ASICs Mediate Pain and Inflammation in Musculoskeletal Diseases

Chronic musculoskeletal pain is debilitating and affects ~20% of adults. Tissue acidosis is present in painful musculoskeletal diseases like rheumatoid arthritis. ASICs are located on skeletal muscle and joint nociceptors as well as on nonneuronal cells in the muscles and joints, where they mediate nociception. This review discusses the properties of different types of ASICs, factors affecting their pH sensitivity, and their role in musculoskeletal hyperalgesia and inflammation.

Overview of Musculoskeletal Pain

About 20% of the adult population suffers from chronic musculoskeletal pain (74, 83), a debilitating condition that is globally recognized as the second leading cause for disability (113). In the U.S., chronic musculoskeletal pain is estimated to cost over $950 billion annually, half of which is due to lost wages (3). Musculoskeletal pain conditions are complex in nature and can arise from traumatic and/or non-traumatic injury to any part of the musculoskeletal system, including muscles, joints, and bones (73, 99). Pain from the musculoskeletal system is often classified as inflammatory or non-inflammatory. Inflammatory musculoskeletal pain conditions are either localized to one site or joint, like osteoarthritis (11, 31), or they are widespread, like rheumatoid arthritis (70). In parallel, pain either can be localized to the site of injury/inflammation (primary hyperalgesia) or can spread to non-injured sites (referred pain or secondary hyperalgesia) depending on the site and nature of the inflammation (34, 99). Non-inflammatory musculoskeletal pain conditions are more complex and much more difficult to characterize or diagnose because of the lack of observable tissue damage or inflammation. Similar to inflammatory conditions, non-inflammatory conditions can be localized to a specific site, like back or neck pain, or they can be widespread, like fibromyalgia (24, 33, 33a, 99).

Musculoskeletal pain conditions are associated with a decrease in local tissue pH (20, 30, 41, 53, 93). For instance, the pH of synovial fluid extracted from joints of rheumatoid arthritis patients is lower than that of normal individuals (20, 30, 41). Similarly, trapezium muscles of myofascial pain patients, with myofascial trigger points, have higher proton concentrations than in individuals with no pain (93). Moreover, fatigued and inflamed muscles both have higher concentrations of protons and lactic acid than non-fatigued muscles (38, 43, 92). Furthermore, when acidic solutions are infused into a muscle in healthy human subjects, it produces both primary and secondary (referred) pain (34, 104, 106), as well as primary and secondary hyperalgesia (i.e., decreases in pressure pain thresholds) (34). Acid-sensing ion channels (ASICs) are the primary sensors of decreased tissue pH (118), are located on muscle and joint nociceptors as well as in nonneuronal cells in local tissue, and are implicated in musculoskeletal pain (2, 98). This review will provide an overview of the properties of different types of ASICs and their role in musculoskeletal nociception.

Biophysical Properties of ASICs

Fluctuations in extracellular neuronal pH occur as a result of multiple pathological and non-pathological conditions that include ischemia (60, 84), inflammation (30, 105), fatiguing exercise (107), and neuronal activity (29). Accordingly, neurons express specific ASICs to detect and respond to these pH fluctuations (63). ASICs are voltage-independent proton-gated sodium channels that belong to the degenerin-epithelial Na\textsuperscript{+} channel (DEG-ENaC) family of ion channels (118). They are activated by a drop in extracellular pH (up to pH 5.0), as well as an increase in extracellular pH (up to pH 8.0) (23). Under acidic conditions, these receptors are activated to produce an amiloride-sensitive, fast-rising, and rapidly desensitizing inward current (117), whereas, under basic conditions, they produce a sustained outward current (23). In addition to pH fluctuations, ASICs are also activated by mechanical stimulation and are suggested to act as mechanoreceptors (14, 82, 85, 86). For example, genetically altering ASIC3 (formerly known as DRASIC or dorsal root ganglia ASIC) enhances the sensitivity of mechanoreceptors that detect light touch but also diminishes the sensitivity of mechanonociceptors that detect noxious pinch (86). On the other hand, genetically altering ASIC2 (formerly known as BNC1 or brain sodium
channel-1) enhances the sensitivity of mechanoreceptors to light touch (85). In addition, ASIC2 is an important regulator of blood pressure and parasympathetic control of circulation by acting through baroreceptors on arterial sensory neurons (69, 75). Moreover, ASICs can be regulated by treatment with compounds like MitTx, amiloride, GMQ, FMRFamide, dynorphin A, big dynorphin, and Mambalgin (reviewed in Ref. 128).

There are six different ASIC subunits discovered to date; these include ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4 (1, 46, 65, 68, 87, 116, 128). These subunits are located throughout the central and peripheral nervous systems (103, 128) where they function as homomeric (identical subunits) or heteromeric (different subunits) trimers to sense changes in extracellular pH (63, 64, 118, 128). All ASIC subunits have the ability to assemble and form functional homomeric and heteromeric channels, except for ASIC2b and ASIC4, which only assemble to form heteromeric channels with other ASIC subunits and modulate channel activity (5, 46, 68).

The subunit composition of ASICs dictates the channel’s properties, including acid sensitivity, ion selectivity, and desensitization kinetics (10, 48). Activation of homomeric ASIC1a, ASIC1b, and ASIC2a receptors, expressed in heterologous CHO (Chinese hamster ovary cells), by rapid application of pH4 produces a transient activation current (48). On the other hand, activation of homomeric ASIC3 produces two different activation profiles. In some cells, it produces a transient activation current similar to that observed in other ASIC homomers (10, 48, 68), whereas in other cells it produces a biphasic inward current composed of a transient activating current followed by slower current (FIGURE 1, A–E) (7, 48). Furthermore, desensitization (τ desensitization) of homomeric ASIC1a, ASIC1b, and ASIC2a is dependent on pH where greater acidic pH results in greater desensitization. This is in contrast to ASIC3, where the desensitization rate is independent of pH (FIGURE 1, G–H) (48).

**FIGURE 1.** Acid-evoked currents and biophysical properties of homomeric ASICs

A–E: CHO-K1 cells transfected with homomeric ASICs were bathed in ringer solution and activated by rapid application of a pH 4 solution. F: proton affinities (pH sensitivity) for homomeric ASICs were measured by lowering the extracellular pH from 7.4 to 4. G and H: the rate of desensitization (τ desensitization) of ASICs was measured at different extracellular pH values and demonstrated that desensitization of all ASIC subunits except ASIC3 is increased when pH is lowered. Figure was adapted from Ref. 48, with permission from The Journal of Biological Chemistry.
Similarly, pH sensitivity is also dependent on ASIC subunit. Homomeric ASIC2a is the least sensitive, requiring the lowest activation pH (pH50 of ~4.5) (10, 12, 48, 128), and ASIC3 is the most sensitive, responding to pH within physiological ranges (pH50 of ~6.6) (10, 27, 108, 128). ASIC1a and ASIC1b have similar proton affinities (pH50 of ~4.1-5.8) (10, 48, 128); however, ASIC1a is activated across a wider pH range (Hill Slope = 0.75) than ASIC1b (Hill Slope = 4.8) (FIGURE 1F) (48).

Studying the functional features of homomeric ASIC subunits in heterologous cells helps explain some, but not all, of the observed acid-evoked currents produced by peripheral sensory neurons. For example, homomeric ASIC3, expressed in COS-7 cells, has similar pH sensitivity (activated at pH 7) and mimics the acid-evoked currents produced by cardiac sensory afferents (108, 124). However, the biophysical properties of H+–gated currents observed in medium to large dorsal root ganglia (DRG) neurons and skeletal muscle afferents differ from those observed in any homomeric receptor combination (10, 36), indicating that these currents are produced by heteromeric ASICs. Thus, in DRG, ASICs form heteromeric channels that have distinct properties and desensitization kinetics that differ from those formed by their homomeric subunits (5, 9, 10, 48, 95, 122). In addition, ASIC subunits also alter receptor trafficking and localization. For instance, ASIC1a localization on dendritic spines of hippocampal neurons is dependent on the presence of ASIC2 and their binding to postsynaptic density protein 95 (PSD-95) (47, 129).

In addition to their pH-dependent activation, ASICs are also activated and modulated by non-proton ligands at normal physiological pH (7.4) (119) that bind to a non-proton ligand-sensing domain (127). For instance, GMQ (2-guanidine-4-methylquinazoline) activates ASIC3 at normal pH and produces pain-related behaviors when injected into the hind paw in an ASIC3-dependent manner (127). Another ligand for this non-proton ligand-sensing domain is agmatine (AGM), a GMQ metabolite. Similar to GMQ, AGM produced hyperalgesia when injected intraplantarly, and inflammatory mediators (arachidonic acid and lactate) potentiate AGM-induced ASIC3 current and hyperalgesia (66). Furthermore, intraplantar injection of MitTx, the active component in Texas coral snake venom, activates both ASIC1 and ASIC3 at normal physiological pH and produces hyperalgesia (12). These data demonstrate that, in addition to protons, ASICs can be activated by non-proton natural ligands to produce pain.

**Distribution of ASICs**

The expression pattern and distribution of ASICs on sensory neurons shows they are ideally localized to play a role in musculoskeletal pain (78, 112, 116, 117). Acid-evoked currents in skeletal muscle sensory neurons are mediated by heteromeric ASICs comprised of ASIC1a, ASIC2, and ASIC3 subunits (2, 10, 36). In unidentified DRGs, ASIC1 is located on small- (<25 μm), medium-, and large-diameter cells (>40 μm), whereas ASIC2 and ASIC3 are mostly located on medium- and large-diameter cells (2). In small sensory DRG neurons, ASIC1a is expressed in 62% of substance P-positive cells and 41% of isolectin B4 (IB4)-positive cells (112), whereas ASIC3 is expressed in 50% of substance P-positive cells, 43% of IB4-positive cells (81, 112), 68% of TrkA-positive cells, 25% of TrkC-positive cells, and 12% of TRPV1-positive neurons (78). Moreover, small sensory neurons innervating skeletal muscles express more ASIC3 than those innervating the skin, and 80% of these ASIC3-positive skeletal muscle afferents co-express CGRP, affirming the role of these receptors in detecting muscle acidity and pain (78). Similarly, in DRG neurons innervating the knee joint, ASIC3 is located on 31% of joint afferents and colocalizes with 20% of CGRP-positive cells (52).

In the CNS, ASIC subunits are also localized in regions that are involved in nociceptive transmission and plasticity (32, 117). For instance, ASIC1a, ASIC2a, and ASIC2b are all expressed in dorsal horn neurons of the spinal cord, a region where nociceptive signals are received from DRGs and relayed to the brain (8, 28, 121). ASIC1a is expressed in the peri-aqueductal grey a primary brain region responsible for descending pain modulation (19). ASICs are also localized to nonneuronal cells like muscles (FIGURE 2A), synoviocytes (FIGURE 3, D AND E), macrophages, and dendritic cells, where they act as pH sensors that participate in nociception and inflammation (42, 61, 62, 111, 114).

**ASICs and Nociception**

**ASICs and Non-inflammatory Muscle Pain**

Skeletal muscle pain is associated with tissue acidosis (53, 93), and infusion of acid into muscle produces pain (34, 104, 106). In humans, intramuscular infusion of an acidic solution (pH 5.2) into the tibialis anterior muscle of the leg produces local muscle pain at the site of infusion and referred pain at the ankle (34). The acid infusion also results in decreased pressure pain thresholds at the site of infusion and in the referred pain area at the ankle (34), demonstrating that human muscle nociceptors are activated by acidic pH to produce...
both primary and secondary hyperalgesia. The pH that activates ASICs on muscle afferents is affected by the presence of muscle metabolites (67). For instance, <4% of group III and IV DRG muscle afferents respond to pH values of >6. However, when these DRG neurons are treated with a combination of ATP and lactate at pH 7.4, the percentage of responding neurons increases to 44% (56, 67). This effect is blocked by the ASICs antagonist A-317567 (67), indicating that ASICs located on muscle nociceptors are activated at neutral physiological pH.

In mice, chronic widespread mechanical hyperalgesia develops after two injections of pH 4 saline, given 5 days apart, into the gastrocnemius muscle (16, 94, 100). The hyperalgesia develops at the site of injection in the muscle, in a referred area in the paw, contralaterally in the muscle and paw, as well as in the viscera (77, 94, 100, 126). This hyperalgesia is not accompanied by observable tissue damage of the muscle or nerve (37, 100). This model mimics chronic widespread pain conditions like fibromyalgia and is sensitive to similar pharmacological and nonpharmacological treatments (4, 25). The first acid injection produces a transient hyperalgesia and primes muscle nociceptors for the chronic hyperalgesia (30 days) produced by the second injection (16, 100). Co-injection of the ASIC3 antagonist APETx2 with the first acid injection attenuates the initial transient hyperalgesia and abolishes the priming activity, such that the second injection of acid only produces a transient hyperalgesia instead of the long-lasting hyperalgesia (15, 16). Moreover, when APETx2 is co-injected with the second injection of acid, it also prevents the chronic hyperalgesia (59), indicating that ASIC3 contributes to the priming of nociceptors as well as development of the chronic hyperalgesia. However, when an ASIC antagonist is given after the development of hyperalgesia (i.e., after the second acid injection) it does not reverse primary hyperalgesia.

**FIGURE 2.** ASICs are expressed on muscle tissue and play a role in carrageenan-induced inflammatory muscle pain

A and B: representative muscle tissue sections from wild-type (A) and ASIC3−/− (B) mice, showing ASIC3 (red) and DAPI (nuclear stain, blue) staining. Figure reproduced from Ref. 114 with permission. C: genetic knockdown of ASIC3 in primary afferent fibers innervating muscle, using HSV-miRNA to ASIC3 (red, HSV-miR844), prevents the development of primary muscle hyperalgesia induced by intramuscular carrageenan injection compared with animals treated with control HSV-miRNA (blue). *Significantly greater than controls (P < 0.05). Reproduced from Ref. 114 with permission. D: muscle withdrawal thresholds (primary hyperalgesia) before and after induction of muscle inflammation in WT (red), ASIC3−/− (blue), and ASIC1−/− (green) mice. ASIC1−/− do not develop muscle hyperalgesia 24 h after inflammation with carrageenan compared with WT and ASIC3−/− mice. *Significantly greater than WT and ASIC3−/− (P < 0.05). Reproduced from Ref. 115 with permission. E: responses to repeated mechanical stimulation of the paw (secondary hyperalgesia) before and after induction of muscle inflammation in WT (red), ASIC3−/− (blue), and ASIC1−/− (green) mice. ASIC3−/− do not develop secondary hyperalgesia 24 h after inflammation with carrageenan compared with WT and ASIC1−/− mice. Reproduced from Ref. 115 with permission. F: genetic knockdown of ASIC3 in primary afferent fibers innervating muscle, using HSV-miRNA to ASIC3 (red, HSV-miR844), prevents development of secondary hyperalgesia induced by intramuscular carrageenan compared with animals treated with control HSV-miRNA (blue). *Significantly greater than controls (P < 0.05). Reproduced from Ref. 114 with permission.
the hyperalgesia, suggesting that ASIC3 is not involved in the maintenance of non-inflammatory hyperalgesia (37, 59).

The hyperalgesia induced by repeated acid injections does not develop in ASIC3−/− mice; however, ASIC1−/− mice still develop hyperalgesia after repeated acid injections (101). Twenty-four hours after the second injection, when animals are hyperalgesic, there are no changes in ASIC-like current properties in DRG neurons innervating the injected muscle (37). On the other hand, sensitization of neurons in the spinal cord parallels the widespread hyperalgesia in this model with expansion of receptive fields to include the contralateral hind limb and enhanced responsiveness to mechanical stimuli. These changes in dorsal horn neurons do not occur in ASIC3−/− mice (101). Once developed, this hyperalgesia is therefore independent of ASICs but appears to be mediated through changes in spinal and supraspinal mechanisms, including activation of the PKA-cAMP-pCREB pathway in the spinal dorsal horn (49), activation of the PKC-ERK pathway in the capsular central amygdaloid nucleus (18), and activation of NMDA and non-NMDA glutamate receptors in the spinal cord and brain stem (21, 90, 97).

Another model of non-inflammatory muscle nociception is induced by subcutaneous injection of reserpine (79, 80, 109). This model shares common aspects of chronic widespread pain conditions, including the long-lasting tactile and deep tissue hyperalgesia, dysfunction of descending pain inhibitory systems, and depression with the absence of observable damage (79, 80, 109). Subcutaneous injections of reserpine for 3 days depletes biogenic amines (dopamine, norepinephrine, and 5-hydroxytryptamine) in the spinal cord, thalamus, and prefrontal cortex. The reserpine-model increases expression of ASIC3 in DRG and produces widespread muscle and tactile hyperalgesia that is reversed by subcutaneous injection of APETx2 (79, 80, 109). Thus ASIC3 plays a role in the development of chronic widespread pain.

ASICs and Inflammatory Muscle Pain

The microenvironment of inflamed tissue is usually more acidic than surrounding normal tissue (53, 105). Both ASIC1 and ASIC3 are expressed on innate immune cells like macrophages and dendritic cells (62, 111), suggesting a possible role for ASICs in the inflammation process. In support, extracellular acidosis increases expression of maturation markers like CD80, CD86, and MHCII on the surface of macrophages and dendritic cells through activation of ASICs (62, 111). Acidosis also stimulates human monocytes to produce IL-1β, a proinflammatory cytokine involved in pain and inflammation (55, 91). In neurons, inflammatory mediators enhance ASIC signaling and expression. For example, proinflammatory mediators like NGF, serotonin, interleukin-1, and bradykinin increase the number of ASIC-expressing neurons and enhance the ASIC-like current intensity in sensory and nociceptive DRG neurons (71, 72, 112). Intramuscular injection of carrageenan results in infiltration of proinflammatory immune cells to the site of injection (22, 35), extravasation of red blood cells, rhabdomyolysis, and vasculitis (125), and enhanced expression of ASIC3 on nerve fibers surrounding blood vessels (125). Both the extravasation of red blood cells and vasculitis are attenuated in ASIC3−/− mice (125), indicating that ASIC3 plays a role in inflammatory pathogenesis. Thus ASICs may alter immune cell function to enhance release of inflammatory mediators that can subsequently activate nociceptors.

Carrageenan-induced muscle inflammation is a commonly used animal model of inflammatory muscle pain and is accompanied by primary hyperalgesia measured directly by squeezing the muscle with tweezers, and secondary hyperalgesia measured by applying noxious stimuli to the paw (35, 88). In animals with muscle inflammation, primary hyperalgesia does not develop in the ASIC1−/− mice, whereas secondary hyperalgesia does not occur in ASIC3−/− mice (FIGURE 2, D AND E) (115, 125), suggesting primary hyperalgesia is mediated by ASIC1 and secondary hyperalgesia is mediated by ASIC3. Reexpressing ASIC3 into primary afferents innervating muscle (using an HSV-1 vector), but not the skin, of ASIC3−/− mice reestablishes the hyperalgesia normally observed in wild-type mice with muscle inflammation. In contrast, selective knockdown of ASIC3 in DRG innervating the inflamed muscle, using miRNA (HSV-miR844), prevents development of both primary and secondary hyperalgesia (FIGURE 2, C AND F) (114). Interestingly, in CHO cells cotransfected with ASIC1 and ASIC3, knockdown of ASIC3 reduces the amplitude of pH-evoked currents, suggesting an overall inhibition of the surface expression of heteromeric channels that contain ASIC3 (i.e., ASIC3 was required for trafficking to the membrane) (114). In parallel, nonselective pharmacological blockade of ASICs using A-317567 reverses both the primary and secondary inflammatory muscle hyperalgesia produced by carrageenan, demonstrating the importance of ASICs in the maintenance of inflammatory muscle pain (115). Furthermore, DRG innervating the inflamed muscle shows increased pH-evoked current amplitudes and a lower rate of recovery from desensitization compared with DRG from control un-inflamed mice (38), and carrageenan-induced muscle inflammation increases mRNA expression of ASIC2 and ASIC3 in DRG (115). Thus alteration in ASIC expression and function mediates the hyperalgesia associated with carrageenan-induced inflammation.
**ASICs and Inflammatory Joint Pain**

Rheumatoid arthritis is a debilitating disease characterized by inflammation, stiffness, and pain of joints (57, 58, 73, 83, 96, 99). Joint inflammation is usually associated with a decrease in local synovial fluid pH to as low as pH 6 (20, 30, 39–41), within the physiological pH range for activation of ASIC3 (10, 48). ASIC3 is present on...
primary afferents innervating the knee joint, articular cartilage, growth plate, meniscus, and Type B synoviocytes lining the knee joint (FIGURE 3, D AND E) (51, 52, 61), suggesting that ASIC3 could be involved in both arthritic pain and inflammation.

Localized inflammatory hyperalgesia is modeled by injection of carrageenan into a single joint (45, 89). This results in infiltration of inflammatory cells that persists for months and is accompanied by primary and secondary hyperalgesia (45, 51, 89). Carrageenan-induced joint inflammation increases expression of ASIC3 and colocalization of ASIC3 with CGRP on primary afferent fibers innervating the synovium. In DRG innervating the knee joint, ASIC3 expression increases from 31% to 50%, CGRP expression increases from 30% to 50%, and colocalization of ASIC3 and CGRP increases from 19% to 31% (51, 52). Interestingly, ASIC3−/− mice with knee joint inflammation only develop primary and not secondary hyperalgesia, indicating that ASIC3 mediates the development of secondary hyperalgesia in this model (51). Thus, like muscle inflammation with carrageenan, there is upregulation of ASIC3 in primary afferents innervating the inflamed tissue, and ASIC3 mediates secondary hyperalgesia.

Another model used to study arthritic pain is the collagen-induced arthritis model (CAIA), which mimics widespread inflammatory arthritis (e.g., rheumatoid arthritis) (45). Injecting mice with an antibody to collagen Type II results in widespread distal joint inflammation, joint destruction, and enhanced joint expression of inflammatory markers [e.g., IL-6, MMP-3 (metalloprotease-3) and MMP-13] (102). In parallel, there is primary hyperalgesia at ankle joint, secondary hyperalgesia of the skin on the paw, and accompanying decreased activity levels that lasts for days after induction of CAIA (102). Similar to carrageenan models, ASIC3−/− mice with CAIA do not develop secondary hyperalgesia of the paw but still develop primary hyperalgesia (102). Furthermore, ASIC3−/− mice with CAIA have higher activity levels than wild-type mice with CAIA.

Interestingly, despite the decreased hyperalgesia, these ASIC3−/− mice show greater inflammation, more joint destruction, and increased inflammatory mediator mRNA than their wild-type littermates (FIGURE 3, A–C) (42, 102). In addition, cultured fibroblast-like synoviocytes (FLS) from wild-type mice show increased release of hyaluronan, increased intracellular calcium concentrations, and reduced phosphorylation of ERK (p-ERK; extracellular signal-regulated kinase) to acidic pH. After treatment of FLS with the inflammatory mediator IL-1β, there is increased expression of ASIC3, and acidic pH enhances intracellular calcium concentrations and p-ERK, and produces cell death (FIGURE 3, F–I) (42, 61, 102). The increases in hyaluronan and intracellular calcium, and decreases in p-ERK to acidic pH do not occur in ASIC3−/− mice. Furthermore, the cell death to the combination of IL-1β and acidic pH also does not occur in ASIC3−/− FLS (42, 61, 102) and is prevented by blockade of intracellular calcium and ERK. Together, these data suggest that ASIC3 plays a protective role in inflammatory arthritis by reducing synovitis and the accompanying inflammation and joint destruction through facilitation of synoviocyte cell death, and thus ASIC3 activation

FIGURE 3. ASIC3 mediates the nociception produced by collagen-antibody-induced arthritis, while protecting the joint from inflammation and destruction

A: ASIC3−/− mice (blue) show enhanced inflammation after induction of collagen antibody-induced arthritis (CAIA) compared with WT mice (red). *Significantly greater than controls (P < 0.05). Reproduced from Ref. 102 with permission. B: CAIA-induced secondary hyperalgesia of the paw (number of responses to repeated mechanical stimuli) was attenuated in the ASIC3−/− mice (blue) compared with wild-type mice (red). *Significantly lower than wild-type mice (P < 0.05). Reproduced from Ref. 102 with permission. C: Representative sections from the ankle joint of animals 12 days after induction of CAIA stained with Safronin-0 from wild-type (ASIC3−/−) and ASIC3−/− mice. Notice enhanced inflammation and damage in tissue section from an ASIC3−/− mouse. Reproduced from Ref. 102 with permission. D: fluorescent micrograph from the synovium of the knee joint immunohistochemically stained for ASIC3 in wild-type mouse. Inset shows ASIC3 immunohistochemistry in synovium from ASIC3−/− mice. Reproduced from Ref. 61 with permission. E: fluorescent immunohistochemical staining for ASIC3 (red) in cultured fibroblast-like synoviocytes (FLS). Nuclei are stained with TO-PRO3 (blue). Reproduced from Ref. 61 with permission. F: intracellular calcium concentration ([Ca2+]i) was calculated in wild-type (black) and ASIC3−/− (red) FLS, loaded with [Ca2+]i-sensitive fluorescent indicator Oregon Green BAPTA-1 AM (OGB-1), in response to exposure to decreasing pH values from 6.8 to 5 (150 s incubation). The response was normalized as percentage change from pH 7.4. Reproduced from Ref. 42 with permission. G and H: live/dead assay of cells of cultured FLS extracted from wild-type (G) and ASIC3−/− (H) mice. FLS were treated with pH 6 after prior incubation with IL-1β. Green staining represents live cells, whereas red staining represents dead cells. Reproduced and modified with permission from Ref. 102. I: the average % dead cells after treating FLS cells with pH 6 and IL-1β in wild-type (red) and ASIC3−/− (blue) FLS. Notice a significant increase in the number of dead cells in wild-type mice treated with pH 6 and IL-1β (•) that did not occur in ASIC3−/− mice treated with pH 6 and IL-1β (•). Reproduced and modified with permission from Ref. 102. J: schematic presentation for the protective role of ASIC3 in collagen-induced arthritis model (CAIA). In this model, the acidic environment associated with inflammation activates ASIC3 on FLS. Activation of ASIC3 enhances intracellular Ca2+ release, leading to cell death, which limits the secretion of inflammatory cytokines and metalloproteases (MMPs) by FLS and limits joint damage (42). ASIC3 located on neurons is also activated by decreases in pH associated with inflammation, which increases pain limiting joint function, to ultimately decrease joint damage. The red arrows show the proposed pathways for activation of ASICs in FLS and in neurons, and how they ultimately lead to reduced joint damage.
may reduce disease severity (FIGURE 3J). In addition, ASIC3 may provide joint protection by increasing the secretion of hyaluronan (61), an important component of articular cartilage that protects it from damage due to compression and inflammation (17). Lastly, ASIC3 activation on nociceptors results in pain and reduced mobility during inflammation that may serve to protect the joint from excessive damage (FIGURE 3J).

Injection of mono-iodoacetate into a single joint is a model of degenerative knee osteoarthritis that results in destructive histological changes that include cartilage degeneration, loss of chondrocytes, thickening of subchondral bone, and osteophyte formation in the knee joint (45, 54). This model induces nociceptive behaviors as measured by reduced weight bearing on the injured limb and enhanced sensitivity to noxious mechanical stimuli of the paw (secondary hyperalgesia) (54). Intra-articular injection of the ASIC3 antagonist APETx2 in this model attenuates cartilage damage and hyperalgesia, indicating that, in this model, ASIC3 contributes to joint damage rather than to joint protection (54).

Fatigue-Induced Muscle Pain

Muscle fatigue is the failure of a muscle to produce force as a result of excessive exercise (110) and can be associated with pain (76, 120). During muscle fatigue, tissue pH drops to ~6.5 (43, 92), within the physiological pH range for activation of ASIC3 (10, 48). Moreover, fatigue is characterized by accumulation of muscle metabolites like lactic acid that can sensitize ASICs on skeletal muscle nociceptors to normal pH values of 7.3 (67, 76, 120). Our laboratory developed a mouse model of fatigue-induced hyperalgesia by combining low-intensity muscle insults (pH 5.0 saline) with 6 min of fatiguing contractions of the gastrocnemius muscle (126). The fatiguing contraction decreases pH, and the combination of muscle insult with fatigue produces muscle hyperalgesia that lasts for 2–4 wk (44). The fatigue-induced hyperalgesia is abrogated by pharmacological blockade of ASIC3 with APETx2 and genetic deletion of ASIC3 using ASIC3−/− mice. Interestingly, downregulating ASIC3 in primary afferents innervating the muscle (using HSV-miR844) has no effect on this hyperalgesia. Instead, there is an upregulation of macrophages after the fatiguing stimulation, and deletion of muscle macrophages using clodronate liposomes attenuates the hyperalgesia (43). Similarly, eccentric exercise produces fatigue and results in hyperalgesia that lasts for days (referred to as delayed-onset muscle soreness); this hyperalgesia is reversed by the nonselective ASIC antagonist amiloride (35). Together, these data suggest that hyperalgesia develops after decreases in pH produced by fatigue combined with a muscle insult and that ASIC3, potentially on local muscle macrophages, mediates the development of hyperalgesia in response to fatigue.

Other Models of Muscle Pain

Postoperative muscle pain. Brennan and colleagues developed a rat model of postoperative pain that produces hyperalgesia after incision of the skin and muscle (13). In this model, the proportion of group III and group IV muscle afferent fibers sensitive to lactic acid (pH 6.0) stimulation increases from 20.8% in sham muscles to 55.4% in incised muscles (123). Incision also increases the expression of ASIC3 on muscle afferent fibers, most of which are small diameter, by 13% (26). In addition, thermal and mechanical hyperalgesia produced by incision are attenuated in ASIC3−/− mice and by blockade of ASIC3 with APETx2 (26). Thus ASIC3 appears to play a role in mediating postoperative pain associated with muscle incision.

Chemotherapy-induced muscle pain. ASIC3 also plays a role in muscle pain induced by the anticancer drug cisplatin. Systemic administration of cisplatin, once a week for 5 wk, produces muscle hyperalgesia that is attenuated by the nonselective ASIC antagonist amiloride. In addition, cisplatin increases ASIC3 expression on muscle primary afferent fibers, indicating that cisplatin-induced muscle pain is mediated by ASIC3 activation (50).

Summary

Tissue acidosis occurs in most musculoskeletal pain conditions, including inflammatory, non-inflammatory, and fatigue-induced musculoskeletal pain. Consistently, injecting or infusing acid into muscle produces both primary and secondary (referred) musculoskeletal pain, and primary and secondary hyperalgesia. ASICs are not only localized in primary afferent fibers innervating muscle and joint tissue but also in nonneuronal cells that can communicate with nociceptors including muscles, cartilage synovium, and immune cells. ASIC expression on skeletal muscle and joint afferents colocalizes with other pain-producing peptides (substance P and CGRP) and receptors (TrkA and TRPV1), and are modifiable by extracellular mediators including cytokines and fatigue metabolites. ASICs clearly play a role in multiple models of musculoskeletal pain as well as in the inflammatory process. Activation of ASICs is clearly pro-nociceptive in musculoskeletal models of hyperalgesia, including non-inflammatory and inflammatory models. Of all the ASICs, ASIC1 and ASIC3 are the most implicated in musculoskeletal hyperalgesia. In non-inflammatory pain models, ASIC3 is required for induction of both primary and secondary hyperalgesia. In inflammation models, ASIC1 is responsible for primary
hyperalgesia, whereas ASIC3 is responsible for secondary hyperalgesia. Inflammatory models are associated with increases in expression of ASIC3 in nociceptors and are involved in both the induction and the maintenance of the hyperalgesia.

Despite mediating nociception, ASICs can also modulate inflammation, and this modulation is dependent on tissue type, cell type, and animal model. On immune cells, protons can enhance release of inflammatory mediators and increase expression of maturation markers. In muscle inflammation, ASIC3 is expressed in mouse tissues, and red blood cell extravasation, and, in a joint inflammation model, blockade of ASIC3 prevents joint destruction. On the other hand, in an inflammatory arthritis model, there is enhanced inflammation and tissue damage. Furthermore, it may be possible to target ASICs with ASIC agonists to alter acidic pH combined with an inflammatory mediator.

Therefore, targeting of ASICs, with ASIC antagonists, for musculoskeletal pain therapy should be done with careful attention to potential effects on non-nociceptive processes, including inflammation and tissue damage. Furthermore, it may be possible to target ASICs with ASIC agonists to alter disease severity by limiting synovitis in those with inflammatory arthritic conditions like rheumatoid arthritis. Thus future experiments will need to be conducted to examine how to best target ASICs for the treatment of pain and inflammation in a disease-specific and tissue-specific manner.

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