Pain mediation by prostaglandin E_2 and leukotriene B_4 in the human masseter muscle

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The pathophysiology behind chronic pain from masticatory muscles is unclear. Our hypothesis was that this pain is of inflammatory origin and associated with release of inflammatory mediators. The aim of this study was therefore to investigate the presence of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) in the masseter muscle and plasma and their relation to myalgia. Nineteen patients with fibromyalgia, 19 with local myalgia of the masseter muscle, and 11 healthy individuals were examined with regard to local muscular pain intensity at rest and pressure pain threshold. Inclusion criteria were masseter muscle pain for at least 3 months and masseter muscle tenderness on digital palpation. Samples were obtained from the masseter muscle by microdialysis, and the dialysates and venous blood samples were analyzed with regard to PGE_2 and LTB_4 concentration. Intramuscular levels were found in all groups, with significantly higher levels of LTB_4 in the patients with fibromyalgia, in whom PGE_2 was positively correlated to muscular pain. In the healthy individuals PGE_2 was negatively correlated to pressure pain threshold. In both patient groups but not in the healthy individuals LTB_4 increased during the consecutive samplings. PGE_2 and LTB_4 were detectable in the plasma of all groups. In conclusion, both PGE_2 and LTB_4 were found in the human masseter muscle. LTB_4 levels are increased on needle trauma in patients with myalgia. PGE_2 levels are related to muscular pain in patients with fibromyalgia. Masseter muscle pain therefore seems to be partly of peripheral inflammatory origin in fibromyalgia. □ *Leukotriene B_4; masseter muscle; microdialysis; pain; prostaglandin E_2*

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Symptoms in fibromyalgia—that is, generalized myalgia—include widespread muscle pain, diffuse tenderness to palpation and other modalities of mechanical provocation, and sleep disturbances. Stiffness, muscle fatigue, and numbness are other common symptoms (1). In addition, patients with fibromyalgia frequently have temporomandibular disorders (2, 3). Local myalgia (myofascial pain) of masticatory muscles is characterized by muscle pain, tenderness, and associated restricted range of mandibular movements (4, 5) and is probably the most common pain condition within the field of temporomandibular disorders (6). The pathophysiology and the etiologic mechanisms of both these chronic muscle pain conditions are still unclear.

Inflammation has been proposed to be a cause of muscle pain (7, 8). Inflammatory mediators are released from damaged tissue during inflammation, and several of them sensitize or activate peripheral nociceptors. One such mediator is prostaglandin E_2 (PGE_2), which is produced in mononuclear phagocytes, fibroblasts, and endothelial cells. The production of PGE_2 depends on the enzymes phospholipase A_2 and cyclooxygenase (8). Phospholipase A_2 catalyzes the breakdown of phospholipids in the cell wall to arachidonic acid, which is the substrate for the synthesis of prostaglandins catalyzed by cyclooxygenase (9). PGE_2 is one of the mediators responsible for the classical signs of inflammation: heat, redness (vasodilatation), and swelling (increased vascular permeability and extravasation of blood cells) and pain (sensitization or excitation of nociceptors).

Leukotriene B_4 (LTB_4) is another potent proinflammatory mediator from the arachidonic acid cascade that induces chemotaxis, degranulation of polymorphonuclear cells, allergic reactions, and sensitization of peripheral nociceptors (10–12). LTB_4 depends for its production on the enzyme 5-lipooxygenase, which catalyzes the synthesis of LTB_4 from arachidonic acid. This enzyme has been found in polymorphonuclear cells, monocytes, macrophages, and mast cells (13). These cells are present in increased amounts in the human muscle during inflammation (14).

One common method used to investigate muscle tissue pathophysiology is to examine muscle biopsy specimens. However, the microdialysis technique provides a method to measure biochemical changes in muscle tissue in vivo with significantly less tissue trauma (15). To our knowledge, microdialysis has not been used previously to study PGE_2 or LTB_4 in the human masseter muscle. Our hypothesis was that chronic muscle pain conditions such as fibromyalgia and localized myalgia are the result of an inflammatory process including peripheral release of the inflammatory mediators PGE_2 and LTB_4. The aim of this study was therefore to investigate the presence of PGE_2 and LTB_4 in the masseter muscle and their relation to local pain and hyperalgesia in patients with fibromyalgia and local myalgia.

Materials and methods

Patients

Two patient groups and a control group of healthy individuals were examined. One patient group included 19 patients with fibromyalgia (FM) in accordance with the criteria of the American College of Rheumatology (1), and the other 19 patients had local myalgia (LM)—that is, myofascial pain in the temporomandibular region in accordance with the definition of the American Association of Orofacial Pain (4). The fibromyalgia patients, 17 women and 2 men, had a mean (standard deviation (*s*)) age of 54 (13) years, duration of general symptoms of 15 (6) years, and duration of symptoms from the masticatory muscles of 14 (8) years. The patients with local myalgia, 14 women and 5 men, had a mean (*s*) age of 57 (16) years and duration of symptoms from the masticatory muscles of 11 (9) years. The criteria for inclusion into either group were presence of masseter muscle pain for a period of at least 3 months and muscle tenderness on digital palpation. Patients with systemic inflammatory connective tissue disease (for example, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthropathy), arthrosis of the temporomandibular joint (TMJ), or masticatory muscle pain due to external trauma were excluded. Patients whose pain could be referred to disease in other components of the temporomandibular system (for example, toothache and neuralgia) or patients subjected to any recent treatment of local muscle pain such as physical therapy or occlusal treatment were also excluded. Local infection of the skin over the muscle was considered a contraindication for intramuscular dialysis. All patients were told not to take any non-steroidal anti-inflammatory drug (NSAID) medication within 24 h before the examination.

The healthy individuals—that is, subjects without symptoms or signs of temporomandibular disorders—comprised seven female and four male healthy volunteers with a mean (*s*) age of 42 (10) years for whom the medical history and clinical examination did not show any abnormality. These individuals were subjected to the same examinations as the patients, except for analysis of acute-phase reactants of the blood.

The methods used and the selection of patients were approved by the local ethical committee at Huddinge Hos-pital, Karolinska Institutet, Huddinge, Sweden (151/93).

Clinical examination

All clinical examinations were performed by the same investigator (B. Hedenberg-Magnusson) immediately before the microdialysis.

A 100-mm visual analogue scale with end points marked 'No pain' and 'Worst pain ever experienced' was used to assess the maximum pain intensity in the masseter muscle region at rest during the week before the examination.

The tenderest point of the masseter muscle on the tenderest side was assessed by digital palpation. If palpatory tenderness of a similar degree was found on the right and left side, pressure pain threshold was used to determine the tenderest side. The pressure pain threshold to linearly increasing pressure over this point was assessed with an algometer (Pain Diagnostics and Thermography Co., Great Neck, New York, USA). The flat rubber tip of the algometer has an area of 1.0 cm^2, and the pressure was applied perpendicularly to the skin surface with a pressure rate of 50 kPa/sec.

Blood examination

A total of 25 mL venous blood was collected in three tubes from each patient immediately before the clinical examination. One tube containing sodium citrate was used to determine the erythrocyte sedimentation rate (ESR), and another tube was used for analysis of serum concentration of C-reactive protein (CRP). Still another tube, containing ethylenediamenetetraacetic acid (EDTA), which also was obtained from the healthy individuals, was immediately centrifuged for 10 min at 1500 *g* and 4°C, with 4.5 µL 4 mM indomethacin. The plasma was then immediately frozen until analysis of PGE_2 (P-PGE_2) and LTB_4 (P-LTB_4). Owing to technical difficulties blood samples were not obtained from two FM patients and one LM patient.

Microdialysis

A standard disposable catheter (Venflon 2, BOC Ohmeda AB, Helsingborg, Sweden; diameter, 1.2 mm) was inserted into the tenderest point of the masseter muscle on the tenderest side of the patients and in the center of the masseter muscle belly on the right side of the healthy individuals after skin surface anesthesia with EMLA® cream (lidocaine, 25 mg/g; prilocaine, 25 mg/g; Astra AB, Södertälje, Sweden) for 20 min. This cream provides skin surface anesthesia, but the anesthesia does not progress into the deeper layers of the skin or into underlying muscle tissue (16).

Intramuscular microdialysis (CMA, Carnegie Medicine, Stockholm, Sweden) was then performed to sample PGE_2 and LTB_4 in vivo. The probe used for dialysis (diameter, 0.65 mm) was inserted via the standard catheter into the muscle and had a polycarbonate membrane with a length of 10 mm, a diameter of 0.5 mm, and a molecular cut-off of 20 ku. The probe was perfused with isotonic saline (NaCl, 9 mg/mL; Kabi Pharmacia, Uppsala, Sweden) at a flow rate of 7 µL/min during 30 min for each sample. Three consecutive samples were obtained from all subjects. In the group with healthy individuals a fourth consecutive sample was taken. In the patient groups the muscle dialysis concentrations of the first to the third samples are denoted M1-PGE_2 to M3-PGE_2 and M1-LTB_4 to M3-LTB_4. In the healthy individuals the first to the fourth samples are denoted M1-PGE_2 to M4-PGE_2 and M1-LTB_4 to M4-LTB_4.

The recovery of PGE_2 and LTB_4 of the microdialysis probes was tested in vitro before the clinical samplings. The recovery was calculated as the ratio between the dialysate concentration and the concentration of the substance in the surrounding medium. The mean (*s*) in vitro recovery of the probes was 22% (4%) for PGE_2 and 30% (21%) for LTB_4. The dialysate levels were used for the statistical analyses in this study.

Analyses of PGE_2 and LTB_4

The PGE_2 concentrations were analyzed in duplicate with a commercially available radioimmunoassay (RIA) kit by (NEN Research Products, DuPont Boston, Massachusetts, USA). The PGE_2 antisera has 30% cross-reactivity with PGE_1 but less than 0.9% with other substances. The detection limit is 0.5 pg/mL, and the sensitivity is 4.4 pg/mL. The standard curve range is 0–500 pg/mL.

The concentration of LTB_4 in the samples was determined with a commercially available RIA kit (NEK-037, NEN Life Science Products, Boston, Massachusetts, USA) with a detection limit of 12 pg/mL and a sensitivity of 50 pg/mL. Specificity for LTB_4 is reported by the manufacturer to be 100%, and the cross-reactivity with other substances to be less than 3.6%. The standard curve range is 0–5000 pg/mL.

All samples were not analyzed simultaneously but were processed within 1 month with the same method and by the same laboratory technician.

Statistics

The Kolmogorov–Smirnov test for continuous variables was used to test whether the variables were normally distributed. The PGE_2 and LTB_4 levels were not normally distributed. One-way ANOVA on ranks with the Dunn multiple comparison test as post-hoc test was therefore used to test the significance of differences between the three groups with regard to PGE_2 and LTB_4 levels. The Friedman repeated measures test was used to test the significance of differences in PGE_2 and LTB_4 dialysate levels between the consecutive samplings within each group. The significance of the difference in distribution of samples with detectable and undetectable levels of PGE_2 and LTB_4 between the three groups was tested with the chi-square test. The significance of correlations between the levels of PGE_2 and LTB_4 and the other variables was tested with the Spearman ranked correlation test. A probability level of less than 0.05 was considered significant.

Results

Masseter muscle dialysate and plasma levels

The clinical variables and the distributions of PGE_2 and LTB_4 in the plasma samples with regard to groups are shown in Table 1. The ESR in the patients varied between 3 and 20 mm/h. CRP was more than 10 g/L in two patients with fibromyalgia (27 and 142 g/L, respectively). Fig. 1 shows the distribution of PGE_2 and LTB_4 in the masseter muscle dialysates.

Table 1. Pain variables, plasma levels of PGE_2 and LTB_4, and blood levels of acute-phase reactants ESR and CRP in 19 patients with fibromyalgia, 19 patients with local myalgia of the masseter muscle (tenderest side), and 11 healthy individuals (right side)

	Mean	IQR	*n*
Fibromyalgia			
VAS	60	35	19
PPT	49	25	19
P-PGE_2	3	1	15
P-LTB_4	0	0	17
ESR	12	11	15
CRP	0	0	15
Local myalgia			
VAS	60	40	19
PPT	74	25	19
P-PGE_2	4	4	18
P-LTB_4	0	19	14
ESR	6	4	16
CRP	0	0	16
Healthy individuals			
PPT	147	123	11
P-PGE_2	4	2	10
P-LTB_4	13	35	9

IQR = interquartile range, *n* = number of observations. VAS = Visual analogue scale (0–100 mm) for masseter resting pain, PPT = pressure pain threshold (kPa) over the masseter muscle. P-PGE_2 = plasma concentration of prostaglandin E_2 (pg/mL), P-LTB_4 = plasma concentration of leukotriene B_4 (pg/mL), ESR = erythrocyte sedimentation rate (mm/1 h) and CRP = serum concentration of C-reactive protein (g/L).

Detectable levels of M-PGE_2 and M-LTB_4 were found in the fibromyalgia patients (84% and 58%, respectively), local myalgia patients (95% and 68%, respectively), and in the healthy individuals (100% and 60%, respectively). M1-LTB_4 differed between the groups ($P = 0.040$), and the post-hoc test showed that it was higher in the fibromyalgia patients than in the patients with local myalgia ($P < 0.050$). M-LTB_4 increased from the first to the third sample in both the fibromyalgia and local myalgia groups ($P = 0.011$ and 0.004, respectively) (Fig. 1).

P-PGE_2 was detectable in all individuals, whereas the presence of P-LTB_4 varied between groups. Detectable levels of P-LTB_4 were most frequent in the healthy individuals (56%), second most in the patients with local myalgia (43%), and third in the fibromyalgia patients (18%). The difference in this distribution was statistically significant ($P < 0.001$).

M1-PGE_2 was positively correlated to P-PGE_2 ($r_s = 0.79$, $P = 0.020$, $n = 8$), and M1-LTB_4 was positively correlated to P-LTB_4 ($r_s = 0.83$, $P = 0.020$, $n = 7$) in the healthy individuals. The M1-PGE_2 and M1-LTB_4 levels were higher than the corresponding plasma levels in the fibromyalgia group ($P = 0.022$ and 0.013, respectively), and M1-PGE_2 was higher than P-PGE_2 in the patients

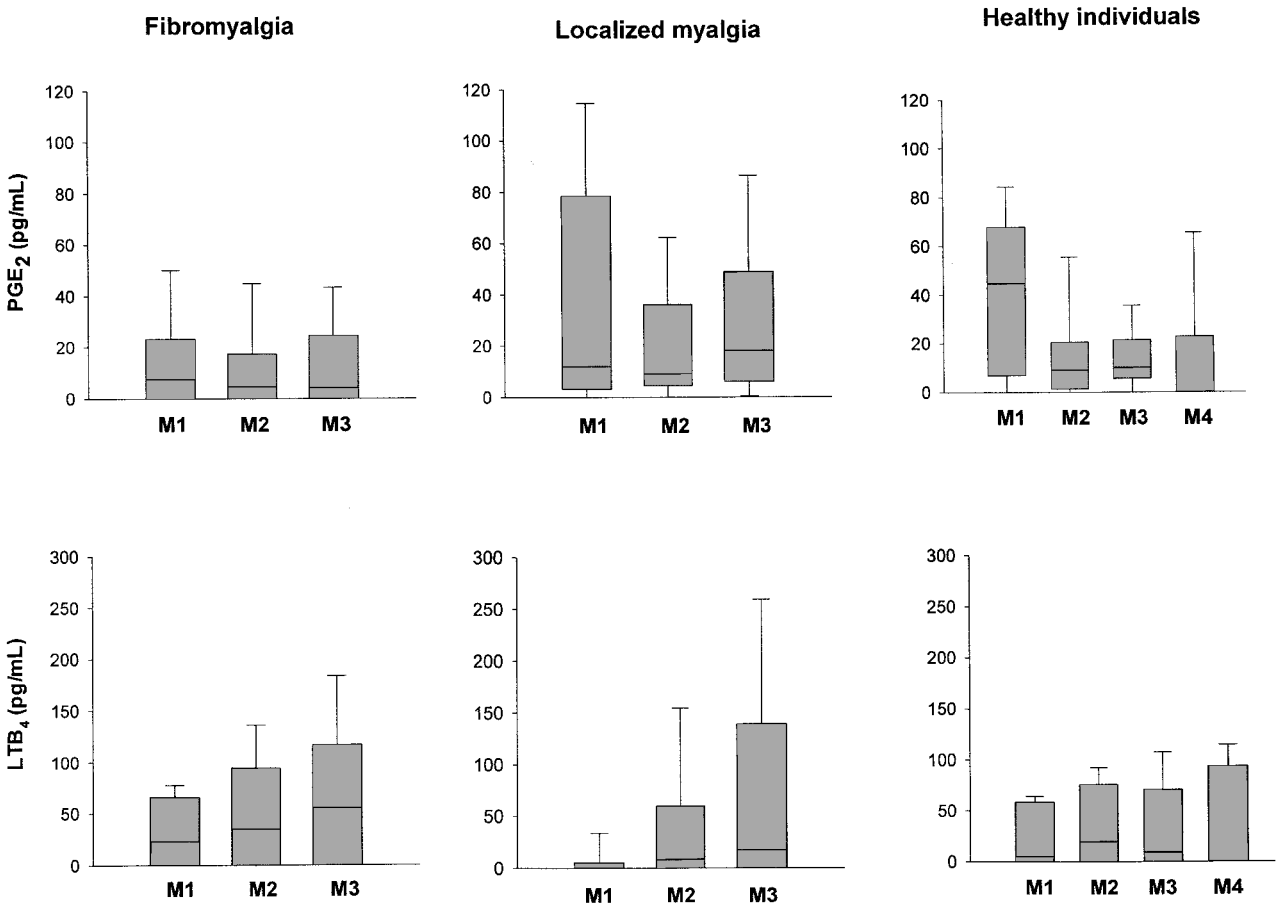

Fig. 1. Box-plots of the distribution of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) concentration (median and 5th, 25th, 50th, 75th, and 95th percentile) in the first (M1) to the third (M3) masseter muscle dialysate in 19 patients with fibromyalgia and in 19 patients with local myalgia and first (M1) to the fourth dialysate (M4) on the right side in 11 healthy individuals. There was an increase of LTB_4 concentration in the two patient groups (fibromyalgia, $P = 0.011$; local myalgia, $P = 0.004$).

with local myalgia ($P = 0.043$) and the healthy individuals ($P = 0.036$).

Relation to pain

The local muscular pain intensity at rest was positively correlated to M1-PGE_2 in the patients with fibromyalgia ($r_s = 0.75$, $P < 0.001$, $n = 18$) (Fig. 2).

In the healthy individuals M1-PGE_2 was negatively correlated to the pressure pain threshold over the masseter muscle ($r_s = -0.87$, $P < 0.001$, $n = 9$) (Fig. 3).

Relation to age and duration of symptoms

Both P-PGE_2 and P-LTB_4 were negatively correlated to age in the patients with local myalgia ($r_s = -0.56$, $P = 0.016$, $n = 18$ and $r_s = -0.56$, $P = 0.038$, $n = 14$, respectively). P-LTB_4 was also negatively correlated to the duration of symptoms from the masticatory muscles in the patients with fibromyalgia ($r_s = -0.62$, $P = 0.008$, $n = 17$) and in the patients with local myalgia ($r_s = -0.56$, $P = 0.038$, $n = 14$).

Relation to acute-phase reactants

M1-LTB_4 was correlated to ESR in the local myalgia group ($r_s = 0.58$, $P = 0.020$, $n = 16$). None of the other mediators was correlated to ESR or CRP.

Discussion

This study indicates that PGE_2 is involved in the mediation of masseter muscle pain in patients with fibromyalgia and in the regulation of the mechanical nociceptive threshold of the masseter muscle in healthy individuals. It also indicates that LTB_4 levels in the masseter muscle increase progressively after needle provocation in chronic muscle pain conditions.

It is assumed that PGE_2 is synthesized and released on

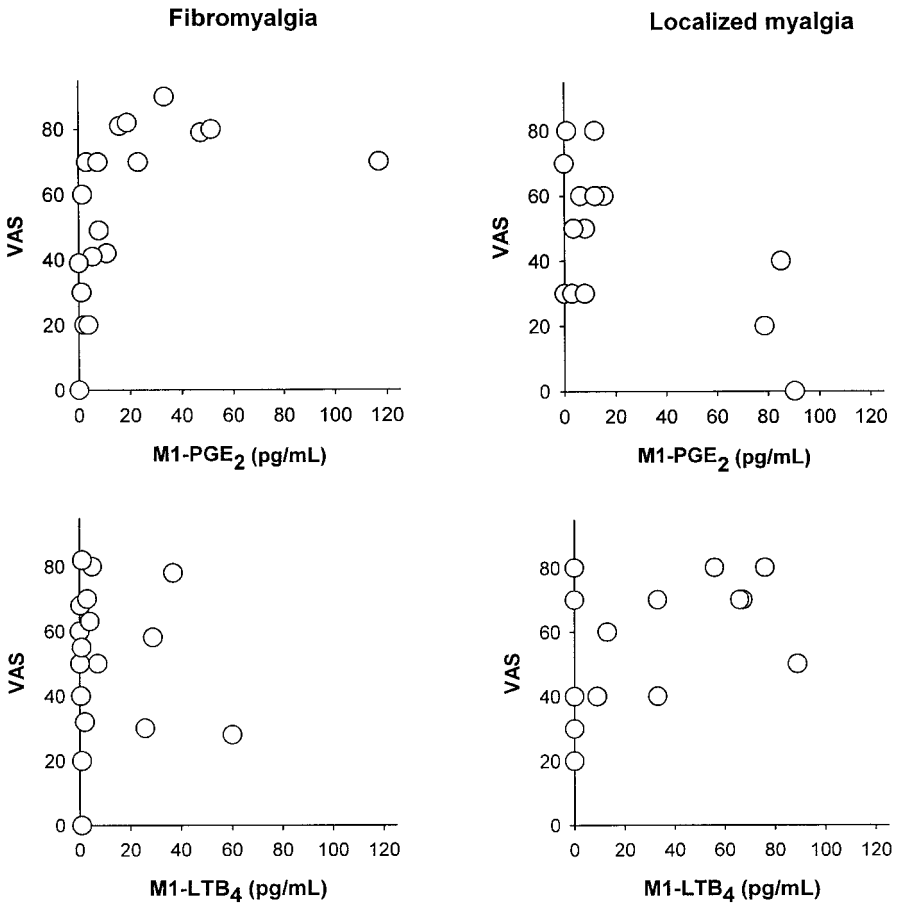

Fig. 2. Scatterplot panels showing the relations between concentration of prostaglandin E_2 (M1-PGE_2) and leukotriene B_4 (M1-LTB_4) in masseter muscle dialysates obtained during the first 30 min of microdialysis versus local muscular pain intensity at rest (VAS) in 18 patients with fibromyalgia and 14 patients with local myalgia of the superficial masseter muscle. There was a positive correlation between M1-PGE_2 and pain in the fibromyalgia group ($r_s = 0.75$, $P < 0.001$).

tissue trauma or during inflammation (17). This study indicates that the trauma caused by puncture of the healthy masseter muscle with a 1.2-mm catheter is severe enough to elicit PGE_2 and LTB_4 production, as observed within 30 min and at a level that is unchanged during 90 min. This is in agreement with the results of Swift et al (17), who found detectable levels of PGE_2 after catheter insertion for microdialysis in the TMJ of the healthy rabbit. Their levels were steady during the whole experiment—that is, for 240 min.

The sources of the high variability of the PGE_2 and LTB_4 levels in the patients are at least of two kinds—that is, basic individual biological variation and methodologic errors. It can be assumed that patients differ with regard to mediator levels if they are correlated to various pain levels. The response to puncture can also be assumed to differ between subjects, including healthy individuals. The interindividual variation among the healthy individuals was most pronounced for M-LTB_4. The microdialysis technique, which has to be adopted in clinical studies, has a disadvantage because absolute tissue levels cannot be expressed, which causes methodological variation. However, it is the only clinically applicable method for in vivo study of human muscle tissue release of pain and inflammatory mediators.

LTB_4 was found in the masseter muscle dialysates at increasing levels during the 90 min of sampling after needle puncture in both patient groups. This is probably a result of the needle trauma. It is remarkable that the synthesis and release was gradually increased on puncture only in the patient groups and not in the healthy individuals. The M1-LTB_4 levels, which were obtained during the first 30 min after puncture, were higher in the fibromyalgia patients than in the patients with localized myalgia and the healthy individuals. This finding suggests that the muscle tissue of patients with fibromyalgia has an increased ability to synthesize LTB_4 which has not been reported previously.

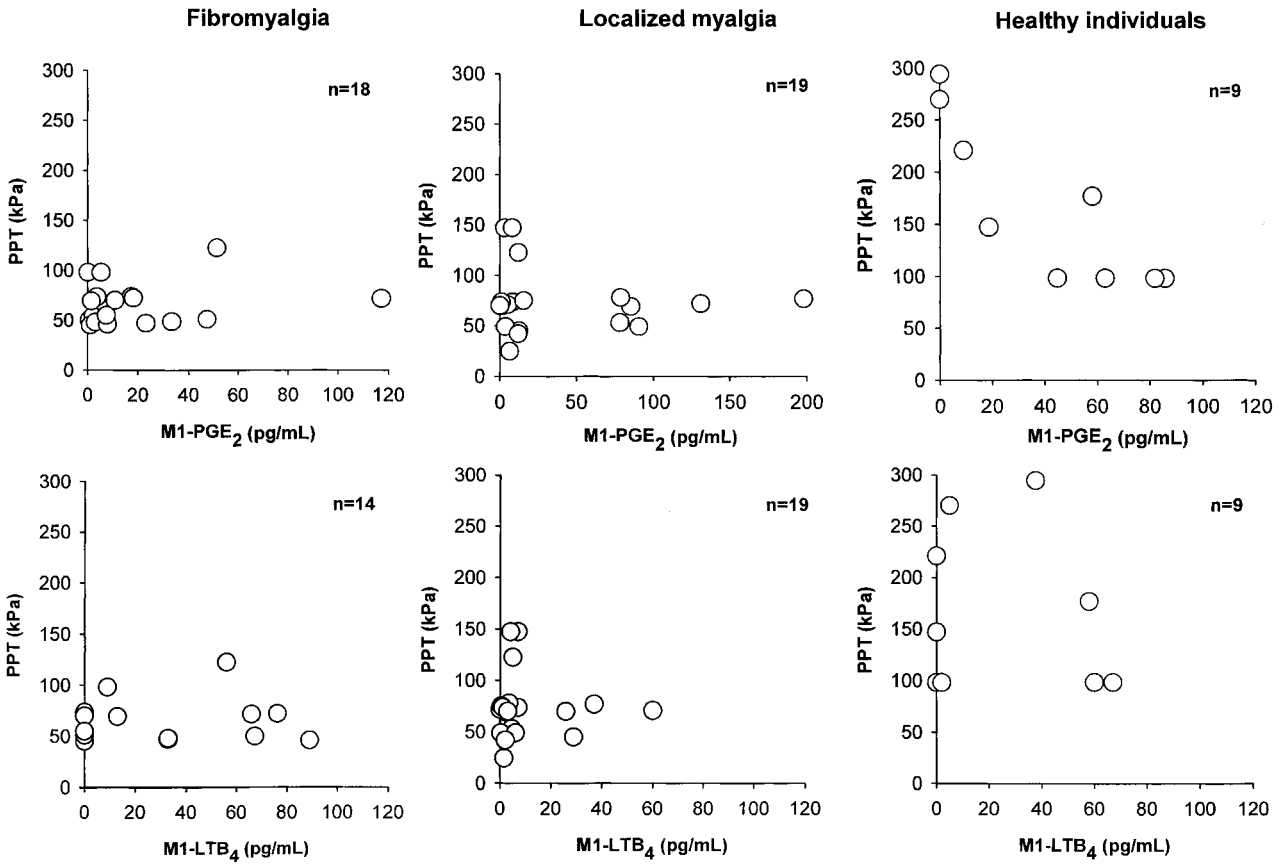

Fig. 3. Scatterplot panels showing the relations between concentration of prostaglandin E_2 (M1-PGE_2) and leukotriene B_4 (M1-LTB_4) in masseter muscle dialysates obtained during the first 30 min of microdialysis versus pressure pain threshold (PPT) over the masseter muscle in patients with fibromyalgia, patients with local myalgia, and healthy individuals. There was a negative correlation between PPT and M1-PGE_2 in the healthy individuals ($r_s = -0.87$, $P < 0.001$). n = number of individuals.

P-PGE_2 was detected in all individuals in this study. This is an unexpected finding, since Davies et al. (18) reported a quick turnover of PGE_2 in plasma. The plasma level was, however, low and close to the detection limit. The frequent presence of P-LTB_4 in the healthy individuals was also a remarkable finding, but P-LTB_4 levels from healthy volunteers in other studies were even higher than in this study (19, 20). Plasma mediator levels were lower than the corresponding masseter muscle dialysate levels, which means that they were even lower than the muscle tissue levels. This indicates a local production of the PGE_2 and LTB_4 in the muscle. The origin of PGE_2 and LTB_4 in plasma is not known, but there were significant correlations between plasma levels and muscle levels (M1-PGE_2 and M1-LTB_4) in the healthy individuals. These findings indicate that the muscle levels are at least partly derived from plasma, since the plasma samples were taken before the microdialysis.

In the patients with fibromyalgia, the local muscle pain intensity at rest in the masseter region immediately before puncture correlated with the first-sample dialysate level of PGE_2. This finding can be due to an upregulation of the synthesis of PGE_2 or its receptors, resulting in continuous activation of peripheral nociceptive fibers. Some patients, however, showed high muscle pain intensity at rest in combination with undetectable PGE_2 levels, which suggests that other mechanisms also are involved. A higher level of 5-hydroxytryptamine (5-HT) was reported in the masseter muscle of patients with fibromyalgia than in healthy individuals (21). It was also reported that high muscle dialysate level of 5-HT was associated with pain and hyperalgesia. Pain mediation by 5-HT could thus partly explain why NSAID lack treatment effect in some patients with chronic orofacial muscle pain conditions (22).

It seems from the results of this study that PGE_2 is involved in the pathogenesis of masseter muscle pain in fibromyalgia, since the level of this mediator after puncture was significantly higher in the fibromyalgia patients with high levels of resting pain than in those with

low levels. The question whether muscle pain in fibromyalgia is of inflammatory nature is not yet answered, but it seems likely that it has an inflammatory component since PGE_2 is involved. This inflammatory component would be local, since neither ESR nor CRP indicated a systemic inflammation.

A fourth microdialysis sample was obtained from the healthy individuals to evaluate the release of PGE_2 and LTB_4 for a longer period. This was not performed in the patient groups, since the prolonged procedure is inconvenient for persons with muscular pain conditions.

As a diagnostic tool the microdialysis method is too complicated for routine use in clinical practice, but the findings indicate that local treatment with anti-inflammatory drugs, such as glucocorticoids, might have an inhibitory effect on local pain in the patients with fibromyalgia. It would be of interest in the future to study this aspect and the influence of neurogenic inflammation—for example, by vasoconstriction due to release of neuropeptide Y from peripheral sympathetic nerve fibers.

With regard to local myalgia, the inflammatory origin of pain is more uncertain. However, the association between LTB_4 in the dialysate from the masseter muscle and ESR indicates an inflammatory component of unknown nature.

The PGE_2 level in the masseter muscle of the healthy individuals was related to the pressure pain threshold over the muscle as assessed immediately before puncture. Those healthy individuals who had the lowest pressure pain thresholds before puncture presented the highest PGE_2 levels during the first 30 min of microdialysis. The finding of high dialysate levels of PGE_2 in normal, non-painful muscle tissue could be due to a high synthetic capacity in some individuals, leading to an increased sensitivity of afferent nerves in the muscle responding with pain on low level of external mechanical pressure. These results show that PGE_2 is synthesized rapidly on needle provocation in human healthy muscle tissue and that it participates in the nociception of the muscle on mechanical provocation.

High P-PGE_2 and P-LTB_4 levels were found in young individuals belonging to the group with local myalgia, whereas short duration of pain was associated with high P-LTB_4 in both patient groups. No such relationships were found with regard to M-PGE_2 or M-LTB_4. These findings indicate that the release into the blood of these mediators decreases with increasing age in patients with localized myalgia and with increasing duration of local pain in both patient categories. They also indicate that plasma and muscle levels of the mediators are independent of each other in patients with myalgia, which is also corroborated by the lack of correlation between dialysate and plasma levels in the patients.

It can be concluded from the results of this study that PGE_2 and LTB_4 can be recovered from the human masseter muscle by microdialysis and that PGE_2 is a putative modulator of resting masseter muscle pain in patients with fibromyalgia. This study also indicates an increased LTB_4 release on puncture trauma in the masseter muscle with chronic myalgia. Peripheral nociceptive muscle pain therefore seems to be partly of local inflammatory nature, at least in patients with fibromyalgia.

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