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R E V I E W

Chronic musculoskeletal pain: review of mechanisms and biochemical biomarkers as assessed by the microdialysis technique

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Abstract: Chronic musculoskeletal pain conditions are multifaceted, and approximately 20% of the adult population lives with severe chronic pain, with a higher prevalence in women and in lower income groups. Chronic pain is influenced by and interacts with physical, emotional, psychological, and social factors, and a biopsychosocial framework is increasingly applied in clinical practice. However, there is still a lack of assessment procedures based on the activated neurobiological pain mechanisms (ie, the biological part of the biopsychosocial model of pain), which may be a necessary step for further optimizing outcomes after treatments for patients with chronic pain. It has been suggested that chronic pain conditions are mainly driven by alterations in the central nervous system with little or no peripheral stimuli or nociception. In contrast, other authors argue that such central alterations are driven by peripheral alterations and nociceptive input. Microdialysis is an in vivo method for studying local tissue alterations and allows for sampling of substances in the interstitium of the muscle, where nociceptor free nerve endings are found close to the muscle fibers. The extracellular matrix plays a key role in physiologic functions of cells, including the primary afferent nociceptor. The present review mainly concerns the results of microdialysis studies and how they can contribute to the understanding of activated peripheral nociceptive and pain mechanisms in humans with chronic pain. The primary aim was to review molecular studies using microdialysis for the investigation of human chronic muscle pain, ie, chronic masticatory muscle pain, chronic trapezius myalgia, chronic whiplash-associated disorders, and chronic widespread pain/fibromyalgia syndrome. Several studies clearly showed elevated levels of serotonin, glutamate, lactate, and pyruvate in localized chronic myalgias and may be potential biomarkers. These results indicate that peripheral muscle alterations are parts of the activated pain mechanisms in common chronic pain conditions. Muscle alterations have been reported in fibromyalgia syndrome and chronic widespread pain, but more studies are needed before definite conclusions can be drawn. For other substances, results are inconclusive across studies and patient groups.

Keywords: algesic, biomarker, human, metabolism, nociception, pain

Background

Living with chronic pain

Chronic musculoskeletal pain conditions are multifaceted, and approximately 20% of the adult population lives with severe chronic pain,[1] with higher prevalence in women and in lower income groups.[2,3] The 12-month prevalence of neck pain in the general population and in the working population is generally between 30%–50% while the 12-month prevalence of activity limiting pain is estimated to 2%–14%.[4–6] Prevalent chronic pain conditions in the population are: neck–shoulder pain, including chronic trapezius myalgia (10%–20%);[7] chronic whiplash-associated disorders (1.5%);[8] and

chronic widespread pain (5%–10%).[9] The prevalence of fibromyalgia syndrome (FMS) – a subgroup of chronic widespread pain – is 1%–4%.[10] Chronic masticatory muscle pain has a prevalence of approximately 10% in the population, with two-thirds being women.

Patients with these pain conditions describe widespread negative consequences: significant pain intensity; depressive symptoms; weakness; sleep-related problems; sick-leave; loss of enjoyment of life in general; and decreased emotional well-being, etc.[11–13] Years lived with disability is a measure of nonfatal health outcomes from diseases and injuries. Pain conditions caused 21% of the years with disability globally.[14] Low back pain was the leading single cause for years lived with disability, followed by major depressive disorder, iron deficiency anemia, and neck pain.[14]

Biopsychosocial model of pain

Acute and chronic pain is influenced by and interacts with physical, emotional, psychological, and social factors, and a biopsychosocial framework is increasingly applied in clinical practice.[15–18] The biopsychosocial framework is seen as increasing the potential for developing better treatments and interventions. However, assessment procedures based on the activated neurobiological pain mechanisms are still lacking (ie, the bio part of the biopsychosocial model of pain); these are necessary to further optimize improvements for patients with chronic pain.

Diagnoses of chronic pain conditions

In clinical practice, physicians are obliged to classify the pain condition using the *International Classification of Diseases, 10th edition* (ICD-10). The diagnoses in the field of pain are vague, reflecting mainly duration and time aspects, such as chronic cervicalgia (chronic pain in the neck). Some studies have attempted to standardize criteria for diagnoses according to ICD-10.[2] Several authors have called attention to the need for diagnoses based on activated pain mechanisms to improve outcomes of treatments.[19,20] To address this need, the first step may be to classify nociceptive, neuropathic, psychogenic, or idiopathic pain even though distinct and validated criteria are lacking, especially for chronic pain.

Generally, chronic muscle pain diagnoses are settled by careful anamnesis and clinical examinations that reveal tender muscle at palpation, corresponding to the reported painful areas. For the diagnostics of chronic masticatory muscle pain, clinicians rely on patient reports, questionnaires, and semi-objective findings. In the Research Diagnostic Criteria for Temporomandibular Disorders, the most recent classifica-

tion for chronic masticatory muscle pain, there are only two chronic masticatory muscle pain diagnoses – myalgia and myofascial pain with referral.[21] The same criteria are used for both diagnoses, but myofascial pain has pain referral as an additional criteria.

The trapezius muscle is often used in morphological, electromyographical, and biochemical studies of myalgia; it is an important clinical structure and is easily accessible for invasive investigations. As trapezius myalgia is not a distinct diagnosis, according to ICD-10, researchers have used various criteria for its definition. Our group has used a standardized process to identify patients with chronic trapezius myalgia.[22]

The following ICD-10 diagnoses have been used to recruit patients with chronic trapezius myalgia: neck myalgia (M 79.1); cervicalgia (M 54); and cervicobrachial syndrome (M 53.1). If there are other simultaneous diagnoses, patients are excluded from the study. The potential research patients are then examined using a standardized clinical examination, using distinct inclusion and exclusion criteria to identify chronic trapezius myalgia and to exclude other conditions.[22] Sometimes, chronic trapezius myalgia is part of a more extensive clinical picture, such as chronic widespread pain and/or FMS.

Chronic whiplash-associated disorders are diagnosed, based upon an anamnesis of trauma and neck pain. The severity is classified using the Quebec Criteria.[23] A revised classification system for all types of neck pain conditions has been presented.[24] A prominent proportion (40%–50%) of those with acute whiplash-associated disorders will develop a chronic pain condition.[25,26]

According to the American College of Rheumatology, the definition of chronic widespread pain is pain that is present in the left and right sides of the body, above and below the waist, and in the axial skeleton, all of which have lasted for at least 3 months.[27] FMS is a subgroup of chronic widespread pain and, additionally, fulfils certain criteria for widespread hyperalgesia (tender point palpation). Comorbidities are common in chronic widespread pain and FMS.[28]

Neurobiological alterations in chronic pain

Acute pain results from a complex integrated series of events at peripheral and central levels. Acute pain mechanisms might not necessarily be valid in intermittent or chronic pain. Pace et al classified chronic pain into: 1) nociceptive/inflammatory pain; and 2) neuropathic pain.[29] There are also differences in the clinical presentation – eg, with respect

to pulse reactions and nausea – of pain located to the skin and to muscles, as pointed out by Mense and Gerwin.[30] The present review mainly discusses nociceptive/inflammatory muscle pain. Chronic pain is more complex than acute pain as extensive plastic changes of the pain transmission system can occur[31,32] and by the modification of psychological and contextual factors.[33–38]

According to the taxonomy of the International Association for the Study of Pain, a nociceptor is defined as: "… a high-threshold sensory receptor of the peripheral somatosensory nervous system that is capable of transducing and encoding noxious stimuli."[39] Small diameter, slowly conducting afferent nerve fibers from skeletal muscle, free nerve endings of group III (Aδ), and group IV afferent (C) fibers transmit nociceptive information and have to be excited to elicit pain.[40] These nociceptors are sensitive to substances released from tissues subject to various types of damages and deformations.[41–43] Nociceptors respond to: single or combinations of stimuli; noxious mechanical stimuli; temperature; and chemical substances, such as H^+, serotonin (5-HT), bradykinin (BKN), glutamate, substance P (SP), nerve growth factor (NGF), adenosine triphosphate (ATP), and potassium (K^+).[42–44] In situations of trauma or inflammation, a combination of substances – the inflammatory "soup" – acts on the nociceptors.[44] The nociceptor is not a static detector as plastic changes can occur, such as peripheral sensitization.[41] A sensitized nociceptor has a lowered threshold for activation and can thus be activated by stimuli that are normally innocuous.[42,43] According to mainly animal studies, H^+, nitrogen oxide (NO), K^+, ATP, NGF, tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), prostaglandin E_2 (PGE$_2$), and glutamate are involved in the process of peripheral sensitization.[43–45] Thus, several substances appear to have both algesic and sensitization effects, eg, 5-HT, glutamate, and K^+.

Studies also report that there are substances with anti-inflammatory roles, eg, certain cytokines (IL-10, IL-4, IL-13, and IL-1 receptor antagonists), fatty acid metabolites (eg, prostaglandins, leukotoxins, resolvins, protectines, endocannabinoids, and N-acylethanolamines [NAEs]) and endogenous opioids.[46–51] The algesic and sensitizing substances act on specific receptors of the nociceptors, eg, G protein-coupled receptors, receptor tyrosine kinases, and ionotropic receptors/ion channels.[52] Sensitization can be accompanied by an increase in the sensitive area.[42] Activation of silent nociceptors has also been reported.[53] Possibly nociceptors, due to induced gene transcription and protein synthesis, can drive pain in the absence of noxious stimuli.[41] In this

review, we will focus on the biochemical muscle alterations associated with chronic muscle pain.

The impulses in the nociceptors are conveyed to second order neurons in the spinal medulla.[54–56] At spinal level, the signal may be modulated, either amplified or attenuated, by different interneuronal pathways. If an adequate excitation of the second-order neuron occurs, the input will be transmitted to different areas of the central nervous system. Different tracts of the spinal cord have the ability to transmit nociceptive information to the central nervous system.[54]

Different structures in the brain – the pain matrix[57–59] – are dynamically involved in processing the nociception and perception of pain.[60] Important areas of pain matrix appear to be anterior cingulate cortex, medial cingulate cortex, insula, prefrontal cortex, and primary and secondary somatosensory cortex (S2). In patients with chronic pain conditions, alterations in connectivity, morphological, and biochemical changes in the pain matrix have been reported.[61–64] The activation of glia cells and neuroglial interactions are now also emerging as mechanisms associated with chronic pain.[65]

Descending supraspinal control of the spinal nociception originates from several brain regions,[60,66] and this control can be altered due to behavioral, emotional, and pathological states.[32,37,60,66–69] A facilitating shift of the descending system has been reported for patients with persistent pain.[32,37,66–70] Alterations in the regulation and activation of inhibitory substances, eg, endorphins, gamma-aminobutyric acid, endocannabinoids, and related substances (eg, NAEs), may also contribute to disturbances in pain inhibition. Due to the above and other processes, patients with chronic pain show a clinical picture associated with signs of hyperexcitability (central sensitization) to nociception and other stimuli. One consequence of central sensitization is that the central nervous system can change pain – eg, amplification, duration, degree, and spatial extent – so that pain no longer directly reflects the peripheral noxious situation.[71]

Several authors have suggested that chronic pain conditions and, in particular, FMS can be driven by the above briefly described alterations in the central nervous system with little or no peripheral stimuli or nociception.[72–74] Other authors have presented data indicating that central alterations are driven by peripheral alterations and nociceptive input.[71,75–77]

Microdialysis technique
Pain is a subjective experience and semi-objective methods like muscle palpation or assessment of pressure pain thresholds have limited sensitivity and often do not correlate

with pain intensity.[78] Microdialysis offers a potential in vivo method for studying local tissue alterations before the substances of interest are diluted and cleared by the circulatory system. The technique allows for continuous sampling of substances in the interstitium of the muscle. It has recently been pointed out that the extracellular matrix plays a key role in physiologic functions of cells, including the primary afferent nociceptor.[79] The trapezius, masseter, vastus lateralis, and gastrocnemius muscles have been investigated in humans with chronic muscle pain conditions. The present review mainly concerns the results of microdialysis studies and how they can contribute to the understanding of activated peripheral nociceptive and pain mechanisms in humans with chronic pain.

The technique is performed in awake subjects and has been described in detail elsewhere.[80–82] Briefly, the skin and the subcutaneous tissues above the muscle under investigation are anesthetized with a local injection (eg, 0.5 mL of Xylocaine® [20 mg/mL]) without adrenaline, and care is taken not to anesthetize the underlying muscle. Thereafter, a thin catheter is implanted in the muscle tissue and slowly perfused with a solution (perfusate) via an infusion pump (Figure 1). The microdialysis catheter consists of a hollow cannula to which the tip (a semi-permeable dialysis membrane) is attached.

During the perfusion molecules in the extracellular space diffuse passively across the membrane and may be collected at the outlet of the microdialysis catheter.[82] This fluid, labeled the dialysate, reflects the composition of the extracellular space.

To determine the concentrations of small molecules (eg, lactate, pyruvate, glutamate, and glucose), a catheter with a 20 kDa cut-off is suitable while a catheter with a 100 kDa cut-off is preferred for determination of larger molecules.[83] Factors that can affect the concentration (recovery) of a substance in the dialysate are: the flow rate; the diffusion rate through the tissue; the area and weight cut-off of the dialysis membrane; and the composition of the perfusate. A low flow rate results in higher relative recovery while a high flow rate results in lower relative recovery. At a very low flow (ie, 0.3 µL/minute), the recovery is near 100%. Detailed descriptions of relative recovery (RR) can be found elsewhere.[81,82,84] Adding dextran or albumin to the perfusate can enhance RR for charged molecules, such as peptides and cytokines. Nutritive muscle blood flow can be estimated by the microdialysis ethanol technique using ^3H_2O instead of ethanol.[85,86] The ratio of ^3H_2O in the dialysate and the perfusate (the outflow-to-inflow ratio) varies inversely with the local blood flow in the tissue.[85,86] The small volumes of dialysate obtained require
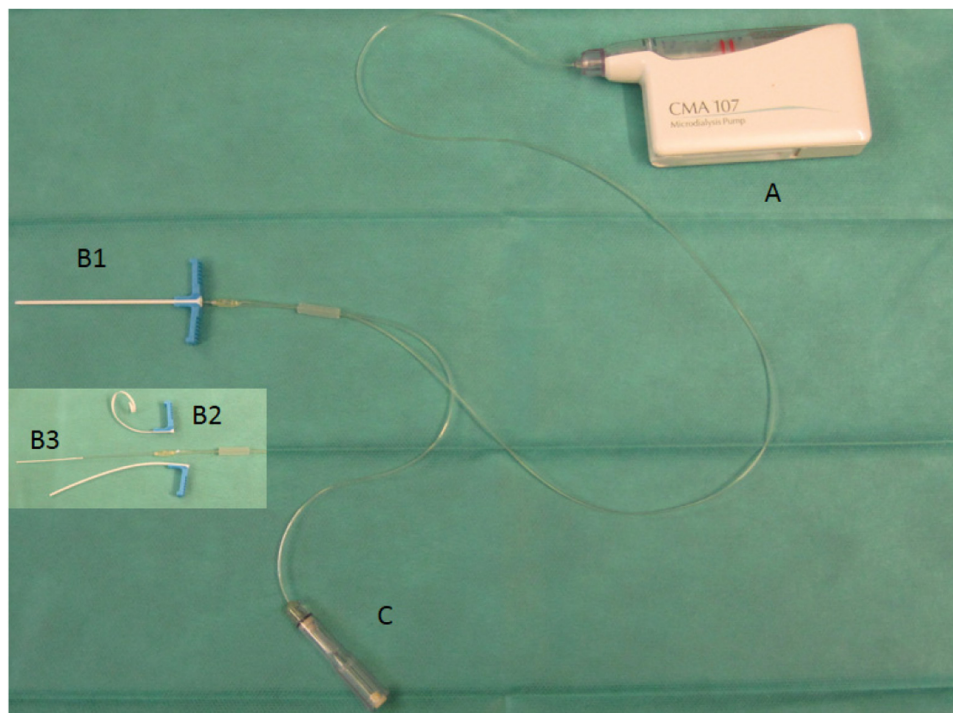

Figure 1 Microdialysis catheter.
Notes: Perfusate is pumped from an infusion pump (**A**) to the microdialysis catheter (**B1**), and the dialysate is collected using a microvial (**C**). When the catheter has been inserted into the muscle tissue, the splittable introducer (with small blue handles, **B2**) is removed, and the catheter (**B3**) is implanted into the tissue during the microdialysis experiment. The catheter (**B3**) consists of a hollow cannula to which the tip (a semi-permeable dialysis membrane [shown in white]) is attached.

both analytical capacity in small volumes and a predetermination of the substances of interest.

Aim

The primary aim is to review molecular studies using microdialysis for the investigation of human chronic muscle pain, ie, chronic trapezius myalgia, chronic masticatory muscle pain, and chronic widespread pain/FMS.

Methods

Criteria for inclusion and exclusion

We have included studies that focused on chronic pain conditions affecting trapezius and masticatory muscles. To be included, the studies had to use microdialysis and had to investigate at least one patient group and one healthy control group. In the present study, we mainly have reported studies with respect to baseline differences between patient groups and control groups.

Search strategy

We have used the following search strategy in PubMed – ([muscle OR pain] AND microdialysis) OR (muscle AND pain AND induced) AND (Humans[MeSH] AND (Clinical Trial[ptyp] OR meta-analysis[ptyp] OR Review[ptyp]) AND English[lang] AND adult[MeSH] AND "last 10 years"[PDat]).[81] From this search, the titles and abstracts were scrutinized. If the articles were relevant and necessary, they were read for further evaluation. We also checked reference lists of these articles and included relevant articles.

Results

Table 1 lists and briefly describes some characteristics for the included studies. Different types of molecules have been investigated – algesic and inflammatory and metabolic and antinociceptive substances. From the studies in Table 1, it has been concluded that an overwhelming proportion of subjects are women. Most studies have investigated the trapezius muscle in local/regional or widespread pain conditions.

Algesic and inflammatory substances

Chronic masseter myalgia

In myofascial temporomandibular disorders, significantly higher glutamate levels were found in the patient group.[87]

Chronic trapezius myalgia

Two out of three studies have reported increased interstitial muscle concentration of glutamate in the trapezius muscle.[22,88,89]

The study that did not report any difference[88] was markedly smaller than the two other studies,[22,89] which may indicate a power problem.

The interstitial muscle concentration of 5-HT in the trapezius was significantly increased in all studies investigating this substance in chronic trapezius myalgia.[22,89–92]

Increased levels of BKN in myofascial pain patients with active trigger points have been reported; the levels were higher in the trapezius (with pain) than in a pain-free distant muscle.[90,91] It should be noted that these studies were small. Two larger studies could not confirm their results.[89,93] In the latter studies, the authors did not investigate active trigger points in the trapezius.

Interstitial levels of kallidin (KAL) have only been investigated in one study and significantly higher levels were found in chronic trapezius myalgia.[93]

No consistent pattern has been reported for the levels of K^+ in chronic trapezius myalgia.[89,94,95]

SP and calcitonin gene-related peptide (CGRP) concentrations were increased in active trigger points in the trapezius, and these levels were higher in the aching trapezius than in a distant pain-free muscle.[90,91]

The two trapezius muscle studies investigating PGE_2 found no increases,[88,96,97] but one of the studies was small,[88] and the other study may have had methodological problems due to the fact that the authors did not compensate for the relative recovery.[96,97]

Several studies of chronic trapezius myalgia have investigated cytokines, but the largest studies have not found elevated levels of cytokines.[89,95] Shah et al found significant differences of cytokines for active trigger points.[90,91] These authors also have compared the levels of cytokines in the myalgic trapezius (trigger points) with a muscle without pain and found higher levels in the aching muscle. These studies, however, have very small sample sizes.

Chronic whiplash-associated disorders

To our best knowledge, only one cohort of patients with chronic whiplash-associated disorders has been investigated and presented in two studies.[93,98] The 5-HT and IL-6 were significantly higher in chronic whiplash-associated disorders than in controls.[98] No group differences were found in the concentrations of K^+, glutamate, BKN, or KAL.[93,98]

FMS

FMS had increased 5-HT levels and leukotriene B4 (LTB4) levels in the masseter muscle.[99,100]

Table 1 Studies of chronic myalgia in humans sorted after muscle investigated

Study	Condition Number of subjects, % W	Substances investigated; flow rate
Masseter		
Ernberg et al, 1999[99]	FMS (n=18), 100% W Localized myalgia of the temporomandibular system (n=17), 76% W Controls (n=10), 60% W	5-HT (corrected for S-5-HT); 7 µL/min
Hedenberg–Magnusson et al, 2001[100]	FMS (n=19), 89% W Localized myalgia of the temporomandibular system (n=19), 74% W Controls (n=11), 64% W	PGE_2 and LTB4; 7 µL/min
Castrillon et al, 2010[87]	Myofascial temporomandibular disorder pain (n=13), 77% W Controls (n=10), 80% W	Glutamate; 2 µL/min
Trapezius		
Flodgren et al, 2005[88]	Chronic shoulder pain (n=9), 100% W Controls (n=9), 100% W	Glutamate and PGE_2; 0.3 µL/min
Flodgren et al, 2010[96]	Chronic trapezius myalgia (n=14), 100% W Controls (compared with controls [n=20] in[97]), 100% W	Lactate, pyruvate, glutamate, PGE_2; 2 µL/min
Rosendal et al, 2004[22]	Chronic trapezius myalgia (n=19), 100% W Controls (n=20), 100% W	Lactate, pyruvate, glutamate, 5-HT; 5 µL/min
Rosendal et al, 2005[95]	Chronic trapezius myalgia (n=19), 100% W Controls (n=20), 100% W	K^+, LDH, IL-6, collagen turnover; 5 µL/min
Gerdle et al, 2008[93]	Chronic trapezius myalgia (n=19), 100% W Chronic whiplash-associated disorders (n=22), 100% W Controls (n=20), 100% W	BKN, KAL; 5 µL/min
Gerdle et al, 2008[98]	Chronic whiplash-associated disorders (n=22), 100% W Controls (n=20), 100% W	Lactate, pyruvate, glutamate, K^+, 5-HT, IL-6; 5 µL/min
Larsson et al, 2008[89]	Chronic trapezius myalgia (n=20), 100% W Controls (n=20), 100% W	Lactate, pyruvate, glutamate, 5-HT, K^+, BKN, GM-CSF, IL-1β, IL-6, IL-8, TNF-α, IL-2, IL-4, IL-5, IL-10; 5 µL/min
Ghafouri et al, 2010[92]	Chronic trapezius myalgia, (n=18), 100% W Controls (n=30), 100% W	5-HT; 5 µL/min
Ghafouri et al, 2011[104]	Chronic trapezius myalgia, (n=11), 100% W Controls (n=11), 100% W	PEA and SEA; 5 µL/min
Sjøgaard et al, 2010[94]	Chronic trapezius myalgia (n=43), 100% W Controls (n=19), 100% W	Lactate, pyruvate, glucose, K^+; 5 µL/min
Shah et al, 2005[91]	Myofascial trapezius pain with active trigger point (n=3) Controls with latent trigger point (n=3) Controls without trigger point (n=3)	BKN, CGRP, SP, IL-1β, TNF-α, 5-HT, norepinephrine, H^+; 1 and 2 µL/min
Shah et al, 2008[90]	Myofascial trapezius pain with active trigger point (n=3) Controls with latent trigger point (n=3) Controls without trigger point (n=3)	BKN, CGRP, SP, IL-1β, TNF-α, IL-6, IL-8, 5-HT, norepinephrine, H^+; 1 and 2 µL/min
Gerdle et al, 2010[102]	FMS (n=19), 100% W Controls (n=19), 100% W	Lactate, pyruvate, glutamate; 0.3 µL/min
Gerdle et al, 2014[103]	Chronic widespread pain (n=17), 100% W (15 of 17 had FMS) Controls (n=24), 100% W	Lactate, pyruvate, glutamate, glucose, glycerol; 5 µL/min
Ghafouri et al, 2011[104]	Chronic trapezius myalgia (n=11) 100% W Controls (n=11) 100% W	PEA and SEA; 5 µL/min
Ghafouri et al, 2013[105]	Chronic trapezius myalgia (n=34) 100% W Chronic widespread pain (n=18) 100% W Controls (n=24) 100% W	PEA and SEA; 5 µL/min
Vastus lateralis		
McIver et al, 2006[101]	FMS (n=8), 100% W Controls (n=8), 100% W	Lactate; 2 µL/min

Note: *Low flow rate associated with 100% relative recovery was defined as 0.3 µL/minute.
Abbreviations: W, women; FMS, fibromyalgia syndrome; 5-HT, serotonin; PGE_2, prostaglandin E_2; LTB4, leukotriene B_4; K^+, potassium; LDH, lactate dehydrogenase; BKN, bradykinin; KAL, kallidin; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor alpha; IL-2, interleukin 2; IL-4, interleukin 4; IL-5, interleukin 5; IL-10, interleukin 10; PEA, *N*-palmitoylethanolamine; SEA, *N*-stearoylethanolamine; CGRP, calcitonin gene-related peptide; SP, substance P; S-5-HT, Serum-5-HT.

Metabolic substances and blood flow

Chronic trapezius myalgia

Most studies concerning chronic trapezius myalgia reported increases in the interstitial concentrations of lactate.[22,89,94,96,97] Methodological problems may exist in the studies conducted by Flodgren et al[96,97] with respect to relative recovery as mentioned previously.[81]

Also, for pyruvate, a majority of chronic trapezius myalgia studies found increased interstitial concentrations of pyruvate.[22,89,94,96,97] Two studies found no significant group differences for pyruvate.[96,97]

Chronic whiplash-associated disorders

No significant alterations were found in the interstitial concentrations of lactate and pyruvate of the trapezius muscle in patients with chronic whiplash-associated disorders.[98]

Chronic widespread pain including FMS

Three studies investigated chronic widespread pain/FMS patients with respect to metabolic substances. The vastus lateralis muscle – not reported if painful at rest or with altered local pain thresholds – was investigated in patients with FMS, and no differences in concentrations of lactate compared to controls were found.[101] Two larger studies have investigated the trapezius muscle in chronic widespread pain/FMS.[102,103] In the first study, significantly higher interstitial concentrations of lactate and pyruvate were found,[102] while the other study reported significantly increased interstitial muscle concentrations of lactate in chronic widespread pain patients.[103] The latter study also investigated concentrations of glucose and glycerol, but no group differences were found. Hence, the metabolic studies indicate alterations in lactate metabolism in the trapezius with chronic widespread pain/FMS.

Blood flow in chronic myalgia

Despite most chronic trapezius myalgia studies reporting significant increases in lactate, there is not a consistent picture with respect to blood flow in this pain condition or in chronic widespread pain/FMS using the microdialysis technique.[22,89,94,103]

Antinociceptive substances

Few studies have targeted muscle levels of pain-inhibitory signalling molecules. The N-acylethanolamines (NAEs) is a family of endogenous lipid mediators that are involved in the regulation of inflammation and pain.[49] The most thoroughly investigated of the NAEs is N-arachidonoylethanolamine (anandamide [AEA]), which interacts with cannabinoid receptors and also can target transient receptor potential (vanilloid-1) receptors. Other examples of NAEs are N-palmitoylethanolamine (PEA), N-stearoylethanolamine (SEA), and N-oleoylethanolamine. Two studies have investigated the interstitial muscle levels of NAEs and reported increased levels of SEA and PEA in chronic trapezius myalgia compared to controls.[104,105] The results for chronic widespread pain indicated alterations in mobilization of PEA and SEA as a consequence of exercise compared to controls.[105] Hence, patients with chronic neck pain showed significantly higher levels of these substances postexercise than patients with chronic widespread pain.

Omics methods for analyzing biochemical milieu of muscle interstitium

Recently, microdialysis studies have applied omics methods to investigate the interstitium of human myalgic muscle. Both proteomic and metabolomics studies have been published indicating that these methods can be used to identify new interesting substances and processes associated with chronic myalgia.[106,107] The proteomic study found that prominent proportions of the 97 identified proteins were at least two-fold higher or lower in chronic trapezius myalgia and in chronic widespread pain (50% and 30%, respectively).[106] Several of the identified proteins are known to be involved in nociceptive/inflammatory processes, eg, creatine kinase, NGF, carbonic anhydrase, myoglobin, fatty acid binding protein, and actin aortic smooth muscle.

Discussion

Important conclusions based on the identified studies were:

- Several studies of local/regional muscle pain conditions indicated that 5-HT, glutamate, lactate, and pyruvate were increased in patients with chronic myalgia.
- Studies of chronic widespread pain/FMS have reported peripheral muscle alterations in 5-HT and lactate.
- Other investigated substances have only been reported in single studies or with conflicting results.

5-HT

All studies investigating 5-HT (Table 1) found significantly increased levels of this substance in the whole spectrum of severity of chronic trapezius myalgia, including chronic whiplash-associated disorders and in chronic masticatory muscle pain. The 5-HT is involved both in central and

peripheral processes of nociception and hyperalgesia.[108] Whether 5-HT has an analgesic or hyperalgesic action depends on the cell type and the type of receptor it targets. In the periphery, 5-HT is released from platelets and mast cells due to tissue damage,[42] and it sensitizes afferent nerve fibers.[108–111] The intramuscular administration of 5-HT into human muscle induces pain.[112] There are several receptor classes and subclasses of 5-HT and especially the 5-HT$_3$ receptor has attracted interest. But also 5-HT$_1$, 5-HT$_2$, and 5-HT$_7$ have been discussed with respect to myalgia. In conclusion, the available microdialysis studies of chronic myalgia indicate that 5-HT is involved in chronic muscle pain.

Glutamate

Glutamate, a pain modulator in the human central nervous system, acts via the N-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA), and kainate receptors[113–115] and appears to influence peripheral pain processing.[116–123] These receptors are also present on peripheral nerve terminals. Glutamate is released from peripheral afferent nerve terminals.[124] Injections of glutamate increase pain intensity.[120,125]

Three studies of chronic myalgia reported significantly higher interstitial concentrations of glutamate,[22,87,89] while a fourth small study found no significant group difference.[88] A possible difference between subjects of these studies may contribute to the inconsistent glutamate finding. Pain history, present pain, and clinical muscular neck status of the subjects were sparsely presented in the fourth study.[88] It has been suggested that the elevation of interstitial muscle glutamate alters pain sensitivity in healthy humans and is associated with pain symptoms in some chronic noninflammatory muscle pain conditions.[126] The available microdialysis studies mainly support the conclusions of that review.

Interstitial concentrations of glutamate were not increased in the trapezius of chronic whiplash-associated disorders.[98] Conflicting results with respect to glutamate have been reported in patients with chronic widespread pain/FMS.[102,103] One difference between chronic trapezius myalgia and the two other conditions might be the more widespread hyperalgesia in chronic whiplash-associated disorders and chronic widespread pain/FMS.[127]

Lactate and pyruvate

The majority of studies concerning chronic trapezius myalgia have reported increases in the metabolites lactate and pyruvate. Often-suggested explanations for the higher concentration of lactate are insufficient oxygen supply and anaerobic conditions, but there is not a consistent picture with respect to blood flow in this pain condition or in chronic widespread pain/FMS using microdialysis or other techniques.[22,89,94,103,128–130] Reduction in tissue oxygenation in FMS[131] and in chronic trapezius myalgia[132] may result in higher pyruvate and higher lactate concentrations, due to a shift toward an anaerobic state. Hence, alterations in oxygen supply cannot be excluded as a factor of importance, but other factors can also contribute, eg, decreased fitness level, increased muscle activation, and/or damaged mitochondria. Changes in the lactate-pyruvate metabolism may result in higher pyruvate levels.[133] A lower fitness level is another explanation as this means more frequent reliance on anaerobic metabolism. However, it is unknown if a general deconditioning in the two pain conditions of chronic trapezius myalgia and chronic widespread pain/FMS involves the postural trapezius. The aerobic capacity of a muscle is largely determined by the number of mitochondria and their enzymes.[134] The mitochondrial density increases as a result of exercise, and an increased density results in enhanced aerobic capacity.[135] Both in FMS and chronic trapezius myalgia, the trapezius muscle fibers can appear with alterations in mitochondrial content and distribution.[131,132,136,137]

Lactate participates in the detection of exercise stress before tissue damage occurs and can be exchanged rapidly among tissue compartments where it may be oxidized as a fuel or reconverted to form pyruvate or glucose.[138–140] Lactic acid is dissociated at body pH.[141] Inflamed as well as ischemic tissues show lowered pH.[142,143] Acid-sensing ion channels (ASIC) are considered transducers for nociception and mechanosensation.[138] Lactate appears to facilitate the response of acid-sensing ion channels-3 (ASIC-3) to low pH.[138] Lactate exposure leads to reactive oxygen species (ROS) generation and can be harmful or beneficial, depending on the level or persistence of ROS.[144–146] Hence, one possibility is that the increased lactate induces, eg, ROS, which directly interacts with the nociceptive system or, in turn, activates the algesics.[147] Other possible receptors for low pH are transient receptor potential cation channel subfamily V member 1 (TRPV1), transient receptor potential cation channel subfamily V member 4 (TRPV4), short transient receptor potential channel 4 (TRPC4) and short transient receptor potential channel 5 (TRPC5).[142,143,148]

To summarize, most studies of myalgic trapezius muscles show significant increases in interstitial levels of lactate and pyruvate.

Conflicting or inconclusive results for other investigated substances

SP and CGRP

Two small studies of active trigger points of myofascial pain reported significant increases in SP and CGRP.[90,91] Both SP and CGRP are involved as mediators of neurogenic inflammation and hyperalgesia.[149] SP stimulation can result in the production of inflammatory mediators and proinflammatory cytokines,[149,150] which – in turn – can stimulate the production of SP.[151] More studies investigating the peripheral roles of SP and CGRP in chronic myalgia are needed.

K+

When reviewing the literature, no consistent pattern of increased K^+ was found in patients with chronic trapezius myalgia[89,94,95] or with chronic whiplash-associated disorders. Increased interstitial K^+ levels may be related to muscle pain.[152] In acute tissue trauma, K^+ is a component of the "inflammatory soup" and is characterized as an algesic substance.[153] The role of K^+ in chronic myalgia pain is unclear at the moment.

BKN and KAL

BKN and KAL are kinins that are produced by the kallikrein-mediated enzymatic cleavage of kininogen.[43,154,155] The two kinins have been suggested as algesic kinins involved in muscle pain. Animal studies have shown that BKN can have both algogenic and sensitizing functions with respect to the nociceptors.[154,156] Four studies investigating the interstitial concentrations of BKN and/or KAL were found.[89–91,93] The difference in results with respect to BKN among these studies could be because the alterations in BKN might be very localized, ie, in the trigger points,[90,91] and not generally found in the aching trapezius muscle.[89,93] KAL was only investigated in one study and increased in chronic trapezius myalgia but not in the trapezius of chronic whiplash-associated disorders.[93] Clearly, more pathophysiological in vivo studies are necessary to understand the roles of BKN and KAL for nociception and pain in patients with chronic muscle pain.

Prostaglandins and leukotrienes

Eicosanoids are substances produced in various cell types in response to tissue trauma by the breakdown of arachidonic acid in the cell wall, leading to the formation of prostaglandins and leukotrienes. Both these substances are suggested as potential pain mediators in chronic myalgia. PGE_2 has been of particular interest for inflammatory pain and in delayed onset muscle soreness. The probable role of PGE_2 is as a sensitizer of nociceptors. However, we only found two studies investigating this substance in chronic myalgia and neither found increased levels.[96,100] In patients with FMS, one study reported significantly elevated levels of LTB4 in the masseter muscle.[100] In conclusion, the roles of prostaglandins and leukotrienes in chronic myalgia are unclear; more studies of these substances may be needed.

Cytokines

Several direct and indirect pathways link cytokines with nociception and hyperalgesia.[43,157,158] Four studies investigated cytokines in chronic trapezius myalgia, but significant differences were only found for active trigger points.[90,91] Larger studies have not found elevated levels of cytokines.[89,95] The latter studies might have had technical problems due to the catheters used. On the other hand, increased IL-6 was found in the chronic whiplash-associated disorders study using the same type of custom-made catheters.[98] The study by Helmy et al indicates the need to carefully review methodology aspects when determining the levels of cytokines.[159]

NAEs

Knowledge about peripheral antinociceptive processes in human chronic myalgia is very insufficient. Two studies, partly using the same subjects, found significant increases in two NAEs in the chronic trapezius myalgia and alterations in the mobilization of these two substances in chronic widespread pain.[104,105] Larger studies also focusing on other NAEs and other myalgic muscles are necessary before any definite conclusions can be drawn with respect to chronic myalgia.

Future perspectives

Several of the microdialysis studies identified in the present review have few subjects, which entails the risk of low power and inconclusive results. Results also need to be confirmed by independent groups to be valid. Patient cohorts need to be better characterized with respect to inclusion and exclusion criteria, diagnoses and their criteria, pain severity, psychological stress, work participation, and sick leave. There is also a need to better characterize the physical fitness level of the subject and, if possible, the daily activity pattern of the investigated muscle, eg, with respect to working tasks. A global measure of the severity of the investigated pain condition can be an advantage when comparing studies.

The subjects of microdialysis studies are nearly exclusively women, which reasonably is a consequence of the higher prevalence of the most chronic myalgias in women.

Hence, future studies ought to include men and compare mediator levels between sexes.

Another factor to consider may be diurnal variations in some biomarker levels, which should be controlled for by taking samples at the same time of the day. Tissue fluid levels of biomarkers may also be influenced by factors such as age, sex, menstrual cycle, food intake, and body mass, which must be considered in future studies.

Reasonably, a number of substances can be released and altered in the milieus of the nociceptors. Only a limited number of substances have been investigated using the microdialysis technique and are reviewed in the present review. Hence, other substances could be of importance for the genesis and maintenance of chronic muscle pain. Even though there are a few studies concerning NAEs and endocannabinoids there is a surprising gap concerning more well-known inhibitory endogenous substances such as opioids. There is also a lack of studies concerning anti-inflammatory cytokines and other fatty acid metabolites, eg, prostaglandins, leukotoxins, resolvins, and protectins. Hitherto, the limited volumes of dialysate obtained have made it impossible to analyze more than certain single biomarkers. The development of more sensitive methods that can combine analyses of several pain-related substances will probably improve knowledge about peripheral mechanisms behind chronic myalgia. Methods from the omics field using nano liquid chromatography or capillary electrophoresis in combination with mass spectrometry may be important tools to understand the complex relationship of the substances involved in chronic myalgia. Pain research will increasingly turn to this powerful technology that could hopefully provide novel and exciting insights in the field.

The development of omics methods also emphasizes the need to use multivariate data analysis. The omics methods are capable of measuring hundreds or even thousands of substances simultaneously. Traditional statistical methods assume markedly higher numbers of subjects than dependent variables, but the omics methods produce the opposite, ie, markedly more dependent and intercorrelated variables than subjects. Hence, using partial least squares regression analyses, cluster analysis, and principal component analysis may represent a complementary approach to the traditional statistical methods for a better understanding of the complex biochemical alterations that may occur in chronic musculoskeletal pain and when sensitization processes may be present in the group with myalgia but not in healthy controls. In future larger studies, it is important to investigate group differences. These studies also need to consider that when comparing a muscle with chronically sensitized nociceptors with a healthy muscle, no group differences may exist for a substance – even though correlations may exist between the substance under consideration and habitual pain intensities and pain thresholds in the patient group.

Conclusion

After reviewing the literature, it can be concluded that several studies clearly show elevated levels of 5-HT, glutamate, lactate, and pyruvate in localized chronic myalgias. Several alterations in metabolites and algesics have been reported in chronic widespread pain/FMS, but more studies using different designs of the microdialysis sessions are needed before more definite conclusions can be drawn about the interstitial muscle milieu in these pain conditions. For other substances, results are inconclusive across studies and patient groups.

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