recordings. Spearman's ranked correlation
test was used to test the significance of the correlations between the variables.

In all studies a probability level of less than 0.05 was considered significant.
Results

Pressure-pain threshold differences between genders (study I and V)
There was no significant difference between genders regarding TMJ pressure-pain threshold in healthy individuals. In study V, female seropositive patients older than 50 years had lower TMJ pressure-pain threshold than males in the same age group (p = 0.010).

Pressure-pain threshold differences between female RA patients and healthy females (study II)
TMJ pressure-pain threshold over the more painful joint in the RA patients was lower than on the less painful joint (p < 0.001) as well as over the TMJ in healthy females (p = 0.006). The 10th to 90th percentile

![Figure 2. The 10th to 90th percentile interval of the pressure pain threshold over the temporomandibular joint in 42 female patients with rheumatoid arthritis (RA) and in 17 healthy females. The more painful TMJ was determined by assessing the number of painful mandibular movements on each side. If the number of movements was equal the pain intensity at rest (VAS) was used to separate the more painful side from the less painful side. The vertical bars denote the mean value for each group.](image-url)
Table 2. Overview of systemic and local modulation of temporomandibular joint (TMJ) pain entities in 60 patients with seropositive and 74 patients with seronegative rheumatoid arthritis. (+) = positive correlation, (-) = negative correlation (study V).

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Systemic modulation:
- Platelet-rich plasma 5-HT
- C-reactive protein
- Erythrocyte sedimentation rate
- Rheumatoid factor
- Pain intensity on mouth opening
- Pain on mouth opening
- Painful mandibular movements
- Painful mandibular movements
- Tenderness to palpation
- Palpebral pain reflex

Local modulation:
- Pressure-pain threshold
- Resting pain
- Pain on mouth opening
- Pain intensity on mouth opening
- Painful mandibular movements
- Tenderness to palpation
- Palpebral pain reflex
- Synovial fluid IL-1sRII in all patients
- Plasma IL-1sRII
- Plasma TNFa
- Plasma 5-HT
- Plasma 5-HT
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- Plasma 5-HT
- Plasma 5-HT
- Plasma 5-HT
interval of TMJ pressure-pain threshold was 64-220 kPa over the more painful joint in the patients, while it was 111-352 kPa over the TMJ in the healthy females. The pressure-pain threshold on the more painful side was within the 10th-90th percentile interval of the healthy females for 28 out of the 42 patients (67%), while 13 (31%) were below the 10th percentile and one (2%) was above the 90th percentile of the healthy females (Fig. 2).

Influence of serotonin on the effect of intra-articular injections of glucocorticoid on temporomandibular pain (study III)
Serotonin was detectable in the TMJ synovial fluid from 11 (55%) patients before treatment and in 12 (60%) after. TMJ pain intensity at rest decreased significantly (p = 0.028) after treatment. The pretreatment synovial fluid level of 5-HT was negatively correlated to the change in TMJ pain intensity at rest and TMJ pain intensity on maximum mouth opening after treatment, i.e. a high pretreatment level of 5-HT was associated with a decrease of TMJ pain intensity at rest ($r_s = -0.52, n = 20, p = 0.018$) and on maximum mouth opening ($r_s = -0.57, n = 13, p = 0.041$). The pretreatment synovial fluid level of 5-HT was also negatively correlated to the change in synovial fluid 5-HT ($r_s = -0.55, n = 20, p = 0.012$), which means that high pretreatment 5-HT was associated with decrease of 5-HT after treatment. The pretreatment plasma level of 5-HT was above the normal limit (15 nmol/L) in 9 out of 10 patients with detectable level of 5-HT before treatment and in 3 out of 6 after treatment and it was correlated to the level of C-reactive protein ($r_s = 0.66, n = 10, p = 0.038$). The pretreatment plasma level of 5-HT correlated to change in TMJ pain intensity at rest ($r_s = 0.66, p = 0.040, n = 10$) and pressure-pain threshold after treatment ($r_s = 0.83, n = 10, p = 0.003$), i.e. a high pretreatment level of 5-HT in plasma was associated with less reduction of resting pain intensity and increase in pressure-pain threshold of the TMJ. The pretreatment thrombocyte particle count was likewise positively correlated to change in pressure-pain threshold ($r_s = 0.59, n = 14, p = 0.024$).
Influence of TNFα on the effect of intra-articular injections of glucocorticoid on temporomandibular pain (study IV)

The pretreatment TMJ synovial fluid level of TNFα was negatively correlated to the change in synovial fluid TNFα level and TMJ pain intensity upon maximum mouth opening, i.e. a high pretreatment synovial fluid TNFα level was associated with a reduction of TNFα in TMJ synovial fluid ($r_s = -0.86, n = 21, p< 0.001$) and TMJ pain intensity upon maximum mouth opening ($r_s = -0.51, n = 21, p = 0.017$) after treatment.

Local and systemic modulation of pressure-pain threshold and other pain entities (study V)

Local and systemic modulation of pressure-pain threshold and other pain entities is shown in Table 2.

Pressure-pain threshold in relation to other temporomandibular joint pain entities (study V)

No significant relation between TMJ pressure-pain threshold and the other TMJ pain entities could be found. The relations between other TMJ pain entities are shown in Table 2.

Data not previously reported

In the patient sample from study IV, the pretreatment C-reactive protein was positively correlated to the change in synovial fluid TNFα level, i.e. a high pretreatment C-reactive protein level was associated with an increase of TNFα in TMJ synovial fluid ($r_s = 0.48, n = 18, p = 0.042$).
Discussion

The main aim of this thesis was to determine the degree of local and systemic modulation of pressure-pain threshold and other pain entities of the temporomandibular joint. TMJ pressure-pain threshold is modulated by systemic rather than local inflammatory mediators and is therefore not valid as a tool for assessment of intra-articular pain conditions of inflammatory nature in the TMJ. Reduction of other TMJ pain entities by intra-articular injection of glucocorticoid is predicted by pretreatment presence of TNFα or 5-HT in the synovial fluid in patients with chronic inflammatory TMJ disorders.

Pressure-pain threshold

TMJ pressure-pain threshold appears to be unrelated or only weakly related to the other TMJ pain entities investigated (study V). Neither TMJ tenderness nor palpebral pain reflex to digital palpation was consistently related to TMJ pressure-pain threshold (study III-V). TMJ pain intensity at rest and tenderness to digital palpation were reduced after intra-articular glucocorticoid but there was no effect on TMJ pressure-pain threshold, which suggest separate mechanisms for modulation of pressure-pain threshold on the one hand and pain at rest and tenderness to digital palpation on the other (studies III and IV).

The TMJ pressure-pain threshold in RA patients seems to be modulated by systemic factors including serotonergic mechanisms. On the other hand, local TMJ inflammation, as indicated by the investigated inflammatory mediators in the synovial fluid, do not influence the TMJ pressure-pain threshold. However, the systemic modulation in seropositive and seronegative patients differ in character regarding the influence of 5-HT in plasma and C-reactive protein. In seropositive patients low TMJ pressure-pain threshold is associated with high levels of 5-HT in platelet-rich plasma. Low TMJ pressure-pain threshold is also related to systemic inflammatory activity, as expressed by C-reactive protein, which supports a systemic inflammatory modulation
of TMJ pressure-pain threshold in this particular group. In seronegative patients, on the contrary, high pressure-pain threshold is associated with high levels of 5-HT in plasma, which in turn was related to high platelet particle count. This can be explained by a higher release of 5-HT from platelets, possibly activated by TNFα, since the platelet-activating rheumatoid factor is not present in these patients. The contrasting influence of systemic serotonergic modulation of pressure-pain threshold in the seropositive and seronegative patients is, remarkable but the same condition was found for glabella pressure-pain threshold.

TMJ pressure-pain threshold is strongly related to glabella pressure-pain threshold (study II-V), probably due to a common systemic modulation since the glabella pressure-pain threshold as well as the TMJ pressure-pain threshold are positively related to plasma 5-HT in seronegative RA patients. Wide-spread decreased pressure-pain thresholds indicate a generalized elevated pain sensitivity (Rollman and Lautenbacher 2001). Indeed, Laursen et al. (2005) reported generalized pressure-pain threshold reduction in four groups of pain patients with fibromyalgia/whiplash, endometriosis, low back pain and RA. All groups showed lower pressure-pain threshold for all eight investigated sites compared to the healthy controls, supporting that the pressure-pain threshold is modulated by systemic mechanisms. TMJ pressure-pain threshold in the RA patients of this investigation was accordingly lower than in the healthy individuals (study II). Female RA patients present lower TMJ pressure-pain threshold than males (study V). The difference between females and males can be a function of sex steroids or gender role (Otto and Dougher 1985, Riley et al. 1998, Isselée et al. 2001, Myers et al. 2001, LeResche 2003, Soetanto et al. 2006). For example, the highest pain levels in female patients of reproductive age with temporomandibular disorders occurred at times of low systemic estrogen levels, i.e. during the menstrual flow, and at times of rapid estrogen change, i.e. late luteal and mid-cycle (Isselée et al. 2001, LeReche et al. 2003).
TMJ pressure-pain threshold cannot be considered as a valid tool for assessment of intra-articular pain conditions of inflammatory nature in the TMJ of RA patients due to its systemic modulation and the absence of investigated inflammatory mediators in the synovial fluid. To our knowledge this is the first time validation of TMJ pressure-pain threshold is based on presence of mediators of pain and inflammation in the synovial fluid. All previous studies investigating the validity of pressure-pain threshold for assessment of TMJ pain have compared TMJ pressure-pain threshold with TMJ tenderness to digital palpation, although tenderness to palpation has not been established as a "golden standard" for TMJ pain or intra-articular inflammation. The weak and inconsistent relation between TMJ pressure-pain threshold and tenderness to digital palpation is in agreement with the results reported by Shaefer et al. (2001) who found that the pressure-pain threshold was approximately two times higher than the pressure needed to elicit tenderness to digital palpation.

**Temporomandibular joint tenderness and palpebral pain reflex to digital palpation**

TMJ tenderness and palpebral pain reflex to digital palpation in RA patients are modulated by a combination of local and systemic mechanisms. However, these pain entities are mainly influenced by systemic inflammatory mechanisms as expressed by the erythrocyte sedimentation rate in the seropositive patients and the plasma level of 5-HT in the seronegative patients. The plasma level of 5-HT is in turn related to TNFα, C-reactive protein and thrombocyte particle count in seronegative patients, which support that systemic inflammatory mechanisms influence pain sensitivity to mechanical stimulation over the TMJ also in seronegative patients. In the seropositive patients tenderness to digital palpation of the TMJ is associated with number of painful mandibular movements, which in turn is associated with synovial fluid levels of 5-HT and TNFα indicating a partial local modulation of tenderness and palpebral pain reflex to digital palpation. In
addition, intra-articular glucocorticoid reduces tenderness and palpebral pain reflex to digital palpation, which supports a partly local modulation. Indeed, TMJ tenderness to palpation has previously been shown to be modulated by local mechanisms involving synovial fluid IL-1β and TNFα (Alstergren et al. 1998, Nordahl et al. 2000).

**Temporomandibular joint pain intensity at rest**

TMJ pain intensity at rest is modulated by local mechanisms involving synovial fluid 5-HT and TNFα. Patients with chronic inflammatory TMJ disorders and high pretreatment levels of synovial fluid 5-HT respond with a decrease in TMJ pain intensity at rest after intra-articular glucocorticoid and patients with high levels of synovial fluid TNFα have higher resting pain intensities than those with low such levels. In addition, TMJ pain intensity at rest is strongly related to TMJ pain intensity on maximum mouth opening, which is a pain entity with a strong relation to inflammatory conditions of the TMJ as determined by presence of inflammatory mediators in the synovial fluid (Alstergren and Kopp 1997, Nordahl et al. 2000). However, there is also additional systemic influence on TMJ pain intensity at rest by plasma 5-HT since patients with high pretreatment plasma level of 5-HT experience less reduction of TMJ pain intensity at rest after treatment with intra-articular glucocorticoid. This can be explained by the higher systemic inflammatory activity in these patients and the association between plasma 5-HT and C-reactive protein (Study III).

**Temporomandibular joint pain on maximum mouth opening**

TMJ pain intensity on maximum mouth opening is modulated by local mechanisms involving synovial fluid 5-HT, TNFα and IL-1sRII. The patients with chronic inflammatory TMJ disorders, who have high levels of synovial fluid 5-HT and TNFα, responded with a greater decrease in TMJ pain intensity on maximum mouth opening after intra-articular glucocorticoid in agreement with the anti-inflammatory effect of glucocorticoid. High 5-HT or TNFα levels indicate high inflammatory activity and the local TNFα level decreased after
glucocorticoid administration. High IL-1sRII in TMJ synovial fluid is associated with TMJ pain on maximum mouth opening in the RA patients, which can be explained by an increased release of IL-1sRII due to inflammation but in insufficient amounts to control the proinflammatory effects of IL-1β (Dinarello 1994). However, the association between this pain variable and high platelet-rich plasma 5-HT and low plasma IL-1sRII in seropositive patients suggests a combination of local and systemic modulation in this patient group (study V). Low plasma IL-1sRII levels in seropositive patients suggest an insufficient endogenous systemic control of IL-1β, i.e. one factor for afferent nerve sensitization (Alstergren et al. 2003). Interestingly, plasma 5-HT and IL-1sRII were not significantly related, which suggests independent influence of these factors. Regarding the seronegative patients, only local factors modulate TMJ pain intensity on maximum mouth opening.

**Prediction of treatment effect of intra-articular glucocorticoid on temporomandibular joint pain**

Presence of 5-HT or TNFα in the TMJ synovial fluid predicts a treatment effect by intra-articular injection of glucocorticoid on TMJ pain in patients with chronic inflammatory TMJ disorders. Patients with detectable levels of synovial fluid 5-HT are more likely to experience a reduction of TMJ pain intensity at rest and on maximum mouth opening after treatment with intra-articular glucocorticoid, although the glucocorticoid treatment had no effect on the level of 5-HT in the TMJ synovial fluid. Patients with high levels of 5-HT in the synovial fluid responded with a better treatment effect, which could be explained by the effect by glucocorticoid on other mediators of pain and inflammation such as TNFα. Patients with detectable pretreatment levels of synovial fluid TNFα experience a reduction of TMJ pain on maximum mouth opening after glucocorticoid treatment. The treatment effect on maximum mouth opening is explained by the inhibiting effect of glucocorticoid on transcription of proinflammatory
proteins like TNFα (Barnes 1998) since synovial fluid TNFα is strongly reduced after treatment in patients with detectable pretreatment TNFα levels.
Conclusions

- TMJ pressure-pain threshold is not valid as a tool for assessment of chronic intra-articular pain conditions of inflammatory nature in the TMJ.

- TMJ pressure-pain threshold in female RA patients is lower than in male RA patients as well as in healthy females. No difference between healthy females and males was observed.

- Pretreatment presence of TNFα or 5-HT in the synovial fluid predicts reduction of TMJ pain by intra-articular injection of glucocorticoid in patients with chronic inflammatory TMJ disorders.

- TMJ pressure-pain threshold is modulated by systemic rather than local inflammatory mediators and is unrelated or weakly related to other TMJ pain entities in RA patients. TMJ pain intensity at rest is mainly locally modulated but with an additional systemic influence. TMJ movement pain is mainly modulated by systemic mediators in the seropositive patients and mainly by local mediators in the seronegative patients. Tenderness and pain reflex to palpation are modulated mainly by systemic mediators in both groups, where TNFα dominate in seropositive and 5-HT in seronegative patients.
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References


Brennum J, Kjeldsen M, Jensen K, Jensen TS. Measurements of human pressure-pain

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Giordano J, Rogers LV. Peripherally administered serotonin 5-HT₃ receptor antagonists


van de Loo AA, Arntz OJ, Bakker AC, van Lent PL, Jacobs MJ, van den Berg WB. Role of


Richardson BP, Engel G. The pharmacology and function of 5-HT₃ receptors. TINS. 1986;424-428.


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