Myalgia! Where does it come from?

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Abstract

Myalgia (also called muscle pain or muscle ache) is a symptom associated with many diseases, including fibromyalgia, neurodegenerative diseases, degenerative spine diseases, etc. Myalgia is a major medical problem affecting 60~85% of the population (lifetime prevalence). However, our understanding of chronic myalgia is still limited and effective treatment for intractable myalgia like fibromyalgia is still lacking. Although multifactorial, one known source of muscle pain is tissue acidosis. Experimental muscle pain can be induced by the intramuscular infusion of a buffered acidic solution in humans. As well, animal studies have revealed that acidic infusion activates chemosensitive nociceptors via the proton-sensing ion channels and receptors. Intriguingly, acid signaling in muscle afferents is promiscuous and could be either pro-nociceptive or antinociceptive, so we have coined the term sngception to describe the somatosensory function of acid sensation. Recent single-cell RNAseq studies have shown proton-sensing ion channels and receptors are expressed in all subpopulations of the somatosensory neurons, including nociceptors and non-nociceptive mechanoreceptors. Here, we address how the acid signaling is integrated in muscle afferents and why muscle pain can be chronic and intractable in mouse models of fibromyalgia. Besides acidosis, we have recently found oxidative stress can be another factor to activate proton-sensing ion channels and thus trigger fibromyalgia-like pain in mice. Together, understanding how the acid signaling works in muscle afferents will provide novel therapeutic strategies for myalgia.

Keywords: **ASIC3, Fibromyalgia, Pain, Sngception, Soreness**

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1. INTRODUCTION

Myalgia, also called muscle pain or muscle ache, is a painful sensation that origins from muscle and commonly occurs in musculoskeletal diseases, fibromyalgia, and other systematic diseases [(1)]. Globally, one third of people

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suffer from myalgia, which causes significantly disability and poor quality of life [2]. Especially, chronic muscle pain is a major medical problem affecting millions of people globally. The lifelong prevalence of this major global health problem affects 60~85% of the population [3]. Myalgia could be caused by irritation of the receptors in muscle and fascia due to muscle overuse, inflammation, infection, or injury, as well as degenerative diseases, stress, and tension [1,4]. However, our understanding how muscle pain become chronic and intractable is still limited and the effective treatment for chronic myalgia such as fibromyalgia is still challenging [5].

Fibromyalgia is the most common intractable myalgia affecting 0.2~6.6% in the general population [6]. Up to date, fibromyalgia is still a mysterious disease characterized with chronic widespread muscle pain and generalized tenderness without knowing the exact disease-causing factors. Although fibromyalgia pain is generally believed as stress-associated and due to the central sensitization of the pain matrix in the brain, the pathophysiology and effective treatments remains controversial. Recent studies have made efforts to elucidate peripheral risk factors that could effectively trigger fibromyalgia pain [7-9]. Here, we aim to review how the peripheral risk factors could lead to the non-inflammatory chronic widespread pain and provide mechanistic insights for further development of effective treatment for fibromyalgia.

2. TISSUE ACIDOSIS, OXIDATIVE STRESS, AND MYALGIA

2.1 Clinical aspects of myalgia

The causes of myalgia can be simple or multifactorial and its classification is based on diffuse myalgia or focal symptoms [1]. Myalgia is commonly diagnosed in fibromyalgia, degenerative spines diseases, Parkinson's disease and other neurodegenerative diseases, viral infection (e.g., Covid-19, influenza), myopathy, rheumatic diseases, metabolic disorders, and the side effects of medication, such as statin [4,6,10,11]. Clinical manifestations of myalgia include pain, soreness, tenderness, sometimes accompany with fatigue, weakness, redness, swelling, or warmth in the areas of muscle pain [12-15]. Myalgia is usually benign and self-limited, but severe chronic myalgia may cause morbidity, poor quality of life, and mood disorder, especially in those with fibromyalgia, myofascial pain syndrome, chronic fatigue syndrome, and phycological somatization disorder [14,16].

Treatment of myalgia usually depends on the underlying causes. Experimental treatment may include physical therapy, heat, rest, pain control, and muscle relaxants. However, there are no established guidelines or expert consensus on how to effectively treat common or mild myalgia. For severe myalgia like fibromyalgia, effective treatments remain an unmet medical need [5]. Moreover, we have recently shown soreness and pain are 2 distinguished dominant symptoms in myalgia associated with fibromyalgia and degenerative diseases [13,15,17-19]. Although morbid soreness is a major complaint among patients, it is not properly treated as pain. Of note, current analgesics (e.g., TCA or pregabalin) are less effective for morbid soreness as compared with pain in fibromyalgia [15].

2.2 Tissue acidosis and myalgia

Local metabolic changes and peripheral nociceptive processes are thought to be involved in the development of muscle pain in humans [20,21]. Especially, protons and ATP are particularly relevant causes of myalgia [22]. Biochemical analyses had shown local acidosis and increased levels of substance P in the trigger points of myofascial pain [23]. A recent study has shown intramuscular pressure is 3 times higher in fibromyalgia patients as compared with rheumatic disease controls [24]. The compressing effects on small capillaries in muscle would lead to an ischemic condition, prevent adequate oxygenation of muscle tissue, and result in tissue acidosis. Indeed, muscle blood flow of fibromyalgia patients is impaired even in relatively low contraction levels, suggesting muscles are frequently forced to work under ischemic conditions and thus sensitize muscle mechanonociceptors [25-27]. Of note, muscle ischemia/acidosis can effectively sensitize muscle mechanonociceptors [28-30]. In humans, the causal relationship between muscle acidosis/ischemia and myalgia have been experimentally proved. Muscle ischemia or intramuscular infusion acidic phosphate buffer (pH5.2) induce muscle pain in healthy volunteers [31,32]. Several studies have indicated acid-sensing ion channels (ASICs) or TRPV1 channels are possible molecular determinants involved in the acid-induced muscle pain in humans [33,34]. However, the role of muscle acidosis in

chronic myalgia remains elusive, as it is not clear whether tissue acidosis can contribute to nociceptor priming and lead to chronic muscle pain or fibromyalgia.

2.3 Mouse models of fibromyalgia

Fibromyalgia is commonly known as a stress-related disease triggered by either physical stress or psychological stress [6]. However, the molecular and neurobiological basis underlying the development of fibromyalgia pain is still elusive. Although central sensitization of the pain

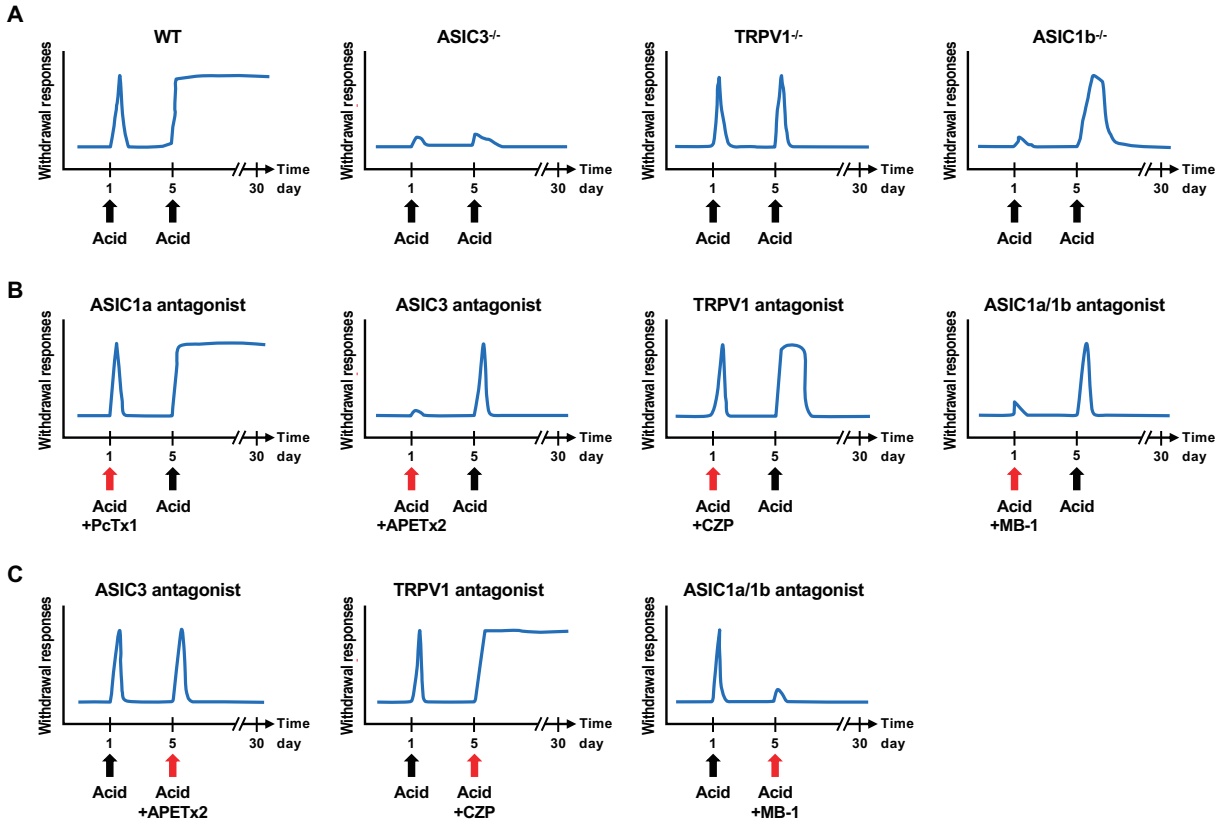

Fig. 1. Proton-sensing ion channels involved in acid-induced chronic widespread muscle pain in the Sluka model. (A) The Sluka model. In wild-type (WT) mice, unilateral repeated intramuscular injections of pH4.0 acidic saline 5 days apart can induce bilateral mechanical hyperalgesia lasting for 4 weeks. The acid-induce hyperalgesia is totally abolished in ASIC3 knockout (ASIC3-/-) mice. In TRPV1 knockout (TRPV1-/-) mice, acid can only induce transient hyperalgesia. In ASIC1b knockout (ASIC1b-/-) mice, the first acid injection evokes a weak priming effect but not transient hyperalgesia, so the second acid injection induces hyperalgesia lasting for 3-4 days. (B) In WT mice, combined ASIC1a antagonist PcTx1 in the first acid injection does not affect the acid-induced transient and chronic hyperalgesia. In contrast, inhibiting ASIC3 (by APETx2) or ASIC1b (by mambalgin-1, MB1) at the first acid injection abolished the transient hyperalgesia and priming, so that the second acid injection only induces a transient hyperalgesia. Inhibiting TRPV1 (by capsazepin, CZP) does not affect the first acid-induced hyperalgesia but shows an effect on priming, so that the second acid injection induces hyperalgesia lasting for 3-4 days. (C) In WT mice, combined APETx2 or MB-1 in the second acid injection prevents the development of chronic hyperalgesia, whereas inhibiting TRPV1by CZP in the second acid injection shows no effect on acid-induced chronic hyperalgesia. (Schematic drawings are based on the data of Chen et al., 2014 [41] and Chang et al., 2019 [42])

matrix is involved in fibromyalgia pain, accumulating evidence has shown the importance of peripheral afferent inputs for the induction and maintenance of fibromyalgia symptoms. Therefore, several animal models have been established based on potential risk factors [35]. Physical stimuli via acidosis or cold are commonly used to induce fibromyalgia-like pain in rodents, whereas psychological stress such as exposures to others pain (empathy) or noisy sound can induce generalized pain mimicking fibromyalgia-like symptoms in mice [8,36-38].

In 2001, Sluka and colleagues developed the acidosis-induced myalgia model (the Sluka model), in which 2 injections of acidic saline (5 days apart) to a unilateral gastrocnemius muscle can induce bilateral mechanical hyperalgesia lasting for 4 weeks in rodents [36]. The first intramuscular injection of pH4.0 saline induces a bilateral transient mechanical hyperalgesia in both hind paws and muscle, whereas a second acid injection to the same muscle in 2-5 days will lead to a bilateral long-lasting hyperalgesia for 4 weeks. The Sluka model provides an insightful concept regarding the possible myalgia origin. As repeated challenges of intramuscular acidosis are required to induce bilateral long-lasting hyperalgesia mimicking fibromyalgia, muscle acidosis would trigger several signal pathways including nociceptor priming and central sensitization [39].

2.4 Roles of ASIC1b, ASIC3, and TRPV1 in myalgia

In the Sluka model, genetic knockout and pharmacological blockade approaches have reveled involvement of ASIC1b, ASIC3, and TRPV1 in different stages of the muscle acidosis-indued pain chronicity (Fig. 1). ASIC3 knockout (KO) totally abolished the acid-induced transient and chronic hyperalgesia, whereas ASIC1b KO abolished the first acid-induced transient hyperalgesia and shortened the second acid-induced

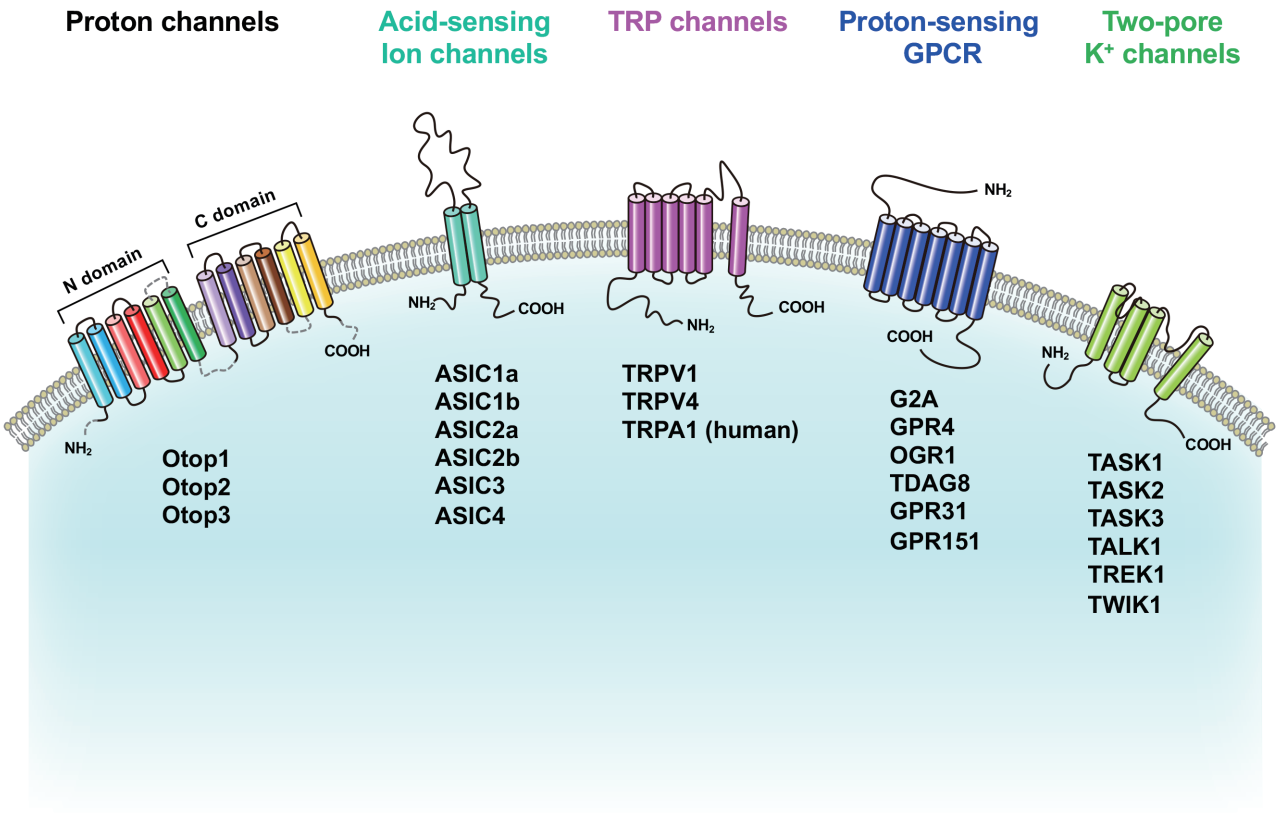

Fig. 2. Acid sensors for sngception. In somatosensory neurons, molecular sensors for tissue acidosis include members of acid-sensing ion channels (ASICs), transient receptor potential (TRP) channels, two-pore potassium (K2P) channels, proton channels, and proton-sensing G-protein-coupled receptors.

chronic effect [40-42]. In contrast, repeated muscle acidosis only induced transient hyperalgesia in TRPV1 KO mice, suggesting a role for TRPV1 in hyperalgesic priming [41] (Fig. 1A). In muscle afferents, pharmacological blockade of ASIC3 or ASIC1b abolished the first acid injection-induced transient hyperalgesia and hyperalgesic priming, so that the second acid injection only induced transient hyperalgesia that declined in 24 hours. While inhibiting TRPV1 did not affect first acid-induced transient hyperalgesia, the second acid-induced hyperalgesia was less than 7 days (Fig. 1B). In the second acid injection, pharmacological blockade of ASIC1b or ASIC3, but not TRPV1, abolished the development of chronic hyperalgesia (Fig. 1C). Taken together, activation of ASIC1b, ASIC3, and TRPV1 is required for the acid-induced hyperalgesic priming, whereas only ASIC1b and ASIC3 are involved in the acid-induced transient hyperalgesia and repeated acidic challenges induced chronic hyperalgesia. Also, these studies suggest muscle afferents are highly heterogenous in response to muscle acidosis and subject to plasticity changes. Of note, apart from muscle afferents, previous studies also showed ASIC3 of resident macrophages in muscle is involved in the acid-induced myalgia [43].

The role of ASIC3 in myalgia has been further demonstrated in 2 other fibromyalgia mouse models induced by either intermittent cold stress (ICS) or repeated intermittent sound stress (RISS). Comparing with intramuscular acid injections, both ICS and RISS treatments deliver systematic stress to mice and evoke chronic widespread thermal and mechanical hyperalgesia lasting for 2-4 weeks [8,37]. Interestingly, ASIC3 KO abolished the ICS-induced mechanical hyperalgesia of muscle, but showed no effect on cutaneous mechanical hyperalgesia. Accordingly, ICS treatment alters several metabolic pathways and reveals ASIC3-depedent metabolites (e.g., LPC16:0) are associated with the ICS-induced hyperalgesia [44]. Although sound stress is considered as psychological stress, RISS-induced chronic hyperalgesia can be attributed to the excessive oxidative stress and lipid oxidation. Excessive oxidative stress causes lipid oxidation and upregulated lipid metabolites are found in fibromyalgia patients [8,15]. In the RISS model, lipidomics analyses reveal significant up-regulation of lysophosphatidylcholines (LPC), phosphatidylcholines, sphingomyelin, and ceramides. Of note, LPC16:0 has been known as a non-proton ligand to activate ASIC3 [45]. Experimentally, repeated intramuscular injections of LPC16:0 can induce chronic mechanical hyperalgesia lasting for 4 weeks as that induced by repeated challenges of pH4.0 acidic saline. Interestingly, intramuscular LPC16:0 or RISS treatment can only induce transient but not chronic hyperalgesia in ASIC3 KO mice. Together, ASIC3 functions as a major molecular determinant to evoke hyperalgesic priming and pain chronicity in response to either physical or psychological stress, which may be a possible disease-causing factor for fibromyalgia.

3. SENSING ACIDOSIS: NOCICEPTION OR SNGCEPTION?

3.1 Proton-sensing ion channels and receptors in somatosensory neurons

Sensing acidosis is one of the most mysterious somatosensory functions in mammals. In rodents, 70~80% of somatosensory neurons are sensitive to tissue acidosis, which numbers are much higher than the total number of nociceptors (~40-50%) [46]. Accordingly, single-cell transcriptomics analyses reveal proton-sensing ion channels and/or receptors are expressed in all types of somatosensory neurons, including those are involved in non-nociceptive functions such as low-threshold tactile receptors, proprioceptors and pruriceptors [47]. At least 5 groups of proton-sensing ion channels/receptors are expressed in the somatosensory nervous system, which include ASICs, transient receptor potential (TRP) channels, two-pore potassium channels (K2P), proton channels (Otop), proton-sensing G-protein-coupled receptors (Figure 2) [48]. Although these proton-sensing molecules are involved in pain-associated with tissue acidosis as shown in many studies, only ASICs and TRP channels are particularly investigated in myalgia.

ASICs are a group of amiloride-sensitive, proton-gated sodium channels widely expressed in the nervous system [49,50]. In humans and rodents, there are 6 subtypes encoded by 4 genes have been identified [51]. ASIC1a and ASIC1b are different at their N-terminals and encoded by accn2 via different promoters; ASIC2a and ASIC2b are different at their N-terminals and encoded by accn1 via different promoters. ASIC3 and ASIC4 are encoded by

accn3 and accn4 respectively. ASICs are trimeric channels assembled either by 3 homomeric or heteromeric ASIC subtypes [52].

3.2 Promiscuous somatosensory functions of ASICs

All ASIC subtypes are expressed in the peripheral nervous system. Among ASICs, homomeric ASIC1a, ASIC1b, ASIC2a, and ASIC3 are functional channels with differential pH sensitivity, whereas ASIC2b and ASIC4 can form heteromeric channels with other ASIC subtypes [53]. ASIC1a and ASIC3 are highly sensitive to pH changes ranging from 6.2 to 6.8. In contrast, the pH sensitivity of ASIC1b and ASIC2a are from 5.1 to 6.2 and 4.1 to 5.0 respectively. Accordingly, ASIC1a, ASIC1b, and ASIC3 are involved in pain associated tissue acidosis in different experimental animal models, such as postoperative pain, ischemic pain, inflammatory pain, arthritic pain, and muscle pain [48].

Intriguingly, tissue acidosis not only evokes pronociceptive effects to induce transient hyperalgesia and hyperalgesic priming, but also mediates an antinociceptive signaling in mouse models of fibromyalgia induced by intramuscular injections of acidic buffer [41]. The acidosis-mediated antinociceptive signaling can be observed, when both ASIC3 and TRPV1 are inhibited. Although the non-ASIC3, non-TRPV1 acid sensors involved in anti-nociceptive signaling are yet to determine, ASIC1a might be a possible candidate, as ASIC1a plays an antinociceptive role in dextrose prolotherapy [54]. Paradoxically, although ASIC3 is well characterized in its pronociceptive role in myalgia and many chronic pain models, activation of ASIC3 via therapeutic ultrasound can induce an antinociceptive effect in mouse models of fibromyalgia [55]. Apart from their acid-sensing properties, evidence has shown ASICs are mechanically sensitive and involved in mechanotransduction of proprioceptors and baroreceptors [56-59]. The roles of ASICs in neurosensory mechanotransduction have also been shown in mechanoreceptors of skin, gastrointestinal tract, urinary bladder, auditory and vestibular system, periodontal ligment and pulp teeth [60,61].

Taken together, the somatosensory functions of ASICs include nociception, antinociceptive signaling, and mechanortansduction

3.3 Sngception

Since acidosis signaling is promiscuous in the somatosensory nervous system and the acid-sensitive sensory neurons are out number of nociceptors, we should consider it is a distinguishable sensation from nociception and the corresponding perception might be not pain either [48]. We have thus coined the term sngception to address the somatosensory function of sensing acidosis, in which sng (pronounced as /səŋ/) is the corresponding perception of acid sensation [46]. Sng is the Romanization form of a Taiwanese word 痠 (or equilibrium to soreness in English), which is commonly used to describe the specific acid-like phenotypes distinct from pain. In Taiwan, sng is typically used in muscle soreness associated with fatiguing exercise, virus infection, adverse effects of statin treatment, fibromyalgia, or degenerative spine diseases [15,18,46]. Of note, soreness is defined as pain in English, so sng would be a better term to distinguish it from pain [46]. In individuals with fibromyalgia, sng and pain can be clearly diagnosed as 2 different symptoms and attributed to different metabolomic and proteomic alterations in serum and urine [13,15,62]. In fibromyalgia, sng is more intractable than pain and the distribution of sng (or morbid soreness) is not associated with pain in most of the body regions [15]. Also, fibromyalgia sng is associated with oxidative stress and LPC16:0 upregulation, suggesting the involvement of ASIC3-mediated signaling.

Taken together, Sng and sngception is a new pathological pathway worthy of in-depth investigation clinically and basically for myalgia associated with tissue acidosis.

4. AN ANTINOCICEPTIVE ROLE FOR SUBSTANCE P IN MYALGIA

In preclinical studies, acid-sensation or sngception is not only pro-nociceptive, but also anti-nociceptive [48]. Especially, in the Sluka model, muscle acidosis can activate the non-ASIC3, non-TRPV1 acid sensors of muscle afferents to induce a prolonged antinociceptive signaling lasting for 2 days. This acid-mediated anti-nociceptive signaling requires the release of substance P from muscle afferents [63]. Interestingly, while proton-sensing ion channels of ASIC1a, ASIC3, and TRPV1 can also mediate an antinociceptive effect in response to

dextrose prolotherapy, therapeutic ultrasound, and low-level laser therapy respectively, the release of substance P from muscle afferents is a determined step shared among these physical therapy approaches [54,55,64]. Substance P is a neuropeptide discovered in 1930s and has been recognized as a pain neurotransmitter since 1960s [65-68]. In many animal models, upon noxious stimuli, nociceptors release substance P from their peripheral terminals to induce neurogenic inflammation and lead to peripheral sensitization; and from their central terminals to potentiate NMDA receptors and facilitate central sensitization [69]. Increased levels of substance P in CSF have been found in fibromyalgia patients [70]. However, the role of spinal substance P in pain modulation remains puzzle. Murphy and Zemlan (1987) showed that intrathecal injection of substance P induced hyperalgesia in naïve rats, whereas substance P could inhibit serotonin-induced spinal nociceptive reflexes [71]. In peripherals, intramuscular injection of substance P neither evokes pain nor induces neurogenic inflammation in humans [28]. In contrast, we have previously shown muscular substance P acts on NK1R to mediate an antinociceptive signaling in muscle afferents by activating Kv7 potassium via a G-protein-independent, but tyrosine kinase-dependent manner [72]. In the Sluka model, intramuscular substance P signaling can prevent the acid-induced hyperalgesic priming, whereas a single acid injection can directly lead to chronic hyperalgesia in mice lacking substance P [63,72].

Together, sngception can be antinociceptive via the release of substance P from muscle afferents, which is a shared signal pathway among many forms of physical therapy. The substance P-dependent antinociceptive signaling would be an ideal opioid-independent solution for intractable myalgia.

5. PERSPECTIVES AND CONCLUSION:

Based on the mouse models and clinical studies, acidosis is an effective risk factor to evoke myalgia associated fibromyalgia. From the molecular aspect, ASIC3 is a major molecular hub to trigger myalgia in response to physical stressors (e.g., acidosis or cold) or psychosocial stressors (e.g., sound stress) and repeated and/or intermittent activating ASIC3 would lead to chronic widespread muscle pain mimicking fibromyalgia symptoms. Also, ASIC1b and TRPV1 are essential acid sensors for myalgia. Paradoxically, activation of ASIC3 on muscle afferents via therapeutic ultrasound would trigger a substance P-dependent antinociceptive signaling to alleviate the fibromyalgia-like pain in mouse models. As ASICs are a trimeric channel, different compositions of ASIC3-containing channels might be differentially expressed in pro-nociceptive and anti-nociceptive muscle afferents. Further investigation of how sngception (acid-sensation) is executed in muscle afferents in a neuron subtype-specific manner would warrant a better understanding of myalgia and provide therapeutic insights for fibromyalgia.

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