Neuroendocrine, Immune, and Local Responses Related to Temporomandibular Disorders

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Orofacial pain frequently originates from pathologic conditions in the masticatory muscles or temporomandibular joints (TMJs). The mediators and mechanisms that monitor pain and inflammation, centrally or peripherally, are of great interest in the search for new treatment modalities. The neuropeptides substance P (SP), calcitonin gene-related peptide (CGRP), and neuropeptide Y (NPY) have all been found at high levels in the synovial fluid of arthritic TMJs in association with spontaneous pain, while serotonin (5-HT) has been found in association with hyperalgesia/allodynia of the TMJ. Interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) have been found in arthritic TMJs, but not in healthy TMJs, in association with hyperalgesia/allodynia of the TMJ as well as spontaneous pain. Anterior open bite, which may be a clinical sign of TMJ destruction, has been found in association with high levels of CGRP, NPY, and IL-1β in the synovial fluid of the TMJ. Interleukin-1β has also been related to radiographic signs of joint destruction. Prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) are both present in the arthritic TMJ, and PGE2 has been shown to be associated with hyperalgesia/allodynia of the TMJ.

Very little is known about pain and inflammatory mediators in muscles. However, we know that 5-HT and PGE2 are involved in the development of pain and hyperalgesia/allodynia of the masticator muscle in patients with fibromyalgia, whereas local myalgia (myofascial pain) seems to be modulated by other, as yet unknown mediators. Interaction between the peripheral nervous system (sensory and sympathetic nerves), the immune system, and local cells is probably of great importance for the modulation of pain and inflammation in the TMJ and orofacial musculature.


Key words: arthritis, cytokines, eicosanoids, inflammation, masticatory muscles, myalgia, neuropeptides, pain, serotonin, synovial fluid, temporomandibular disorders, temporomandibular joint

Pain in the temporomandibular joints (TMJs) and orofacial muscles is a significant part of the symptomatology of patients with temporomandibular disorders (TMD) and a common source of chronic orofacial pain. However, little is known about the pathophysiologic mechanisms that underlie the development of these pains. Regarding the TMJ, joint fluid analysis has recently provided valuable information about local joint pathology and associated pain. This may help to further develop specific diagnostic and prognostic tools by identifying markers of disease. In addition, more specific treatment alternatives may be developed and tried by adopting antagonists to mediators of pain and inflammation.
The author’s research of the TMJ has focused on the clinical interpretation of the roles played by neuropeptides, ie, substance P (SP), calcitonin gene-related peptide (CGRP), and neuropeptide Y (NPY), as well as serotonin (5-HT), interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and the prostanoids prostaglandin E2 (PGE2) and leukotriene B4 (LTB4). Their relationships to TMJ pain, mandibular mobility, and inflammatory joint tissue destruction have been studied.1 The sampling of synovial fluid (SF) has been a major problem in the past. The techniques have varied between direct SF aspiration, saline washing, and saline washing with vitamin B12 as an internal standard.2,3 The last method is a step forward, since it permits quantification of substances in the SF by spectrophotometric determination of the saline dilution (Fig 1).

Regarding orofacial muscle pathophysiology, study of the mediator mechanisms underlying inflammation and pain has long been a problem. Muscle biopsies are difficult to perform in the orofacial region for several reasons, including esthetics, high neural and vascular density, and small muscles. The introduction of the microdialysis technique has made it possible in vivo to obtain most molecules from soft tissue without danger of tissue damage. The collection of data regarding 5-HT as well as the prostanoids PGE2 and LTB4 has just started.

The pathophysiology of pain and inflammation is certainly complex, and a large number of interacting mediators are involved. This review will describe some of these mediators relevant to orofacial pain and inflammation as they relate to TMD.

**Neuroendocrine Peptides**

There is now clear evidence that peripheral terminals of primary afferent nociceptors not only respond to noxious stimuli and mediate pain but also release inflammatory mediators.4 Similarly, the sympathetic nerves of the peripheral nervous system contribute to joint inflammation by increased activity of the postganglionic sympathetic nerve fibers.

**Substance P**

Substance P (molecular weight 1,348 Da, 11 amino acids) belongs to the tachykinins, which are released from nociceptive afferents (eg, C-fibers), and has local circulatory effects that cause vasodilation and increased capillary permeability as well as the release of histamine from mast cells.5,6 In addition to its vasoactive properties, SP has been claimed to elicit hyperalgesia by local or intrathecal injection.7 On the other hand, SP has also been claimed to be involved in antinociception at the spinal or supraspinal levels.8 It activates macrophages,9 B-lymphocytes,10 polymorphonuclear (PMN) cells,11 leukocytes and platelets,12 and synoviocytes.13 Platelets are thereby stimulated to release 5-HT. Substance P also stimulates IL-1 secretion or amplifies the action of this cytokine.14 The effects by SP on the cells above are mediated by the neurokinin 1 (NK1) receptor located on the cell membrane.

Substance P-immunoreactive nerve fibers have been demonstrated in, among other tissues, the TMJ capsule, the disc attachment, and the interfascicular...
connective tissue of the lateral pterygoid muscle of the monkey.15 These nerve fibers are found in the adventitia of the arteries or as free nerve endings. In the rat lip, SP is released from sensory nerve terminals and has been shown to act on the vascular system partly via histamine and serotonin from mast cells, but in the tooth pulp, it may cause a direct vascular response.16

Release of SP from the peripheral terminals of afferent C-fibers is assumed to be the main cause of neurogenic inflammation. The vasodilatation and plasma protein extravasation caused by infusion of SP can be decreased or inhibited by the SP receptor (NK₁) antagonist CP-96, 345.17 The severity of joint inflammation and pain has been correlated to intra-articular SP release in experimental studies. Very little is known, however, about SP and muscle pain.

Clinically, SP has been found in the SF from arthritic TMJs at concentrations high above plasma levels in patients with rheumatoid arthritis (RA).18 However, SP has not been associated with TMJ pain or restricted mandibular function in this patient category19; it was even found to be negatively correlated to TMJ pain in patients with inflammatory disorders and positively correlated to pressure-pain threshold (PPT) and tolerance level over the lateral aspect of the joint.20 The results of this study also showed that increased levels of SP were correlated with increased intra-articular temperature, which in turn was associated with increased PPT. Intra-articular temperature was used as an indirect measure of joint blood flow.21,22 Substance P is probably involved in the local pathophysiology of the TMJ with inflammatory disorders but perhaps has an anti-nociceptive effect. This might be the result of an increased synovial blood flow. Its presence is strongly correlated to the presence of both CGRP and NPY,23 of which the former has a strong vasodilatory effect.

Calcitonin Gene-Related Peptide

Calcitonin gene-related peptide (molecular weight 3,952 Da, 37 amino acids) is co-localized with SP in nociceptive C-fibers24 and has a strong vasodilatory effect in joints and muscles that leads to increased blood flow. In contrast to SP, it does not increase vascular permeability and has little or no ability to induce edema.25,26 In agreement with these known vascular effects, high levels of SF CGRP have been associated with high intra-articular temperature in TMJs of subjects with inflammatory disorders.27

Calcitonin gene-related peptide is assumed to participate in pain perception and to potentiate hyperalgesia produced by SP,28 but anti-inflammatory effects have also been reported.26 Clinically, CGRP has been found in higher concentrations in the knee joint of arthritic patients than in controls with degenerative joint disease29 and in concentrations high above plasma levels in RA of the TMJ.18 The level of CGRP in plasma has been found to be about 5% of the SF level in patients with inflammatory disorders of either a local or a systemic nature.27 The CGRP level in the TMJ has been associated with spontaneous pain and restricted mandibular mobility in patients with RA.19 A feature of severe RA and other inflammatory disorders in the TMJ is a progressive anterior bite opening, resulting from bilateral destruction of the mandibular condyles and the corresponding part of the temporal component of the joint. The mandible is thereby rotated posterosuperiorly around the molars. The destruction of the joint is caused by the inflammation and is usually, but not always, associated with pain. Anterior open bite has been found to be associated with high concentrations of CGRP in the TMJ SF.19

Neuropeptide Y

Neuropeptide Y is a 36-amino-acid peptide with a molecular weight of 3,960 Da that is found, together with catecholamines, in peripheral sympathetic nerve fibers. It is released from these fibers together with norepinephrine30 and has a strong, long-lasting vasoconstrictive effect, particularly on the arterial blood vessels, that is much stronger than that of norepinephrine.

Three NPY receptor subtypes have been identified: Y1, Y2, and Y3. The receptor type Y1 mediates a vasoconstrictive effect as well as an inhibitory effect on vasodilatation.31 In striated muscles, NPY causes constriction of arterioles by activation of receptor Y1 on vascular smooth muscle cells.32 No competitive Y1 receptor antagonist has been found, but Ins [1, 2, 6] P3 has a non-competitive antagonistic effect directly on the Ca²⁺ channels of the recipient cell. Sensory, sympathetic, and parasympathetic nerves have receptor subtype Y2.31 It has been shown that NPY inhibits neurogenic inflammation by inhibition of SP release and neurokinin A (NKA) from airway sensory nerve terminals.33 In addition, NPY may, via its Y2 receptor, inhibit neuropeptide release from sympathetic as well as sensory nerve terminals. Neuropeptide Y (amino acids 18-36) is a selective Y2 antagonist.34
In the TMJ of the rat, this neuropeptide has been found around blood vessels in the capsule, but not in the disc or joint surfaces, which normally are avascular. Neuropeptide Y has been demonstrated to have an important role as a regulator of joint inflammation in experimentally induced adjuvant arthritis in rats. Clinically, NPY has been found in significantly higher concentrations in the SF from patients with arthritis of the knee than in controls with non-inflammatory arthritis induced adjuvant arthritis in rats. Clinically, NPY has been found in significantly higher concentrations in the SF from patients with arthritis of the knee than in controls with non-inflammatory arthritis. NPY has been found in significantly higher concentrations in the SF from patients with arthritis of the knee than in controls with non-inflammatory arthritis.

Serotonin has long been known to be an important endogenous mediator of inflammation in peripheral tissues and to sensitize or excite peripheral sensory nerve endings. In peripheral tissues, 5-HT is stored in mast cells and platelets and is released simultaneously with histamine upon degranulation induced by several substances, eg, SP. Platelets are likely to be the major source of 5-HT in serum as well as in SF and have been found in human knee SF from patients with several rheumatic diseases, including RA. In comparison to platelets from healthy individuals, the platelets from rheumatic patients are activated, ie, a release of 5-HT from the platelets has occurred. Serotonin, as well as other substances released locally from activated platelets, has been suggested to contribute in several ways to the inflammatory response in RA, and the platelet content of 5-HT has been found to be decreased during inflammatory episodes of RA. Several studies of the neuroimmune system have shown that 5-HT is released from mast cells in response to interactions with NPY. Spatial associations between mast cells and peptidergic nerves containing neuropeptides have been found, indicating a functional relationship.

Serotonin has been shown to produce hyperalgesia by a direct action on the 5-HT \(_{1A}\) receptors of the primary afferent sensory neurons, but it may also sensitize sensory neurons via the 5-HT \(_{2}\) receptor. In addition, 5-HT participates in the mediation of spontaneous pain from inflamed peripheral tissues by exciting small-diameter afferents via the 5-HT \(_{3}\) receptor, and 5-HT effects in inflammatory pain in humans have been associated mainly with actions on the 5-HT \(_{3}\) receptor on primary afferents. The 5-HT \(_{3}\) receptor is widely distributed in the peripheral nervous system but is located only on neurons. On peripheral sympathetic nerve terminals, the 5-HT \(_{3}\) receptor causes release of norepinephrine and NPY. The peripheral 5-HT \(_{3}\) receptor has been suggested to play a role in chemical, but not in thermal or mechanical
nociceptive mechanisms. However, activation of 5-HT3 receptors causes a long-lasting sensitization of high-threshold mechanosensitive afferents as well as a brief excitation of chemo- and mechanosensitive afferents in joints. Peripherally administered 5-HT3 receptor antagonists reduce inflammatory pain, especially during chronic inflammation. Cutaneous pain involves the 5-HT3 receptor but also the 5-HT2 receptor. In addition, 5-HT3 as well as 5-HT1A and 5-HT2 receptors have been shown to be involved in hyperalgesia in several animal studies.

Little is known about the relationship between the SF level of 5-HT and pain in generalized arthritis and TMJ arthritis, although the relationship between SF-5-HT, pain, and functional impairment of the TMJ has been investigated. Pain in the TMJ that is provoked during mandibular movements (internal mechanical stimulus) has been positively correlated to SF-5-HT, while the maximum voluntary mouth opening capacity has been negatively correlated with SF-5-HT. The mandibular movement capacity is influenced by pain during movement and constitutes an indirect estimate of pain, although there are other causes of restricted movement, eg, adhesions in the joint or muscle pain. Pain in the TMJ on mandibular movement is most probably a result of hyperalgesia or even allodynia of the joint, because it appears during normal, non-painful, mechanical loading of the joint. Pain localized to the TMJ as a response to mandibular movement might be a clinical parameter for verification of intra-articular pain conditions of the TMJ, although Taiwo and Levine suggested that pain and hyperalgesia could be 2 different entities, spontaneous pain being mediated by the 5-HT3 receptor and hyperalgesia by the 5-HT1A receptor. Serotonin seems to be undetectable in samples of TMJ SF from healthy individuals, which is important from a diagnostic point of view.

The highest blood serum levels of 5-HT (S-5-HT) are found in patients with seropositive RA, and the lowest are found among patients with fibromyalgia. There have been several studies of the relationship between S-5-HT and pain, but they are partly in disagreement. Tenderness to digital palpation has been found to be both negatively and positively correlated to S-5-HT in patients with fibromyalgia. The explanation for this discrepancy is as yet unknown. A study of seropositive RA found a strong correlation between S-5-HT and TMJ pain upon mandibular movement (Fig 2). Circulating unbound 5-HT (P-5-HT) should have the greatest potential to influence 5-HT receptors, but the associations found in most studies have been between S-5-HT and pain.

There are 2 major forms of chronic myalgia: generalized myalgia, such as fibromyalgia, and local myalgia. Fibromyalgia is characterized by generalized pain and tenderness, muscle stiffness, fatigue, and sleep disturbances, while local myalgia is characterized by local muscle pain and tenderness. The majority of patients with TMD suffer from chronic muscle pain. The pain is mostly of the local type, affecting only the orofacial muscles. However, patients with fibromyalgia often complain of symptoms from the orofacial muscles and thus frequently show signs of TMD. The etiology and pathophysiology of these 2 different muscle pain conditions are largely unknown.

Microdialysis sampling has revealed that 5-HT is released in the masseter upon puncture as well as during a steady state (Figs 3 and 4). The level of 5-HT is higher immediately after puncture than during the steady state. Serotonin can be detected in the masseter muscle of patients with fibromyalgia and local myalgia as well as in healthy individuals. However, the level of 5-HT in the masseter muscle in relation to the level of 5-HT in the blood.

![Fig 2](image.png)
serum is higher in patients with fibromyalgia than in healthy individuals (Figs 5a and 5b), and a high intramuscular level of 5-HT is associated with pain as well as allodynia of the masseter muscle. The main origin of the intramuscular 5-HT is probably the blood, but peripheral release may also occur. Peripheral sources could be mast cells or perhaps sensory nerves, which have been shown to contain 5-HT in animal studies. Ernberg et al positively correlated the intramural 5-HT level in the initial sample taken immediately after puncture to S-5-HT, but this was not the case with samples taken during a steady state. The S-5-HT in their study did not differ between patients with fibromyalgia, local myalgia, or healthy individuals, in contradiction to others.

The effects of intramuscular glucocorticoid administration on the level of 5-HT in the masseter muscle (M-5-HT) have been investigated in patients with fibromyalgia and local myalgia, along with associated changes in local pain, tenderness, and microcirculation. Ernberg et al estimated the latter by intramuscular temperature (IMT). Intramuscular microdialysis was used for 5-HT sampling at 2 visits, 2 to 3 weeks apart, with local glucocorticoid injection at the first visit. The ratio between the initial 5-HT level during stabilization and the steady state level was used as a relative measure of the intramuscular release of 5-HT. This ratio decreased significantly after treatment in fibromyalgia patients, occurring simultaneously with an increase in IMT. In general, patients with muscle pain have been reported to show decreased muscle microcirculation. Also, patients with fibromyalgia present a decreased IMT. Thus, according to the study by Ernberg et al, it is possible that intramuscular release of 5-HT in fibromyalgia patients results in vasoconstriction of arteries, which in turn leads to ischemia. This may occur either directly via the 5-HT$_2$ receptor or indirectly via the release of other vasoconstrictors, eg, noradrenaline or NPY. An experimental study by Kurita et al showed that the 5-HT$_2$ receptor antagonist ritanserin inhibits the decrease in blood flow induced by 5-HT in the rabbit masseter muscle, indicating that the 5-HT$_2$ receptor is directly involved. No significant change in S-5-HT occurred after treatment, and it is unlikely that this local glucocorticoid administration would have any significant systemic effect on the serum level of 5-HT. Serotonin thus seems to be involved in the modulation of local muscle microcirculation in patients with fibromyalgia and in the modulation of hyperalgesia in patients with local myalgia. Patients with fibromyalgia show a decrease in M-5-HT at steady state after glucocorticoid injection, which is associated with an increase in IMT. In addition, the patients with local myalgia showed a decrease of M-5-HT at steady state that was associated with an increase of PPT and pressure-pain tolerance level. Reductions in pain and hyperalgesia in these patient groups after glucocorticoid administration have been reported previously, which also might be a result.
**Fig 4** The microdialysis probe is inserted into the masseter muscle.

**Figs 5a and 5b** Box plots (median and 10th, 25th, 75th, and 90th percentile) showing the intramuscular 5-HT level in 18 patients with fibromyalgia (FM) and 17 with local myalgia (LM) of the temporomandibular system, as well as in 10 healthy controls (C). (Left) Level of 5-HT during stabilization after muscle puncture. (Right) Level of 5-HT during steady state. $S_{1\text{corr}}$ and $S_{2\text{corr}}$ express the percentage of intramuscular 5-HT in relation to 5-HT in blood serum. The samples were collected from the most tender masseter muscle in the patients and from the right masseter muscle in the healthy individuals. There was a significant difference between the FM and C groups regarding $S_{1\text{corr}}$ ($P < 0.05$). No significant difference was found between groups regarding $S_{2\text{corr}}$. From Ernberg et al.\(^{64}\); reprinted with permission from Life Sciences/Elsevier.
of other effects, such as prostaglandin synthesis inhibition.

Injection of 5-HT into the human masseter muscle to study whether local pain or allodynia/hyperalgesia develop was performed by Ernberg et al in patients with fibromyalgia and age-matched healthy female individuals. Three concentrations of 5-HT (10^-3, 10^-5, and 10^-7 mmol/L) or isotonic saline were injected in a randomized, double-blind manner. In the fibromyalgia patients, there was a nonsignificant increase in pain intensity that lasted during the entire 30-minute study period, irrespective of whether 5-HT or saline was injected. In the healthy individuals, significant pain developed after injection irrespective of whether 5-HT or saline was injected, but significantly more after injection of 5-HT at 10^-3 mmol/L. Injection of 5-HT at both 10^-5 mmol/L and 10^-3 mmol/L caused a greater reduction in PPTs than injection of saline. Thus, 5-HT injected into the masseter muscle of healthy female subjects elicits both pain and allodynia/hyperalgesia, while no such 5-HT-specific responses seem to occur in female patients with fibromyalgia. These results differ from the results of Jensen et al, who found no effect on these pain parameters by injection of
5-HT $10^{-5}$ mmol/L into the temporal muscle. A concentration of at least $10^{-3}$ mmol/L therefore seems to be necessary to exert a nociceptive effect. It is remarkable that no difference has been found in the response to 5-HT and saline in the fibromyalgia patients. One explanation could be that the 5-HT receptors for pain and allodynia/hyperalgesia already were occupied with endogenous 5-HT in the patients with fibromyalgia and therefore were not available for the injected 5-HT.

The pain increase and allodynia/hyperalgesia induced by injection of 5-HT in healthy subjects was investigated by Ernberg et al by simultaneous intramuscular injection of the 5-HT$_3$ receptor antagonist granisetron or the 5-HT$_{1A}$ receptor antagonist propranolol (eg, Fig 6). Both granisetron and propranolol reduced the maximum pain intensity and granisetron alone antagonized the lowered PPT induced by local administration of 5-HT. The effect of granisetron was stronger than that of propranolol. The median intensity of pain was reduced only by granisetron, especially in females. This result might depend on several factors. One is that 5-HT$_{1A}$ receptors in the human masseter muscle may not be expressed strongly enough to mediate substantial 5-HT effects, or it may be that these receptors are not greatly involved in the development of peripheral pain or allodynia/hyperalgesia in the human masseter muscle. Since the molar concentration of the injected granisetron and propranolol was similar, the difference in effect between the compounds might also have been the result of a lower affinity of the non-specific antagonist propranolol for 5-HT$_{1A}$ receptors, compared to the affinity of the selective antagonist granisetron for 5-HT$_3$ receptors.

Granisetron has been shown to eliminate allodynia and hyperalgesia. The PPT over the masseter muscle increases after injection of granisetron and 5-HT, ie, by blocking of 5-HT$_3$ receptors, compared to 5-HT alone (Fig 6). This result indicates that the 5-HT$_3$ receptor influences the PPT. Topical administration of the 5-HT$_3$ receptor antagonist ondansetron was recently reported to attenuate inflammatory pain induced by the intradermal injection of capsaicin in healthy subjects. In addition, systemic treatment with ondansetron has been reported to reduce pain intensity in patients with fibromyalgia. The differences found between injection of 5-HT alone and in combination with granisetron for median pain intensity and PPT are significant only in females; several studies have reported on gender differences in responses to noxious stimuli.

The level of S-5-HT in patients with TMD of muscular origin, ie, local myalgia and fibromyalgia, was investigated by Ernberg et al, along with its relationship to pain parameters. Age- and sex-matched healthy individuals were included for comparison. The S-5-HT did not differ significantly between the groups. However, in the patients with local myalgia, a low S-5-HT was associated with a high tender point index of the orofacial muscles. In the patients with fibromyalgia, a low S-5-HT was associated with a high number of painful musculoskeletal body regions in general as well as a low PPT over the masseter muscle. The levels of S-5-HT resemble those found by Alstergren et al in patients with RA. However, the results of the former study differ from those reported by Alstergren et al, where an association was found between high S-5-HT and allodynia (PPT and pain during mandibular movement) of the TMJ in patients with seropositive RA. The reason for this difference is unknown at present, but it might be a result of pathophysiologic differences between patients with fibromyalgia and patients with RA. The positive relationship between S-5-HT and allodynia is in agreement with a previous study by Wolfe et al but contradicts the results of studies by Moldofsky and Warsh, Russel et al, and Stratz et al. The latter study was an epidemiologic population survey, while the others comprised patients receiving treatment. There is thus reason to believe that the intensity and character of pain differs between these 2 types of subjects. There is thus some evidence that allodynia/hyperalgesia of orofacial muscles is somehow related to S-5-HT concentration.

When platelets are activated, they release 5-HT into the plasma compartment (P-5-HT), which then can bind to receptors. The relationship between P-5-HT and S-5-HT versus orofacial pain and anxiety was investigated in patients with fibromyalgia and RA as well as in healthy individuals. The ratio between P- and S-5-HT was thereby calculated to estimate the relative plasma fraction of serotonin. Patients with fibromyalgia showed lower S-5-HT than did patients with RA, which is in agreement with a previous study, while the relative plasma fraction of serotonin was similar in fibromyalgia and RA patients and the healthy individuals, which also is in agreement with a previous study. The patients with fibromyalgia in that study showed higher scores on the Spielberger State and Trait Anxiety Inventory scale, a higher tender point index of the orofacial muscles, and a lower PPT over the masseter than the healthy individuals. A high relative plasma
fraction of serotonin was thereby associated with a high level of pain in daily living activities and a high Spielberger State and Trait Anxiety Inventory score. Many reports present associations between low S-5-HT and depression in conditions that include fibromyalgia, as well as associations between chronic pain and anxiety, including depressive symptoms.

The results hitherto presented indicate that a high level of P-5-HT in relation to S-5-HT is associated with pain and increased anxiety in fibromyalgia patients, and that high P-5-HT is associated with low PPT in healthy individuals. The latter finding suggests that the pain threshold upon mechanical stimulation/provocation of non-painful muscles is determined to a certain extent by the free amount of 5-HT in the blood.

Cytokines

Interleukin-1

Cytokines play an important role in the pathology of RA, for example, by taking part in the mediation of acute and chronic inflammation as well as the ensuing destruction of connective tissue. Interleukin-1 (IL-1), which like TNF is derived mainly from macrophages, plays a key role in amplifying and perpetuating inflammation in conditions other than RA. Antigen presentation in the joint tissues by macrophages expressing the genetic factor HLA-DR 1/4 appears to be an important component in the etiology of arthritis. Interaction in the synovial membrane between the antigen-presenting cells and specific receptors on the surface of T-cells activates the T-cells to produce cytokines, including IL-1, which results in an inflammatory reaction. The presenting macrophages secrete IL-1 following antigen recognition. Interleukin-1 then induces several inflammatory events: it activates lymphocytes, it stimulates the production of prostaglandin and collagenase in connective tissue cells, and it stimulates the breakdown of cartilage proteoglycans. It is known that IL-1 and TNF mediate cartilage destruction by stimulating chondrocytes to produce proteinases and possibly oxidative radicals. Furthermore, IL-1 blocks the synthesis of proteoglycans and type 2 collagen by chondrocytes, but these cells continue to produce types 1 and 3 collagen. In addition, IL-1 stimulates fibroblast proliferation and metaplasia of chondrocytes to fibroblast-like cells, which both promote pannus formation. It also has systemic effects by stimulating the acute phase response, with induction of production and release of C-reactive protein, eliciting fever and enhancing muscle protein catabolism.

To date, 2 subtypes of IL-1 have been identified: IL-1α and IL-1β. Most of the IL-1α remains intracellularly on the surface of the cell membrane, where it functions more as an autocrine messenger rather than as an extracellular mediator, while most IL-1β is transported out of the cell, where it acts locally or enters the blood circulation. Both are involved in inflammatory reactions, but only IL-1β is found in the SF of patients with RA. The IL-1 family also includes an IL-1 receptor antagonist (IL-1Ra). This receptor antagonist, which is produced in much higher concentrations than IL-1, does not elicit a biologic response when coupled to an IL-1 receptor and has therefore been proposed to be anti-inflammatory. There are 2 IL-1 receptors, IL-1RI (high affinity) and IL-1RII (low affinity); IL-1RII causes no signal transduction when stimulated. Soluble IL-RI (IL-1sRI) and IL-1RII (IL-1sRII) circulate in the blood in both healthy individuals and patients with inflammatory disorders. Elevated levels, especially of IL-1sRII, are found in the blood plasma and SF of patients with RA. Up-regulation of these soluble receptors has been proposed to have an anti-inflammatory effect. The presence of endogenous soluble IL-1 receptors, inactive forms of receptors, or the IL-1 antagonist thus blocks the effects of IL-1.

The SF level of IL-1β in human knees has been correlated with local disease activity, as measured by the Ritchie score and joint circumference. Interleukin-1β is capable of decreasing nociceptive thresholds in peripheral tissues. Given systemically to rats, IL-1β is a potent hyperalgesic agent, possibly by a peripheral site of action. Jeanjean et al. showed that intraplantar injections of IL-1β in rats were able to sensitize nociceptors by a long-term increase of neuronal synthesis and axonal transport of SP, as well as the receptors that control its release. In chronic inflammation, this effect could increase the sensitivity to stimuli by peripheral neurogenic inflammation. Interleukin-1β may also cause a general decrease of nociceptive thresholds by a central action, either directly, or indirectly via actions on the hepatic vagus nerve. Because of the potent effect of IL-1β in inducing bone and cartilage resorption and of the stimulating effect on fibroblasts, IL-1 is involved in joint destruction by pannus formation and in the development of fibrous or bony ankylosis.

Interleukin-1β is seldom detectable in the TMJ SF of healthy individuals, while patients with polyarthritides have significantly higher TMJ SF.
concentrations of IL-1β than healthy individuals. Alstergren et al found detectable levels of SF-IL-1β in 34% of their patients and 37% of joints. The SF-IL-1β levels found in arthritic TMJs were within the range for SF levels found in other joints with RA. In a study of joint damage and local disease activity in relation to SF-IL-1β levels in patients with RA and other arthritides, Holt et al found IL-1β in 16% of knee joints. The IL-1β found in the TMJ SF of patients with an inflammatory disorder did not seem to originate from the plasma but from local production, since the correlation between the 2 was poor and the SF level was much higher than the plasma level. There was a strong positive correlation between the right and left sides regarding SF-IL-1β, indicating that the inflammatory conditions of the TMJ involving IL-1β often are bilateral. The same has been reported in a study by Rooney et al, who found almost identical levels of IL-1β in the SF from the right and left knee joints in RA patients with clinical signs of symmetric joint involvement.

The presence of IL-1β in both plasma and TMJ SF occurs more frequently in RA patients than in non-RA arthritis patients; this finding has also been documented for several other joints. Recent investigations found that IL-1β in the SF from patients with arthritic TMJs showed significant positive correlations to pain (Fig 7) and tenderness to digital palpation and a negative correlation to PPT. It therefore seems that IL-1β is one of the determinants of pain and allodynia/hyperalgesia of the TMJ. There are no results hitherto indicating a difference between patients with specific (eg, RA, psoriatic arthritis, ankylosing spondylitis) compared to unspecific inflammatory joint conditions with respect to IL-1β levels. Detectable levels of IL-1β in the SF of the TMJ are thus present in specific as well as non-specific inflammatory joint diseases or pain conditions, which is in agreement with recent studies by Kubota et al and Imamura et al (unpublished data).

The SF-IL-1β level is also related to radiographic signs of TMJ destruction. Radiographic change, such as erosion of the cortical outline, is a common finding in RA patients (Fig 8). Erosion of the TMJ is related to decreased mandibular mobility, anterior open bite, and difficulty in chewing. The extension of radiographic erosion has been found to be significantly greater in joints with IL-1β than in those without. Bilateral TMJ inflammation with extensive destruction, eg, resulting from RA, may lead to the development of an anterior open bite and frequently restricted joint movement. Patients with an inflammatory disorder of the TMJ and anterior open bite have been found to have high levels of SF-IL-1β, while patients without anterior open bite have shown very low levels of SF-IL-1β. SF-IL-1β might thus be an indicator of an active immune-related inflammatory process of a destructive nature in the joint and perhaps be of clinical diagnostic or prognostic significance. Interleukin-1β released in the synovial tissues of the joint that has diffused into the SF may signal the occurrence of tissue destruction.

Alstergren et al found detectable plasma levels of IL-1β (P-IL-1β) in 79% of 14 patients with rheumatoid factor–positive RA and in 33% of 9 patients with rheumatoid factor negative RA, versus 22% in healthy individuals. Plasma levels of IL-1β have been associated with general inflammatory joint disease activity, as indicated by the Ritchie score, pain, erythrocyte sedimentation rate, and hemoglobin level in patients with RA. The study by Alstergren et al did not support a relationship between circulating IL-1β and TMJ pain, although patients with seropositive RA showed...
significantly higher P-1L-1β than did healthy individuals. Both the extension of erosion and grade of radiographic changes of the TMJ are greater in patients with detectable P-1L-1β than in patients without it. In a study of P-1L-1β and the year-long progression of radiographic changes of joints in the hand and foot, North et al found a correlation between the 2 and suggested that P-1L-1β could be one of the factors responsible for radiographic progression.

The IL-1Ra and IL-1sRII are also present in the SF of TMJs with chronic arthritis (Alstergren et al, unpublished data). Plasma levels of both are reported to be higher in polyarthritic patients than in healthy controls. The IL-1Ra has also been found to correlate positively with indices of disease activity and joint destruction, while IL-1sRII was found to be negatively correlated with indices of joint destruction.

Tumor Necrosis Factor α

Tumor necrosis factor α, or cachectin, is a pleiotropic cytokine that is produced by a number of cell types, including activated macrophages and monocytes. It consists of 157 amino acids and its molecular weight is 17,000 Da. It is not considered to be produced by normal cells, but rather by cells stimulated by, eg, neoplastic or infectious disease. Pretreatment with TNFα causes increased cytotoxicity in monocytes. Tumor necrosis factor α has been detected in the synovium and SF of patients with RA and of patients with other inflammatory diseases, such as psoriatic arthritis, pselvospondylitis, osteoarthritis, and reactive arthritis. It has also been found in the SF of patients with internal derangement and degenerative joint disease of the TMJ as well as in patients with unspecified TMJ disorders. In our clinic, TNFα was detected in 33% of the TMJ SF samples taken from patients with chronic connective tissue diseases involving joints, eg, RA, psoriatic arthropathy, pselvospondylitis, chronic unspecific arthritis, Marfan’s syndrome, osteoarthrosis, and Sjögren syndrome. The SF-TNFα level significantly exceeded the corresponding P-TNFα level. The concentration of circulating TNFα in the blood is usually very low. The frequency of SF samples with detectable levels of TNFα in our clinic is in agreement with the results of Di Giovine et al, who also found detectable levels of TNFα in 30% of knee SF samples from 93 individuals with chronic connective tissue diseases. In a study of patients with a diagnosis of internal derangement of the TMJ without systemic disease, TNFα was detected in 8% of 62 SF samples.

Tumor necrosis factor α is a very potent cytokine that acts as an endogenous mediator of inflammatory immune and host defense reactions. Its effects include activation of macrophages, neutrophils, and eosinophils, as well as induction of prostaglandin and metalloproteinase synthesis. Like IL-1β, TNFα has been shown to induce cartilage cells to degrade proteoglycans and to suppress proteoglycan synthesis. These effects of TNFα on porcine articular cartilage in culture were weaker than those of IL-1, but the effects seemed to be additive. In human cell cultures, a synergistic
action between TNF\(_\alpha\) and IL-1\(\beta\) caused plasminogen activator production, and plasminogen activator may have an important role in the destructive processes in arthritis through its ability to convert plasminogen to plasmin.\(^{131}\) Tumor necrosis factor \(\alpha\) induces macrophages to synthesize IL-1\(\alpha\) as well as IL-1\(\beta\), which in turn causes bone resorption. In RA, TNF\(_\alpha\) stimulates the production of PGE\(_2\) and collagenase in addition to regulating the production of IL-1.\(^{132}\) An immunohistochemical study has shown the presence of TNF\(_\alpha\) in the synovium, synovial membrane lining cells, endothelial cells, and in the cells at the junction between cartilage and pannus. Tumor necrosis factor \(\alpha\) produced by pannus cells thus degrades cartilage by inhibiting proteoglycan synthesis and stimulating production of proteases by macrophages and fibroblasts, as well as by a direct effect on chondrocytes.

There are 2 different TNF\(_\alpha\) receptors: the 55-kDa (p55 TNF-R) and the 75-kDa (p75 TNF-R). They activate different intracellular signaling pathways, although no significant functional difference between the 2 receptor types has yet been revealed. The receptors have been found on most cells.\(^{132,133}\) Naturally occurring soluble fragments of the 2 TNF receptors have been found in human urine, blood serum, and SF. These TNF-binding peptides may function to regulate the bioavailability of TNF in the body. The diverse biologic actions of TNF can be attributed to its ability to activate a variety of genes in multiple target cells, such as c-fos, IL-1, collagenase, and haptoglobin genes. Different signal transduction pathways are likely to be involved in TNF actions, such as prostaglandins.

Studies of TNF\(_\alpha\) in relation to nociception have shown that experimental hyperalgesia can be caused by local or systemic administration of the cytokine.\(^{134,135}\) It may have an indirect effect in receptor sensitization by initiating increased production of IL-1\(\alpha\), which has been shown to cause pain and hyperalgesia.\(^{136,137}\) The production of IL-1\(\beta\) results in up-regulation of the neurotrophic nerve growth factor that mediates inflammatory hyperalgesia.\(^{138}\) Tumor necrosis factor \(\alpha\) was also shown to induce ectopic activity in nociceptive primary afferent fibers in rats when applied directly to the sciatic nerve trunk.\(^{139}\) Synovial fluid TNF\(_\alpha\) levels were shown to be significantly higher in individuals with TMJ pain than in those without such pain.\(^{130}\) In addition, a correlation was shown between SF-TNF\(_\alpha\) levels and tenderness to palpation of the posterior aspect of the TMJ. Either TNF\(_\alpha\) or agents induced by this mediator could thus be possible contributors to pain of the arthritic TMJ.

Of the many cytokines present in the SF of patients with RA, IL-1\(\beta\) and TNF\(_\alpha\) are believed to have particular importance in the inflammatory disease process, and blocking of the production of TNF\(_\alpha\) has been introduced as a new therapeutic approach.\(^{140}\) Monoclonal anti-TNF antibodies have been shown to attenuate collagen-induced arthritis in mice.\(^{132}\) In preliminary clinical trials that included patients with RA, anti-TNF antibodies appear to have a significant effect on disease activity, including reduced C-reactive protein and serum amyloid-A production.\(^{132}\) Therefore, TNF\(_\alpha\) seems to be a possible therapeutic target in patients with RA. Administration of soluble p75 TNF-R has also shown promising results in RA.

**Local Cell-Derived Mediators of Pain and Inflammation**

Eicosanoids are derived from the unsaturated fatty acid arachidonic acid, which is a major lipid component of the cell membrane, and comprise prostaglandins, thromboxanes, and leukotrienes. These substances are short-lived after synthesis and have paracrine effects, ie, they influence the activities of the cells in which they are synthesized as well as those of adjoining cells.\(^{141}\) The synovial membrane is the major source of eicosanoids in SF, and a wide range of stimuli increase the synthesis of eicosanoids.\(^{142}\) Arachidonic acid is liberated by an enzymatic reaction catalyzed by the enzyme phospholipase A\(_2\). Some of the free arachidonic acid is then converted to prostaglandins by the enzyme cyclo-oxygenase (cox).

**Prostaglandin E\(_2\)**

Prostaglandin E\(_2\) (molecular weight 352.5 Da) is released as a result of tissue damage.\(^{143}\) Trauma causes release of the enzyme phospholipase A\(_2\), which causes a breakdown of phospholipids in the cell wall to arachidonic acid, which further breaks down to prostaglandins.\(^{144}\) There are several prostaglandins, which produce various effects. Prostaglandin E\(_2\) is one of the substances responsible for the classic signs of inflammation: heat, redness (vasodilatation), swelling (increased vascular permeability and extravasation of blood cells), and pain (sensitization of C- and A-delta fibers). The endothelial cells of the arterioles appear to be a major source of PGE\(_2\) in mature skeletal muscle.\(^{145}\) It has been reported that release of PGE\(_2\) contributes to the arteriolar vasodilatation and hyperemia that occurs in skeletal muscle during
exercise. The synthesis of prostaglandins is inhibited by glucocorticoids, which block the activity of phospholipase A₂, and by non-steroidal anti-inflammatory drugs (NSAIDs) through inhibition of the COX pathway. Studies of knee joint SF from patients with RA subjected to NSAID therapy have shown that NSAIDs reduce SF levels of prostaglandins.

The actions of the prostaglandins are usually manifested locally around the site of prostaglandin synthesis, where PGE₂ acts as a potent pro-inflammatory, immunoregulatory molecule. Prostaglandin E₂ modulates platelet and leukocyte reactivity via the EP receptor, stimulates bone resorption, promotes sensitization of peripheral nociceptors, and elicits erythema as well as edema. It has been found in the TMJ SF of healthy individuals, while the corresponding level of PGE₂ was about 12% of the muscle dialysate level. PGE₂ was about 12% of the muscle dialysate level in all 3 groups. However, PGE₂ has recently been detected by microdialysis in painful masseter muscle tissue (M-PGE₂) as well as in the plasma of patients with fibromyalgia and local myalgia, and it was not found among the local myalgia patients.

Leukotriene B₄

Leukotriene B₄, which has a molecular weight of 336.5 Da, is released by leukocytes during inflammation. Phospholipids in the cell wall are broken down to arachidonic acid by phospholipase A₂ and are broken down further to leukotrienes by the action of the co-enzyme lipoygenase. Leukotriene B₄ is a potent proinflammatory mediator that induces chemotaxis, degranulation of PMN cells, allergic reactions, and sensitization of peripheral nociceptors. The enzyme 5-lipoxygenase is found in only a few cell types, such as PMN cells, monocytes, macrophages, and mast cells. These cells, however, are present in the synovial membrane, especially during inflammation, and LTB₄ is present in the knee joint SF of patients with RA and in the TMJ SF. LTB₄ has been shown to induce production of the strong proinflammatory substance IL-1β by rheumatoid synovial cells.

Leukotriene B₄ is a potent chemotactic agent, causes adherence of neutrophils to endothelial cells, and stimulates the release of lysosomal enzymes and the generation of superoxide anion in neutrophils. Furthermore, LTB₄ has been shown to induce hyperalgesia in behavioral experiments and to act as an inflammatory mediator in several immune-mediated diseases, eg, RA, psoriasis, and chronic inflammatory bowel disease. The release of leukotrienes is inhibited by gluco-
corticosteroids, which block phospholipase A₂, but not by NSAIDs.

Leukotriene B₄ was found to be undetectable in the TMJ SF of healthy individuals (Alstergren and Kopp, unpublished data), an important finding regarding a potential clinical diagnostic value. In a study by Quinn and Bazan,¹⁵⁴ 95% of the TMJ SF samples from patients with chronic TMJ internal derangement and associated pain had detectable LTB₄ levels. In a study by Prete and Gurakar-Osborne,¹⁵⁶ LTB₄ in knee joint SF was significantly higher in patients with RA than in patients with osteoarthritis and was positively correlated to phospholipase A₂ activity in the SF. Knee joint SF-LTB₄ was detected in all samples after direct aspiration. Leukotriene B₄ has thus been found in SF from the knee joints of patients with several disorders with an inflammatory component,¹⁶⁶ including RA,¹³⁹ as well as in synovial tissue specimens from patients with osteoarthritis.¹⁴²

In the tender masseter muscle of fibromyalgia patients, as well as in patients with myalgia, LTB₄ was detected in samples obtained from both groups by microdialysis (Hedenberg-Magnusson et al, unpublished data). The LTB₄ levels in the tender masseter muscle (M-LTB₄) were higher in fibromyalgia patients than in local myalgia patients and healthy individuals. Hedenberg-Magnusson et al also found detectable plasma levels of LTB₄ (P-LTB₄) most frequently in healthy individuals (56%), less so in local myalgia patients (43%), and least frequently in fibromyalgia patients (18%) (unpublished data). No correlation was found between M-LTB₄ and P-LTB₄, but there was a significant correlation between P-PGE₂ and P-LTB₄, suggesting that these mediators are interrelated and frequently synthesized at the same time. The P-LTB₄ levels from healthy individuals were studied by Takamoto et al¹⁶⁷ and Shindo et al,¹⁶⁸; both studies found detectable levels of this mediator. Present knowledge thus indicates that LTB₄ is detectable in the plasma of healthy individuals.

Conclusion

• There is considerable evidence that neuropeptides take part in the modulation of TMJ arthritis and pain.
• None of the mediators IL-1β, 5-HT, or PGE₂, which are associated with local pain, hyperalgesia/allodynia, and tissue destruction, seem to be detectable in the TMJ SF of healthy individuals, which indicates that they are specifically related to inflammation, suggesting in turn that they might become useful as diagnostic tools or therapeutic targets.
• Peripheral 5-HT and PGE₂ seem to be involved in the pain pathophysiology of fibromyalgia.
• Despite conflicting results, blood levels of 5-HT seem to be a factor involved in the modulation of pain or hyperalgesia/allodynia in the TMJ and orofacial muscles, as well as anxiety, and may become a prognostic factor for local versus systemic treatment.

References


