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MYOFASCIAL PAIN RESEARCH

Uncovering the biochemical milieu of myofascial trigger points using in vivo microdialysis: An application of muscle pain concepts to myofascial pain syndrome

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Summary This article discusses muscle pain concepts in the context of myofascial pain syndrome (MPS) and summarizes microdialysis studies that have surveyed the biochemical basis of this musculoskeletal pain condition. Though MPS is a common type of non-articular pain, its pathophysiology is only beginning to be understood due to its enormous complexity. MPS is characterized by the presence of myofascial trigger points (MTrPs), which are defined as hyperirritable nodules located within a taut band of skeletal muscle. MTrPs may be active (spontaneously painful and symptomatic) or latent (non-spontaneously painful). Painful MTrPs activate muscle nociceptors that, upon sustained noxious stimulation, initiate motor and sensory changes in the peripheral and central nervous systems. This process is called sensitization. In order to investigate the peripheral factors that influence the sensitization process, a microdialysis technique was developed to quantitatively measure the biochemical milieu of skeletal muscle. Biochemical differences were found between active and latent MTrPs, as well as in comparison with healthy muscle tissue. In this paper we relate the findings of elevated levels of sensitizing substances within painful muscle to the current theoretical framework of muscle pain and MTrP development.

Introduction

Myofascial pain syndrome (MPS) is a major progenitor of non-articular local musculoskeletal pain and

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tenderness that affects every age group, and is commonly recognized as "muscle knots" (Kao et al., 2007). MPS has been associated with numerous pain conditions including radiculopathies, joint dysfunction, disk pathology, tendonitis, craniomandibular dysfunction, migraines, tension-type headaches, carpal tunnel syndrome, computer-related disorders, whiplash-associated disorders, spinal dysfunction, and pelvic pain and other urologic syndromes, post-herpetic neuralgia, and complex regional pain syndrome (Borg-Stein and Simons, 2002).

Characterized by a physical finding and symptom cluster, MPS lacked demonstrable pathology and attracted little research attention until recently. Although the specific pathophysiological basis of MTrP development and symptomatology is unknown, several promising lines of scientific study (i.e. histological, neurophysiological, biochemical, and somatosensory) have revealed objective abnormalities (Reitinger et al., 1996; Windisch et al., 1999; Mense, 2003; Shah et al., 2005, 2008; Kuan et al., 2007; Niddam et al., 2007). These findings suggest that myofascial pain is a complex form of neuromuscular dysfunction consisting of motor and sensory abnormalities involving both the peripheral and central nervous systems. MPS is not to be confused with fibromyalgia syndrome, which is ascribed to a collection of complaints including chronic widespread pain, accompanied by tactile allodynia, fatigue, sleep disturbance, and psychological distress (Wolfe et al., 1990).

Historical terminology

Since muscle pain and particularly MPS is described as diffuse and can often refer to deep somatic tissue, terminology regarding muscle pain has been controversial. The first descriptions of "muscular rheumatism" were made by a French physician, de Baillou, in the 16th century (Stockman, 1904). Later observations by the British physician Balfour in 1816 described nodular tumors and thickenings (Stockman, 1904). In the early 20th century, literature on muscle pain used several terms that described similar conditions: myalgic spots, fibrositis, and myogeloses—all used to identify painful areas of hardened muscle. In 1940, Steindler introduced the term "trigger point" in a series of papers on gluteal myofascial pain (Steindler and Luck, 1938; Steindler, 1940). In the 1950s, Travell and Rinzler observed that fascia referred pain patterns appeared similar to underlying muscle referred pain patterns, leading them to alter their terminology to "myofascial pain" to highlight the interaction between these elements (Travell and Rinzler, 1952; Travell, 1968).

Myofascial trigger point diagnostic criteria

Myofascial pain is identified by palpating skeletal muscle for myofascial trigger points (MTrPs). A MTrP is classically defined by Simons and Travell as "a hyperirritable spot in skeletal muscle that is associated with a hypersensitive palpable nodule in a taut band" (Simons et al., 1999). Figure 1 illustrates the trigger point complex. MTrPs are sensitive to pressure and are stiffer than surrounding tissue. Palpation of a MTrP produces local pain and sensitivity, as well as diffuse and referred pain patterns away from the affected area. Trigger points are classified in two ways. An "active" MTrP will elicit pain locally and at some distance from the MTrP and generate seemingly spontaneous pain complaints. "Latent" MTrPs show similar physical characteristics as active MTrPs only when palpated, and can cause muscle dysfunction. Both active and latent MTrPs are responsible for motor dysfunction, such as stiffness and restricted range of motion, as well as autonomic dysfunction, though to a lesser

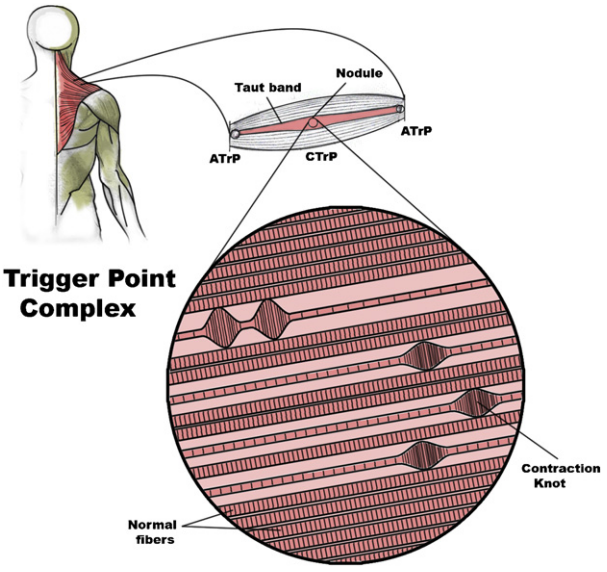

Figure 1 Schematic of a trigger point complex. CTrP identifies the central trigger point that is found in the endplate zone and contains numerous contraction knots and electrically active loci among normal fibers. A taut band of muscle fibers extends from the trigger point to the attachment (ATrP) at each end of the involved fiber. (Adapted from Simons, D.G., Travell, J.G. Myofascial Pain and Dysfunction: The Trigger Point Manual, vol. 1; second ed., and Användare: Chrizz.)

degree for latent MTrPs (Travell and Simons, 1983; Mense and Simons, 2001). Healthy muscle tissue does not contain MTrPs. The cause of MPS and the development of active MTrPs are often linked to postural problems, muscle overload and overwork fatigue, as well as emotional stress (Mense and Simons, 2001). While the pain associated with MTrPs sometimes resolves without intervention, the mechanism(s) that underlies this change is not fully understood. Clinical observations support that MPS may become chronic if perpetuating factors are present (Edwards, 2005).

One of the most important characteristics found in clinical examination that confirms the presence of a MTrP is the local twitch response (LTR). Strumming or snapping the taut band in a direction perpendicular to muscle fibers produces a quick contraction in the muscle fibers of the taut band. The origin of the LTR is not yet fully understood, though this response may be due to altered sensory spinal processing resulting from sensitized peripheral mechanical nociceptors (Mense and Simons, 2001).

There are several widely accepted treatment methods for MPS and soft tissue pain, and although there is no single accepted standard of care, dry needling is an effective non-pharmacologic treatment that is thought to induce changes in the MTrP's surrounding fascia (Hong, 1994; Langevin, 2008). In this technique, a fine gauge acupuncture needle is inserted into the MTrP and manipulated until several LTRs are elicited. Direct mechanical stimulation through dry needling may induce connective tissue remodeling and plasticity to interrupt the pathogenic mechanism of MTrPs. Other needling therapies, such as superficial dry needling, as well as manual therapies including massage and stretching, are targeted at releasing contractured muscle fibers and surrounding connective tissue (Mense and Simons, 2001).

Motor abnormalities of the myofascial trigger point

Electrophysiology

The pathophysiology of MTrPs is incompletely understood. MTrPs are hypothesized to be a result of physiological dysfunction within the neuromuscular junction and the surrounding connective tissue. There is evidence that motor endplates of neurons terminating at the muscle fibers of a MTrP have abnormal activity. Electromyographic studies have revealed spontaneous electrical activity (SEA) generated at MTrP loci that was not seen in surrounding tissue (Hubbard and Berkoff, 1993). Originally attributed to dysfunctional muscle spindles, the excess electrical activity was later identified as an increase in miniature endplate potentials and excessive acetylcholine (ACh) release (Hubbard and Berkoff, 1993). Figure 2 displays a comparison of endplate potentials and noise. The dysfunctional motor endplates within the MTrP tissue is one piece of evidence that may explain the taut band phenomenon. Wang and Yu (2000) and others have hypothesized that the excessive ACh release perpetuates a contracture of associated muscle fibers, resulting in increased metabolic demands in the muscle (Wang and Yu, 2000; Mense and Simons, 2001). However, there is still much controversy as to whether SEA represents normal muscle endplate activity. There is disagreement in electromyography and physiology literature on the significance of abnormal motor endplate potentials and "endplate noise." According to Simons, investigators who lack training in examining muscles for MTrPs may misinterpret a MTrP's abnormal "endplate noise" as a normal finding (Wiederholt, 1970; Simons, 2004).

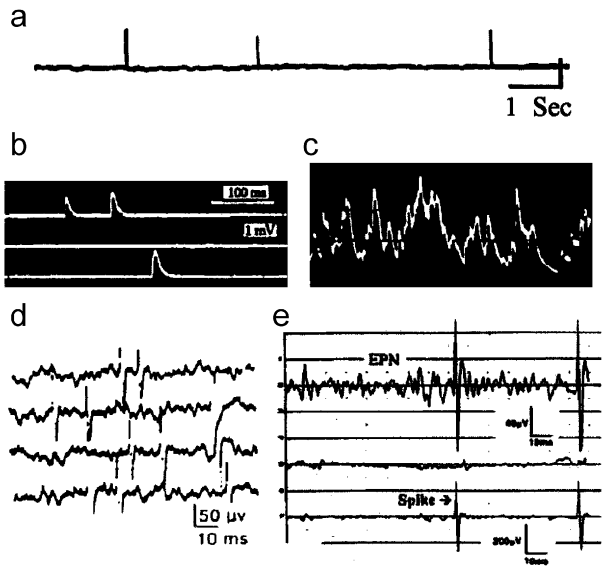

Figure 2 Comparisons of normal miniature endplate potentials (MEPP, a result of random release of ACh packets) and endplate noise (EPN, thought to be a sign of abnormal and increased motor endplate activity). (A) Normal human MEPPs. (B) Normal rat MEPPs. (C) Experimentally induced endplate noise. This method produced a thousand time increase of ACh release. (D) Textbook "normal" endplate potentials, with evidence of EPN. (E) Endplate noise and spikes from a human trigger point. (Reproduced by kind permission of Elsevier Ltd., from Simons, 2004.)

The Integrated Trigger Point Hypothesis

Encompassing the pathophysiology of the motor endplate activity is the Integrated Trigger Point Hypothesis introduced by Simons, which brings together several findings of MTrPs to describe a possible sequence of MTrP development (Simons et al., 1999). Included in this sequence is an ''energy crisis'' that perpetuates an initial sustained contracture at the muscle fibers near an abnormal endplate. Due to excessive ACh release from the motor endplate, it is hypothesized that sustained sarcomere contracture leads to increased local metabolic demands and compressed capillary circulation. With reduced blood flow and diminished sources of adenosine triphosphate (ATP), muscle fibers are locked in a contracture without sufficient energy to return Ca^{2+} to the sarcoplasmic reticulum and restore a polarized membrane potential. Additionally, the local hypoxic conditions and energy crisis may elicit the release of neuroreactive substances and metabolic by-products that could sensitize peripheral nociceptors (Huguenin, 2004).

The Cinderella Hypothesis

The Cinderella Hypothesis (Hagg, 1988) provides a possible explanation of MTrP development that complements the Integrated Trigger Point Hypothesis (Simons et al., 1999). The Cinderella Hypothesis describes how musculoskeletal disorder symptoms may arise from muscle recruitment patterns during sub-maximal level exertions with a moderate or low physical load. According to Henneman's ''size principle'', smaller type 1 muscle fibers will be recruited first and be derecruited last during these static exertions, using only a fraction of motor units available. As a result, these ''Cinderella'' fibers are continuously activated and metabolically overloaded, while larger motor units do not work as hard and spend less time continuously activated. Sub-maximal exertions, such as postural maintenance, can lead to possible muscle damage and disturbance of Ca^{2+} homeostasis, suspected features that may contribute to MTrP pain. A study by Treaster et al. (2006) supports the Cinderella Hypothesis. The study demonstrated that low-level, static, continuous muscle contractions in office workers during 30 min of typing induced the formation of MTrPs. Their findings suggest that ''...a MTrP may provide a useful explanation for muscle pain and injury that can occur from low level static exertions'' (Treaster et al., 2006).

Sensory abnormalities of the myofascial trigger point

Nociceptor properties

Sensory processing and pain perception are key aspects in the description of MPS, along with the abnormal motor findings mentioned above. Transduction of local pain sensation often begins with the sensitization and activation of nociceptive sensory receptors. Nociceptors are located at free nerve endings in muscle, joint, skin, viscera, and blood vessels. Furthermore, muscle nociceptors may make up 50% of the composition of muscle nerves (Willard, 2008). The abundance of these nociceptors may explain the severity of pain and exquisite tenderness in the muscle upon palpation. Nociceptors also innervate the surrounding connective tissue of muscle fibers (Langevin, 2008; Willard, 2008). A preliminary study in mice indicates that sensory afferent and nociceptive terminals are located in subcutaneous perimuscular fascia (Corey et al., 2007). Neurons involved in pain processing can be polymodal, meaning they can be activated by several stimuli, depending on whether they contain chemoreceptors, mechanoreceptors, or thermoreceptors. Continuous activation of muscle nociceptors is very effective at inducing neuroplastic changes and central sensitization in dorsal horn neurons (Wall and Woolf, 1984).

Chemical activation of afferent nerves

Muscle nociceptors monitor the sensitizing or pain-producing substances, as well as the strength of the stimuli present in the peripheral environment. Chemical activation of nociceptors by substances released from surrounding damaged tissue or immune cells is responsible for the muscle soreness and pain associated with MPS (Gerwin et al., 2004). Chemical activation is specific at the nociceptor, where there are distinct receptors for substances including bradykinin (BK), prostaglandins (PG), 5-hydroxytryptamin/serotonin (5-HT), protons (H+), adenosine triphosphate (ATP), and glutamate, a primary excitatory neurotransmitter. There are also purinergic and vanilloid receptors. Purinergic receptors bind ATP, which is released during muscle tissue trauma (Cook and McCleskey, 2002). Vanilloid receptors respond to low pH, and therefore are activated under ischemic conditions where pH is acidic (Caterina and Julius, 1999). 5-HT is released from platelets and mast cells following tissue injury. Nociceptor terminals also contain the

neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP), which cause vasodilation, plasma extravasation, and stimulation of an inflammatory cascade within the peripheral milieu.

The biochemicals that are released from injured tissue stimulate a unique cascade of cytokines that are integral to the inflammatory response. For example, BK and 5-HT are two agents that are released immediately at damaged tissue and stimulate cytokines that are involved in complex pain pathways. Pro-inflammatory cytokines involved in these pathways, such as tumor necrosis factor alpha (TNF-α), Interleukin 1-beta (IL-1β), Interleukin 6 (IL-6), and Interleukin 8 (IL-8), have been shown to induce hypernociception when administered to peripheral tissue in animal models (Verri et al., 2006). Additionally, anti-inflammatory mediators are released in parallel to this pathway.

Endogenously released pain and inflammatory mediators not only carry nociceptive signals for central processing, but also alter the local conditions at the site of tissue damage. SP, in particular, alters the local microcirculation and vessel permeability, leading to local edema. Several biochemicals, including BK, PG, 5-HT, CGRP, and SP, have both nociceptive and vasodilatory effects. Therefore, the release of these substances can increase local blood flow and pressure, activating mechanoreceptors and nociceptors, leading to increased local tenderness and pain. In addition, a persistent barrage of algogenic substances leads to changes in nociceptor responsiveness. For example, inflammation in peripheral tissue changes the number and population of BK receptors at the nociceptor terminal (Cunha et al., 2007). Thus, the biochemical cascade of inflammation makes primary afferent neurons susceptible to abnormal depolarization activity by various means, enhancing peripheral and central sensitization.

Peripheral and central sensitization

Sensitization of both peripheral and central afferents is responsible for the transition from normal to aberrant pain perception in the central nervous system that outlasts the noxious peripheral stimulus. In animal models of pain, nociceptive input from skeletal muscle is much more effective at inducing neuroplastic changes in the spinal cord than noxious input from the skin (Wall and Woolf, 1984). Continuous input from peripheral muscle nociceptors may lead to changes in function and connectivity of sensory dorsal horn neurons via central sensitization. For example, sustained noxious input from an active MTrP may sensitize dorsal horn neurons, leading to hyperalgesia and allodynia, as well as generate expanded referred pain regions. A possible explanation for this phenomenon is increased synaptic efficiency through activation of previously silent (ineffective) synapses at the dorsal horn.

This concept was demonstrated in a rat myositis model, in which experimentally induced inflammation unmasked receptive fields remote from the original receptive field, indicating that dorsal horn connectivity expanded beyond the original neurons involved in nociceptive transmission (Hoheisel et al., 1994). In this study, nociceptive input resulted in central hyperexcitability, which helps to explain referred pain patterns common to MPS. Central sensitization may also facilitate additional responses from other receptive fields due to convergent somatic and visceral input at the dorsal horn (Sato, 1995). Afferent fibers can also sprout new spinal terminals that broaden synaptic contacts at the dorsal horn, and may contribute to expanded pain receptive fields (Sperry and Goshgarian, 1993). This change in functional connectivity occurs within a few hours, before metabolic and gene induction changes in dorsal horn neurons (Mense and Hoheisel, 2004).

There is a biochemical basis to the development of peripheral and central sensitization in muscle pain. Continuous activation of muscle nociceptors leads to the co-release of glutamate and SP at the pre-synaptic terminals of the dorsal horn. In addition to activation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by glutamate at the post-synaptic terminal, SP facilitates activation of previously dormant *N*-methyl-D-aspartate (NMDA) receptors. This leads to maximal opening of calcium-permeable ion channels, which hyperexcites nociceptive neurons and induces apoptosis of inhibitory interneurons (Mense, 2003), as seen in Figure 3. Consequently, a persistent noxious barrage from the periphery can create long-lasting alterations in the central nervous system. Metabolic and gene induction changes, such as cyclo-oxygenase 2 (COX-2) induction in dorsal horn neurons, are maximal at several hours after an initial noxious stimulation and bolster functional changes after peripheral tissue injury (Woolf, 2007).

In addition, glial cells that surround primary afferent neurons can contribute to central sensitization in the dorsal horn. In particular, astrocytes and microglia are activated by SP, and can produce cytokines (such as TNF-α, IL-1, and IL-6) that sensitize neurons and generate hyperalgesia (Watkins et al., 2007). Activated glial cells also induce a rise in SP release from central terminals of

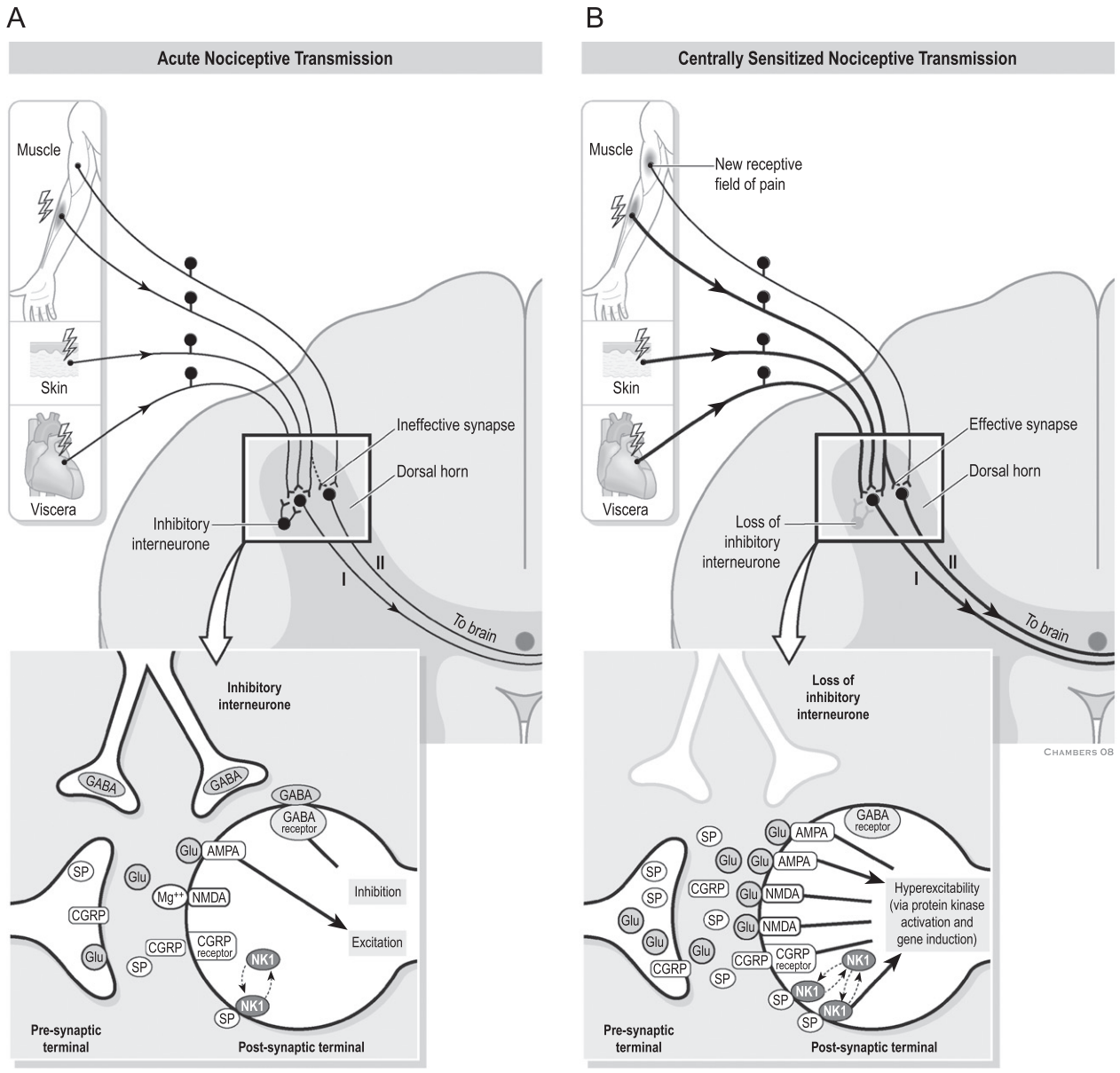

Figure 3 Transition from normal to pathological pain perception, via central sensitization at the spinal cord dorsal horn. Insets of central synaptic transmission. (A) Acute nociceptive transmission. Nociceptive signals may originate from muscle, cutaneous, or visceral afferent neurons. (B) Centrally sensitized nociceptive transmission. Convergence of muscle, cutaneous, and visceral afferents can be responsible for referred pain patterns. Activation of ineffective synapses in the dorsal horn may create additional receptive pain fields of pain. For example, input from the extensor carpi radialis longus normally activates neuron I. With intense and/or continuous noxious input from the extensor carpi radialis longus, another previously ineffective (silent) synapse may be converted into an effective (active) synapse. Here, a synapse to neuron II, which normally receives input from the biceps brachii, becomes effective. The expansion of effective spinal connections at neuron II can create a new receptive field of pain at the biceps brachii, where no nociceptor is being activated and the tissue is normal. Increased neurotransmitter release at the dorsal horn alters receptor ion channel activity. All of these factors contribute to central hyperexcitability via protein kinase activation and gene induction. Glu: glutamate, SP: substance P, CGRP: calcitonin gene-related peptide, GABA: gamma-aminobutyric acid, AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionate, NMDA: *N*-methyl-D-aspartic acid, NK1: neurokinin 1. (Adapted from Mense, 2003; Vadivelu, N., Sinatra, R., 2005. Recent advances in elucidating pain mechanisms. Current Opinion in Anaesthesiology 18, 540–547; Willard, 2008.)

primary afferent neurons, thus contributing to the excessive calcium influx and the subsequent central nervous system alterations described above (Inoue et al., 1999).

Though experimental mechanisms have implicated endogenously released substances in muscle pain, the pathogenesis of MPS is still unclear (Mense, 2003). As a result, standard treatments of MPS are largely empirical and suboptimal. Treatments may improve symptoms, though may not resolve all symptoms or eliminate the MTrP (Bennett, 2007). Eliciting an LTR through dry needling often has a therapeutic benefit (Hong, 1994). As mentioned above, the initial peripheral conditions (inflammation, ischemia, and hypoxia) within muscle seem to be the source of feed-forward mechanisms that transform and intensify central processing of muscle pain. Therefore, assaying the peripheral milieu of a MTrP before, during, and after an LTR could disclose changes in bioactive substances that may contribute to myofascial pain.

Uncovering the biochemical milieu of myofascial trigger points

We developed a microanalytical system to sample the unique biochemical milieu of substances related to pain and inflammation in muscle tissue with and without MTrPs (Shah et al., 2005). This system employed a minimally invasive 30-gauge needle capable of in vivo collection of small volumes ($\sim$0.5 μl) at sub nanogram levels (<75 kDa). The needle (Figure 4) has the same size and shape as an acupuncture needle and allows simultaneous sampling of skeletal muscle tissue when an LTR is elicited by advancement of the sampling needle. The complete sampling setup includes a microdialysis pump and Terasaki plate for fluid collection.

Clinicians use various dry needling techniques to induce multiple LTRs in order to achieve therapeutic benefit (Chen et al., 2001; Dommerholt et al., 2006; Shah, 2008). The LTR is an involuntary spinal reflex contraction of muscle fibers within a taut band, and occurs during needling of a taut band. As this event is associated with pain relief and reduction of stiffness (Hsieh et al., 2007), sampling at the muscle during this event could reveal aspects of the LTR's biochemical basis.

Microdialysis sampling of the trapezius

In one study, the microanalytical system was used to measure the local biochemical milieu at a standardized location, the acupuncture point GB-21, at the upper trapezius muscle (Shah et al., 2005). Based on patient history and physical examination, nine subjects were classified into one of three groups:

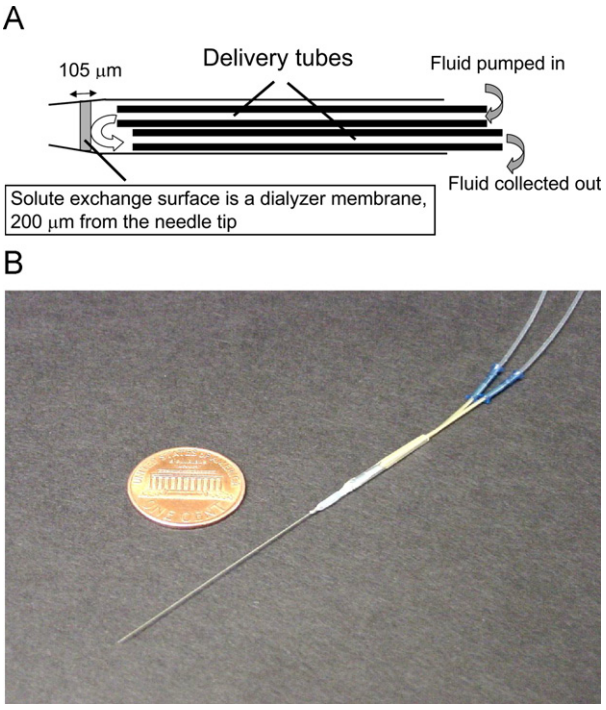

Figure 4 (A) Microdialysis schematic and (B) photo of needle. (Reproduced with kind permission by the American Physiological Society and Elsevier, Ltd., from Shah et al., 2005, 2008.)

- Group 1—Normal (no neck pain, no MTrP);
- Group 2—Latent (no neck pain, MTrP present);
- Group 3—Active (neck pain, MTrP present).

Samples were obtained at regular intervals before needle movement, during needle advancement and LTR, and after the LTR, for a total of 15 min. After collection, dialysate samples were analyzed by immunoaffinity capillary electrophoresis (ICE) and capillary electrochromatography (CEC). Outcome measures were levels of pH, and concentrations of SP, CGRP, BK, 5-HT, norepinephrine (NE), TNF-α, and IL-1β.

The results showed that overall, the concentrations of SP, CGRP, BK, 5-HT, NE, TNF-α, and IL-1β were higher in the Active group than in the Latent and Normal groups ($p<0.01$). In addition, pH levels were significantly lower in the Active group, indicating a greater concentration of protons than in the Latent and Normal groups ($p<0.03$). There were no overall differences between the Latent and Normal groups. At post-LTR for the Active group, concentrations of SP and CGRP were significantly lower than "pre" (2 min following

needle insertion) or "peak" (about 5 min following needle insertion) values ($p<0.02$). These results showed that the biochemical milieu of active MTrPs is different from latent MTrPs and normal tissue. Also, the milieu changes with the occurrence of a LTR, corresponding to clinically observed decrease in pain and tenderness after MTrP release by dry needling. Changes in analyte levels after an LTR might result from increasing local blood flow to the MTrP region, leading to a "wash out" of the pain and inflammatory mediators.

Microdialysis sampling of the trapezius and gastrocnemius

In a second study, we sought to investigate whether the differences in levels of inflammatory mediators, neuropeptides, catecholamines, and cytokines are present not only at the site of the MTrP, but also in an uninvolved site remote from the MTrP (Shah et al., 2008). Accordingly, samples were collected from nine additional subjects using the same procedure as the previous study at the upper trapezius. Additionally, samples were also collected from a site in the upper medial gastrocnemius at the acupuncture point LV-7. The site was examined before sampling to verify that none of the subjects had active or latent MTrPs present at this location in the muscle.

Results from the second study confirmed that in the upper trapezius muscle, concentrations of biochemicals associated with pain and inflammation agreed with levels found in the previous study. These findings verify that the selected analytes are elevated in soft tissue in the vicinity

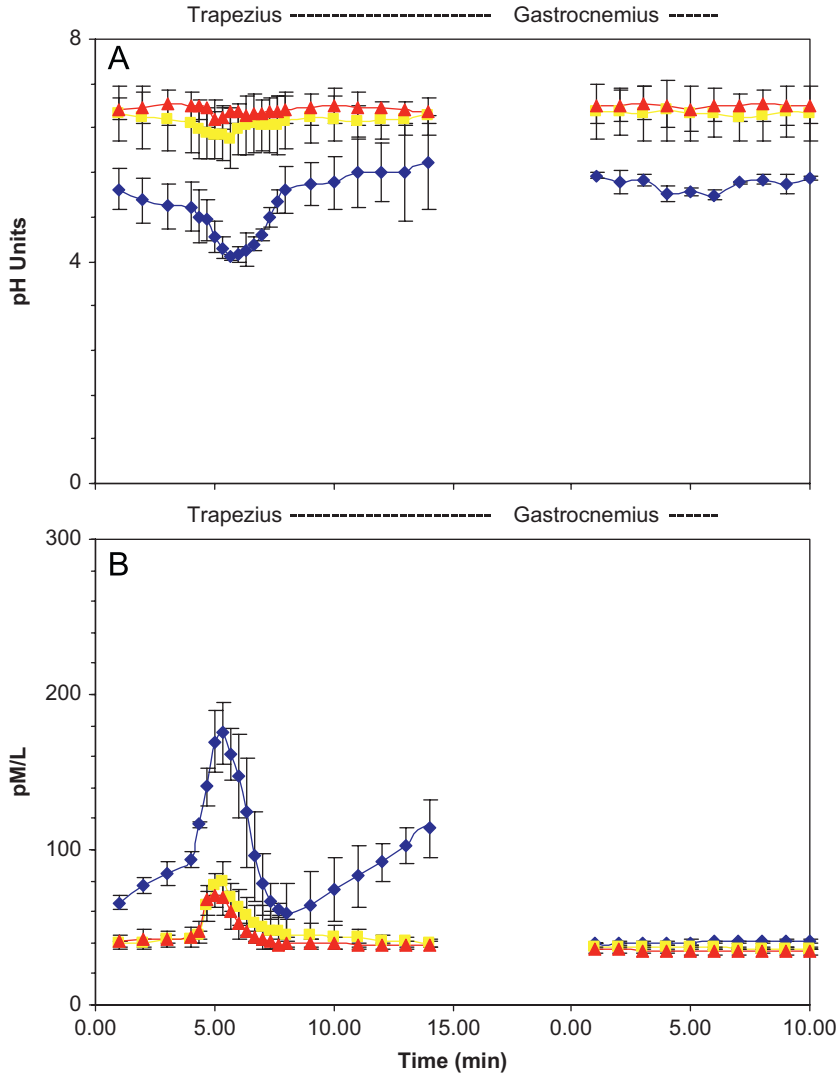

Figure 5 Analyte concentrations for the trapezius compared to the gastrocnemius for (A) pH and (B) BK. (Reproduced with kind permission by Elsevier Ltd., from Shah et al., 2008.)

of active MTrPs. Two additional analytes known to be associated with inflammation and intercellular signaling, IL-6 and IL-8, were also measured. These analytes were overall significantly elevated in the upper trapezius of the Active group compared to the Latent and Normal groups ($p < 0.002$). As in the previous study, each of the groups demonstrated different responses to needle insertion in the trapezius. The Active group exhibited the largest and most elevated response, the Latent group an intermediate response, and the Normal group exhibited the smallest.

Comparisons between the trapezius and the gastrocnemius showed differences in levels of analytes, as seen in Figures 5–7. Within the Active group, almost all measurements of concentrations for the gastrocnemius were lower than concentrations for the trapezius muscle. In the Latent group, most gastrocnemius concentrations were significantly lower than trapezius peak values, though not for other measurements in the trapezius. The only exception was pH, for which levels were similar within the trapezius and gastrocnemius muscles. This information showed that the biochemical milieu of active MTrPs in the upper trapezius differs quantitatively from a remote, uninvolved site in the gastrocnemius muscle.

Analyte levels in the gastrocnemius were also compared among the Active, Latent, and Normal groups. Although there were no MTrPs in any of the subjects at the upper medial gastrocnemius, the analyte concentrations of the Active group were significantly higher than in the Latent and Normal groups ($p < 0.05$). In the Active group, the pH was lower ($p < 0.01$). This suggests that analyte abnormalities may not be limited to local areas of active MTrPs in the upper trapezius, but may also be present in unaffected muscle remote from the

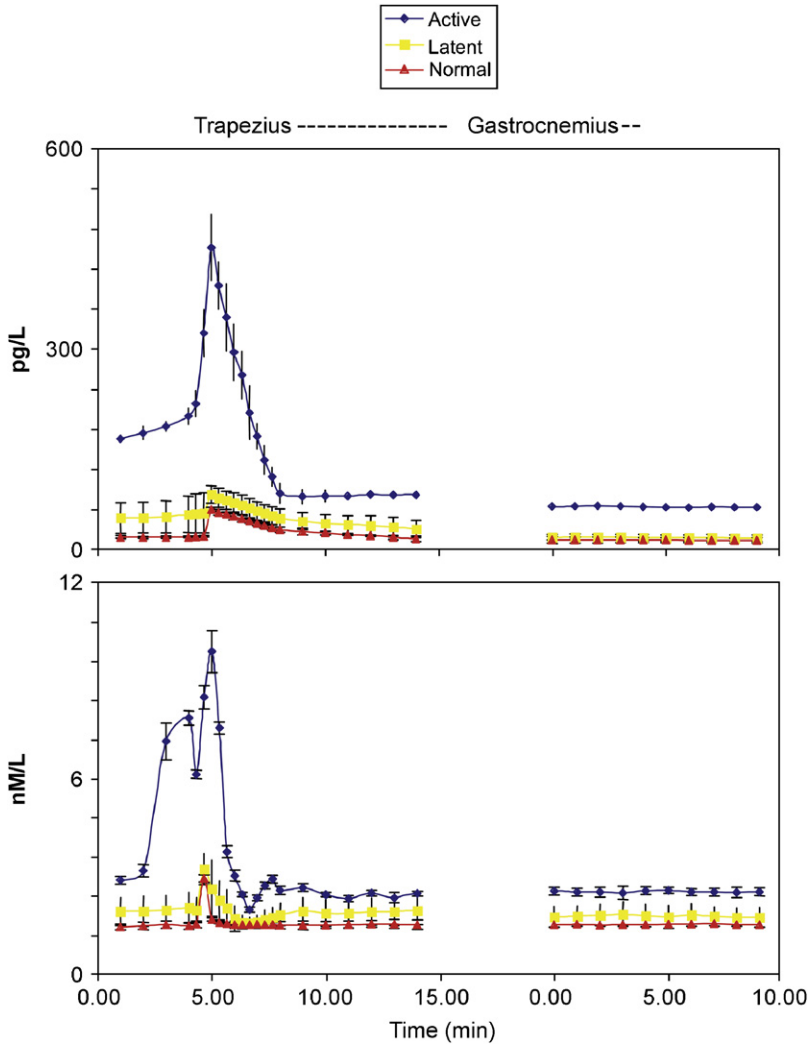

Figure 6 Analyte concentrations for the trapezius compared to the gastrocnemius for (A) SP and (B) NE. (Reproduced with kind permission by Elsevier Ltd., from Shah et al., 2008.)

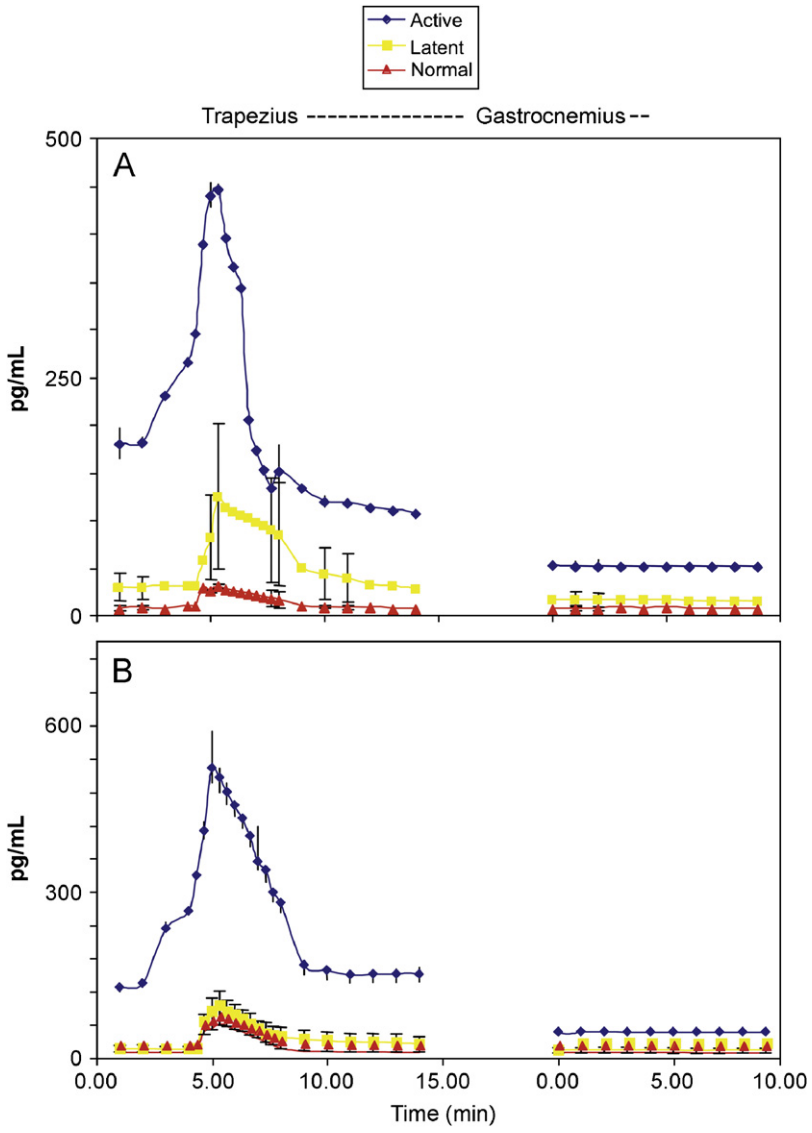

Figure 7 Analyte concentrations for the trapezius compared to the gastrocnemius for (A) TNF-α, and (B) IL-6. (Reproduced with kind permission by Elsevier Ltd., from Shah et al., 2008.)

active MTrPs, albeit lower in concentration than in the trapezius. The elevated levels of analytes in the Active group at the gastrocnemius may be related to central sensitization within these subjects. One explanation could be that widespread elevation of substances associated with pain and inflammation follows initial development of MTrPs. Conversely, individuals who are susceptible to developing MTrPs may have preexisting elevated levels of these analytes. These findings present questions about what makes individuals susceptible to possibly widespread elevations of biochemicals. An impaired ability to clear metabolites from injured tissue could make some individuals prone to MTrP development, though the basis of such a condition is currently unknown.

Though both the trapezius and gastrocnemius muscles displayed elevated concentrations for subjects in the Active group, these muscles exhibited different biochemical responses to needle insertion. In the trapezius, analytes from all groups reached a sharp peak value (in the case of pH, a minimum value) at about 5 min. In the gastrocnemius, no peak concentrations were noted for any of the groups. As the trapezius (involved in posture maintenance) and gastrocnemius (involved in locomotion) muscles have different functions and fiber compositions, this may explain the difference in responses to needle insertion.

The temporal changes in analyte concentrations can also provide information about the specific biochemical response of the trapezius muscle to

needle insertion. As an analyte's concentration rises, its presence could influence the activity of other biochemical mediators. Detailed analysis of the temporal sequence of analyte changes may characterize a possible inflammatory cascade. Further study is needed to understand the mechanism(s) underlying the myofascial tissue's response to needling procedures. In the following sections, we will discuss the properties of the biochemicals measured in these studies and their involvement in muscle pain and inflammation.

Roles of biochemical substances associated with pain and inflammation

pH

Acidic pH levels within muscle have been shown to be associated with pain and lowered nociceptor threshold sensitivity (Issberner et al., 1996). This association is supported by the microdialysis studies above, which found acidic pH levels in muscles containing active (painful) MTrPs. In a study of mouse model hyperalgesia, Sluka et al. (2001) showed that unilateral injections of acidic saline into the gastrocnemius resulted in long-lasting bilateral mechanical hyperalgesia. Contralateral hyperalgesia was not affected by lidocaine injections or dorsal horn rhizotomy on the contralateral side. This study demonstrated that contralateral pain perception could be maintained without constant afferent input or muscle tissue injury, suggesting that neuroplastic changes may have occurred at the central nervous system, generating secondary hyperalgesia.

An acidic milieu is observed during ischemia and hypoxia, and after exercise. The release of protons from physically stressed or injured muscle tissue is likely to activate acid sensing ion channels (ASICs) and vanilloid nociceptors that signal hyperalgesia. In light of the capillary constriction and increased metabolic demands of the muscle contracture proposed by the Integrated Trigger Point Hypothesis, ischemia and hypoxia may result at the site of the MTrP, sensitizing peripheral and central nociceptors (Gerwin et al., 2004). Expanding on Simons' Integrated Hypothesis, Gerwin et al. (2004) suggested that acetylcholine esterase (AChE) is inhibited by an acidic pH, leaving an excess of ACh in the synaptic cleft.

Neuropeptides

Stimulation of nociceptive neurons can also mediate the orthodromic and antidromic release of neuropeptides, such as SP and CGRP. Direct actions of SP include sensitization of nociceptors, vasodilation, increased vascular permeability, and mast cell degranulation, leading to release of other inflammatory mediators. While SP has known algesic effects, it has been identified as a neuromodulator that brings about slow changes at the NK1 receptor and interacts with opioid transmission (Snijdelaar et al., 2000). CGRP appears to modulate nociceptive terminals. In an experimental rat model of inflammation, noxious stimulation induced increased CGRP mRNA and numbers of primary afferent neurons containing CGRP, which was associated with nociceptive behaviors (Ambalavanar et al., 2006). Furthermore, Gerwin et al. (2004) hypothesized that CGRP intensifies the response to excess ACh at the nerve terminal by enhancing ACh receptor activity and synthesis, supporting the role of neuropeptides in the MTrP pathophysiology. On the other hand, a study by Ambalavanar et al. (2007) found that CGRP expression in the rat is muscle-specific; e.g. craniofacial muscles react differently to noxious stimuli than hindlimb muscles. Neuropeptide expression in muscle may also differ from that in cutaneous or connective tissue.

Catecholamines

Significantly elevated levels of neurotransmitters NE and 5-HT were found to be elevated in active MTrPs. 5-HT is a pro-nociceptive substance with vasoconstrictive properties. In an area of tissue damage, 5-HT is released from platelets, mast cells, and basophils that infiltrate the damaged area. Activation of the various 5-HT receptors has direct and dose-dependent nociceptive effects on the vascular bed (Giordano and Schultea, 2004). The increased levels of NE, the sympathetic neurotransmitter, may be associated with increased sympathetic activity in the motor endplate region of MTrPs. In one study, sympathetic activity was recorded from rabbit myofascial trigger spots, which is a model of the human trigger point (Chen et al., 1998). Intra-arterial injection of phentolamine, an α-adrenergic antagonist, decreased the SEA from a locus of a myofascial trigger spot in rabbit skeletal muscle (Chen et al., 1998). Effects of NE have also been linked with depressed feedback control of muscle length and increased SEA at motor endplates, pointing to the possible role of NE in MTrP pathophysiology (Bukharaeva et al., 2002; Roatta et al., 2002).

Cytokines

Following injury and inflammation, a specific cascade of cytokines is initiated. Stimulation of

this cascade is suspected in the development of muscle pain associated with MPS, and elevation of the cytokines TNF-α, IL-1β, IL-6, and IL-8 was observed in the studies by Shah et al. Two major cytokine pathways employ prostaglandins and sympathetic amines as final mediators that directly sensitize nociceptors. Studies of experimentally induced cutaneous and muscle hypernociception in rats have shown that TNF-α regulates both pathways, including the intermediary pro-inflammatory cytokines IL-6, IL-8, and IL-1β (Sachs et al., 2002; Mense, 2003; Verri et al., 2006). IL-1β and IL-6 stimulate cyclo-oxygenase (COX) mediated pathways, which terminate with prostanoid activation (Verri et al., 2006). In an *in vitro* experiment with skeletal muscle, IL-1β was shown to stimulate the release of IL-6, perhaps suggesting a synergistic effect of IL-1β and IL-6 (Luo et al., 2003).

IL-8 and the rat homologue cytokine-induced neutrophil chemoattractant 1 (CINC-1) mediate the sympathetic amine pathway. In a study by Loram et al. (2007) of experimentally induced rat muscle hypernociception, CINC-1 demonstrated a unique ability to induce primary hyperalgesia. While TNF-α, IL-1β, IL-6, and IL-8 have demonstrated time- and dose-dependent effects of injection in the skin, these cytokines had different effects in rat muscle (Verri et al., 2006; Loram et al., 2007). The study showed that primary hyperalgesia corresponded temporally with high measurements of CINC-1. However, maintenance of secondary hyperalgesia might be attributed to actions of IL-1β and IL-6, which were elevated at times later than initial inflammation (Loram et al., 2007). Additional study is needed to clarify the cytokine cascade unique to muscle pain and MPS, in order to investigate possible pharmacologic targets.

Conclusion

Myofascial trigger points are a very common and complex component of non-articular musculoskeletal pain and dysfunction. However, they are also regularly found in asymptomatic individuals. Therefore, our studies sought to determine if there are biochemical aspects that differentiate active MTrPs from latent MTrPs, and muscle without MTrPs. Our microanalytical technique permits direct sampling of the biochemical milieu of MTrPs, including bioactive substances (e.g., inflammatory mediators, neuropeptides, catecholamines, and cytokines) that are released from and act on muscle, nerve, and connective tissue. We have confirmed that biochemicals associated with pain, inflammation, and intercellular signaling are elevated in the vicinity of active MTrPs. Furthermore, subjects with active MTrPs in the upper trapezius have elevated levels of these biochemicals in a remote, unaffected muscle, suggesting that these conditions are not limited to localized areas of active MTrPs. A natural history study, following similar procedures to the biochemical studies discussed in this paper, is underway to determine whether MTrPs resolve spontaneously or evolve into the active forms from latent or normal conditions. Further research with these microanalytical techniques could improve characterization and validation of the temporal cascade initiated during noxious stimulation or dry needling treatment.

The recent lines of scientific investigation suggest that it may be useful for clinicians and scientists to develop a model of MTrP pathophysiology as a type of neuromuscular dysfunction. From this perspective, future clinical research studies should focus on identifying the mechanisms responsible for the etiology, amplification, and perpetuation of MPS. The development of successful treatment approaches depends upon identifying and targeting these mechanisms and addressing the perpetuating factors that maintain this ubiquitous pain syndrome.

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