Ion channels involved in inflammation and pain in osteoarthritis and related musculoskeletal disorders

Running title: Ion channels in osteoarthritis pain

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Abstract

Osteoarthritis (OA) is a currently incurable, chronic, progressive and debilitating musculoskeletal condition. One of its hallmark symptoms is chronic nociceptive and neuropathic pain, which significantly reduces the quality of life of OA patients. While research into the pathomechanisms of OA pain is ongoing and several pain pathways are well understood, the true source of OA pain remains unclear. Ion channels and transporters are key mediators of nociceptive pain. In this narrative review article, we summarize the state-of-the-art in relation to the distribution and function of ion channels in all major synovial joint tissues in the context of pain generation. We provide an update on the ion channels likely involved in mediating peripheral and central nociceptive pathways in the nervous system in OA pain, including voltage-gated sodium and potassium channels, members of the transient receptor potential (TRP) channel family, and purinergic receptor complexes. We focus on ion channels and transporters that have a potential to be candidate drug targets for pain management in OA patients. We propose that ion channels expressed by the cells of constituent tissues of OA-afflicted synovial joints including cartilage, bone, synovium, ligament and muscle, should be more thoroughly investigated and targeted in the context of OA pain. Based on key findings from recent basic research articles as well as clinical trials, we propose novel directions for the development of future analgesic therapies to improve the quality of life of OA patients.

(234 words in abstract)

New & Noteworthy

- Pain in osteoarthritis involves complex mechanisms, some of which are currently not well-understood.
- Ion channels are involved in pain generation and transmission.

- While current knowledge regarding the role of ion channels in pain pathways is convincing, their involvement and specific functions in peripheral joint tissues are less clear.
- A better understanding of how ion channels contribute to OA pain may offer new insights into the development of more specific, effective, safer and personalized analgesic treatment.

Keywords

osteoarthritis; pain; channelome; ion channels; analgesics; synovial joint; nociception

List of abbreviations

AA, adjuvant arthritis; ASIC, acid-sensing ion channel; BDNF, brain-derived neurotrophic factor; CIP, congenital insensitivity to pain; CNS, central nervous system; DALY, disability-adjusted life years; DCT, distal convoluted tubule; DEG, differentially expressed genes; DMOAD, disease-modifying OA drugs; DRG, dorsal root ganglion; ECM, extracellular matrix; GDNF, glial cell line-derived neurotrophic factor; HCN, hyperpolarization-activated cyclic nucleotide-gated channel; JNK, Jun N-terminal kinase; MIA, monoiodo-acetic acid; MSK, musculoskeletal; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; NSAID, non-steroidal anti-inflammatory drugs; OA, osteoarthritis; PNS, peripheral nervous system; PTHLH, parathyroid hormone-like hormone; RA, rheumatoid arthritis; TRP, transient receptor potential; VGCC, voltage-gated calcium ion channel; VGSC, voltage-gated sodium ion channel; ZOL, zoledronic acid.

1. Introduction

Musculoskeletal disorders, which include rheumatoid arthritis (RA), osteoarthritis (OA), low back pain, neck pain and gout, are ranked 5th among all diseases in disability-adjusted life years (DALYs), and ranked 1st in years lost due to disability in the Global Burden of Disease study in 2017 (1, 2). Nevertheless, musculoskeletal (MSK) disorders have generally received little attention by governments and key decision makers, as they are rarely fatal, and they are assumed to be mostly irreversible age-related conditions (3). However, the global burden of MSK disorders is steadily rising, mainly due to the increasing life expectancy and growing levels of obesity (4).

OA is the most common form of inflammatory joint disorders. It is one of the leading causes of chronic pain, resulting in long-term physical disability (5). OA primarily affects the major weight bearing joints. The knees and hips are most frequently involved, but hand OA is also an important and severe condition (6). The etiology of joint damage in OA is multifaceted. Damage may be inflicted by repeated excessive or inappropriate mechanical loading on the joint through injury or by the cumulative impact of low-grade inflammation over time caused by inflammaging (7). One of the hallmarks of OA is loss of articular cartilage structure and function (8), which leads to joint pain and structural changes, affecting mobility (9). In addition to causing severe physical impairment, such debilitating symptoms can also affect the psychosocial wellbeing of patients; therefore, OA is not only a disease of articular cartilage, but a disorder of all synovial joint tissues, affecting the individual as a whole, and impacting on quality of life (9). In light of the lack of disease-modifying OA drugs (DMOADs), the management of OA must be multi-modal, multi-disciplinary and personalized, focusing on integrative pharmacological and non-pharmacological treatments including self-management through education, weight loss, exercise, biomechanical interventions, acupuncture, and electrotherapy (10).

Despite the limitations in our current understanding of OA pathogenesis and underlying biology (11), it is now increasingly accepted that OA is a heterogeneous and multifaceted disease with several subtypes, featuring multiple anatomical morphotypes, clinical phenotypes and molecular endotypes (9, 12). There is now a general consensus that chronic low-grade inflammation is a key pathophysiological process in OA (13). Pro-inflammatory mediators including cytokines, proteases, neuropeptides, chemokines, prostaglandins, neurotrophins, gaseous mediators and lipids are released (14), resulting in joint tissue damage and inflammation (synovitis), which induce a cascade of events that leads to peripheral sensitization, triggering nociception and joint pain (15). Indeed, pain is one of the hallmark symptoms of OA and is the main reason patients consult with their general practitioner or specialist, adding to the rising cost burden of healthcare systems (16). According to its most recent definition, pain is "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (17).

OA patients often experience different types of pain including a dull aching pain or intermittent pain with varying intensity. At first, pain is activity-related and subsequently becomes constant over time (18). Chronic pain negatively influences mental health, sleep, and social activity, affecting the overall quality of life, thereby imposing a great socio-economic burden on individuals, families, employers and the society as a whole (16, 19). Conventionally, non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and opioid analgesics are the most commonly prescribed drugs for the management of OA pain. However, there are significant side-effects associated with the long-term use of these symptom-modifying drugs. However, because the pathogenesis of OA pain is not completely understood, the current strategy for the management of OA pain is insufficient for delivering satisfactory pain relief for patients (16). Furthermore, the chronic use of analgesics is often associated with significant side effects (20) and could potentially even worsen OA symptoms (21).

While pain is the main symptom in OA, the source of pain is unclear (*Figure 1*). One of the greatest unresolved challenges in OA research is understanding the underlying pathogenic processes and the initial mechanisms involved in nociceptive pain (22). It is worth noting that the synovium, the joint capsule, ligaments and muscles, and the subchondral bone are all heavily innervated with peripheral nerves; articular cartilage, in contrast, has gained little attention in this regard and has been largely dismissed as the potential source of OA pain. However, chondrocytes may play a role in inflammatory pain by producing mediators which can sensitize and activate peripheral nerves in adjacent tissues (23). These inflammatory mediators can in turn influence ion channels on nociceptive sensory nerve endings. Damage to articular cartilage, particularly towards the basal layer, causes upregulation and release of nerve growth factor (NGF) and other pain-inducing molecules, to sensitize local pain fibers and induce neoinnervation of the tissue (24). Ion channels and transporters, located in the chondrocyte cell membrane, in osteocytes, synoviocytes, tenocytes, muscle fibers or in afferent nerve endings innervating the joint, could be safe, effective, promising candidate targets for drugs to reduce pain and improve the quality of life of OA patients (*Figure 2*).

Ion channels are undoubtedly key mediators of nociceptive pain. In this narrative review, we summarize the current knowledge on the distribution of ion channels in all major synovial joint tissues in the context of pain generation and provide an update on the ion channels that are involved in mediating peripheral and central nociceptive pathways in the nervous system in OA pain. We highlight the ion channels and transporters with a potential to be targeted for pain management in OA patients.

2. Ion channels involved in inflammation and pain in OA joints

Ion channels are transmembrane proteins that allow for the passage of various ions into or out of the cell (25). They are often made of multiple proteins forming a central aqueous pore

that opens and closes through conformational changes. Ion channels are classified based on their gating mechanisms, i.e., the mechanism through which they open or close. Conformational change is thus mediated depending on the type of gating of the channel—this may be voltage, chemical or mechanical gating (26). Patch clamp techniques were developed long before molecular biology techniques for studying ion channels in excitable membranes (27). Chondrocytes, although conventionally classified as non-excitable cells, are characterized by a plasma membrane that is rich in ion channels and transporters—the chondrocyte channelome. To date, several classes of ion channels have been identified and partially characterized in chondrocytes, including sodium channels (epithelial sodium channels, voltage activated sodium channels), potassium channels (e.g., ATP-dependent potassium (K(ATP)) channels), non-selective cation channels or transient receptor potential (TRP), as well as calcium and chloride channels (26). These ion channels, being responsible for ion conductance, serve various functions, including regulation of the resting membrane potential, pH sensing, mechanotransduction, cell volume regulation, and cell proliferation. Ion channels serve as biomarkers in synovial joints—through protease cleavage that releases by-products that act as biomarkers in the serum and synovial joint. They also act as specific receptors for ligands and are instrumental components of larger molecular assemblies in signaling cascades (28).

OA pain is a classic example of nociceptive pain, which arises from the abnormal loading of a damaged joint. In this setting, altered joint biomechanics open mechanosensitive ion channels, leading to pain sensation (15, 29).

2.1. Ion channels in the cells of musculoskeletal tissues

The musculoskeletal system is composed of tissues with various characteristics and functions, including cartilage, muscle, bone, synovium, tendons, and ligaments (30). The cells that make up the musculoskeletal system are also varied, and only muscle cells are electrically

excitable cells. Still, bioelectric signaling, controlled by ion channels in the plasma membrane, is fundamental to both excitable and non-excitable cells. Such bioelectrical signals are involved in the excitation generation and impulse conduction of muscle fibers, regulation of proliferation, migration, differentiation, apoptosis, and matrix production, and act as key sensors and transducers of extracellular signals in non-excitable cells including chondrocytes, bone cells, and synoviocytes. Multiple functional ion channels have been reported in different types of cells of the musculoskeletal system. The chondrocyte channelome is well characterized (26, 28, 30), and much is known about ion channels that control the functions of osteoblasts and osteocytes (31, 32). Current knowledge is more restricted in terms of the channelome of fibroblast-like synoviocytes (33-35), and even less is known about ion channels in the cells of ligaments and tendons (36). The plasma membrane ion channel complement in muscles, however, is better characterized (37).

2.2. Ion channels involved in inflammation and chronic pain

Genetic studies on blood samples from OA patients have demonstrated associations with genes encoding channels or transporters. These include the phospholipid transporter TMEM30A; the parathyroid hormone-like hormone PTHLH, which also inhibits voltage-gated calcium channel activity in neurons; the osteogenic transcription factor RUNX2, which regulates chondrocyte pannexin channels; and SMAD3, which inhibits the transcription of an acid-sensing ion channel (ASIC3) in cells of the nucleus pulposus (23). Members of the pannexin family, i.e., pannexin 1 (Panx1) can form non-selective, large-pore plasma membrane ion channels that act in conjunction with ligand-gated NMDA and P2X receptors (38). Pannexin channels are involved in the release of ATP from cells, and the activation of cell death pathways and the inflammasome (39).

Another set of genome-wide association studies (GWAS) carried out on blood samples identified five genes that are directly associated with OA pain, three of which encode ion channels: the alpha subunit of voltage-gated sodium channel $Na_V1.7$ (SCN9A); the transient receptor potential channel vanilloid subtype 1 (TRPV1); and the purinergic ligand-gated ion channel P2X7 (40). In sensory nerve cells, both TRPV1 and ASIC3 have been implicated in mediating OA-related pain (41, 42). The TRPV1 antagonist APHC3 significantly improved the symptoms of monoiodo-acetic acid (MIA) induced OA in mice; it reduced inflammation and pain and prevented cartilage degradation (43). P2 purinergic receptor-mediated signaling is known to be altered in OA (44). In particular, P2X7 is involved in regulating inflammation and chronic pain in the central nervous system (45), and it is an important modulator of OA-associated cartilage inflammation by targeting the NF-κB pathway (46).

2.3. Ion channels involved in inflammation and pain in articular cartilage and synovium

Several ion channels and transporters have been implicated in mediating inflammation and pain in cartilage and the synovium in musculoskeletal disorders (*Table 1*). In a global gene expression analysis of human OA articular cartilage (47), certain transporter genes were differentially expressed among the top differentially expressed genes (DEGs), including the facilitated glucose transporters 1 and 3 (GLUT-1 & 3, SLC2A1 & SLC2A3), the sodium- and chloride-dependent creatine transporter 1 (SLC6A8), the sodium-coupled neutral amino acid transporter 3 (SLC38A3), zinc transporter 5 (SLC30A5), multidrug and toxin extrusion protein 1 (SLC47A1), and UDP-xylose and UDP-N-acetylglucosamine transporter (SLC35B4). Taken together, these findings suggest that altered transport processes expressed in articular chondrocytes in an inflammatory micro-environment need to be further considered as initiating factors and targets for pharmaceutical therapies.

Since glutamate concentrations are significantly elevated in the synovial fluid in OA and RA patients (48), it is logical to hypothesize the involvement of glutamatergic signaling in these diseases. Indeed, in addition to *N*-methyl-D-aspartate (NMDA) glutamate receptors (GluRs), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (KA1) glutamate receptors are also expressed in human OA chondrocytes; furthermore, intra-articular injections of GluR antagonists alleviate cartilage and bone destruction in arthritis (49). Transient receptor potential ankyrin 1 (TRPA1), an ion channel known to be involved in nociception, pain and inflammation, was found to mediate acute inflammation and degenerative changes in articular cartilage and joint pain in a murine model of OA (50, 51). TRPA1 regulates the synthesis and release of the pro-inflammatory cytokine IL-6 in chondrocytes (52). TRPA1 is also implicated in mediating inflammation and degeneration in the annulus fibrosus of the intervertebral disc (53). Furthermore, DNA methyltransferase 3 beta (DNMT3B) may suppress inflammation and alleviate the effects of intervertebral disc degeneration by methylating the TRPA1 promoter (54).

A strong correlation has been established between synovitis and the progression/severity of OA (55). The role of macrophages is gaining increased attention as mediators of OA, which is a low-grade inflammatory disease (56). The two main types of macrophages are inflammatory (M1) and anti-inflammatory (M2) (57). Accumulating evidence links the polarization of M1 macrophages in the synovium to being a prominent aspect of OA progression (58). Attempts at conditional depletion of macrophages for the mitigation of OA were unsuccessful (59), which further highlights the complexity of the immunological microenvironment. It has recently been demonstrated that TRPV1 regulates M1/M2 macrophage polarization, while its activation decreases levels of various M1 macrophage markers (such as inducible nitric oxide synthase (iNOS) and interleukin-6 (IL-6)) in a Parkinson's disease model (60). TRPV1 is also capable of reducing synovitis and

alleviating OA through the inhibition of M1 macrophage polarization (61). These effects are mainly attributable to the Nrf2/ARE pathway, which is also active as a self-adaptive mechanism to promote cell survival in the OA microenvironment (62). TRPV1 stimulation can enhance the activity of Nrf2 *via* the increased phosphorylation of CaMKII (61).

Emerging evidence suggests that ASIC1a is a central player of RA pathogenesis. Activation of ASIC1a appears to promote synovial hyperplasia, inflammation, and destruction of articular cartilage/subchondral bone (63). ASIC1a is highly expressed in RA synovial tissues and RA synovial fibroblasts (RASF), and induces synovial inflammation and invasion, which can be downregulated either by its selective inhibitor (PCTX-1), or specific RNA interference (64, 65).

Treatment of adjuvant arthritis (AA) rats with the ASIC1a inhibitor amiloride resulted in the significant reduction of several RA symptoms, such as synovial hyperplasia and thickening, pannus formation, and infiltration of inflammatory cells. Articular cartilage ECM genes (*COL2A1* and *ACAN*) were also upregulated by the same treatment (66). There is also recent evidence that ASIC1a might be related to tumor proliferation and migration (67, 68). A similar role has been hypothesized about ASIC1a in fibroblast-like synoviocytes (FLS) that invade the synovium in RA, thereby contributing to disease progression and cartilage destruction (69). The inhibition of ASIC1a can reduce FLS invasion of the synovium and the resulting destruction in RA, making it a desirable therapeutic target (63). Extracellular acidification is a common feature of RA. Evidence is available that this signal activates ASIC1a in a RA setting, which is in turn responsible for the nuclear translocation of NFATc3 by regulating $[Ca^{2+}]_i$ (63). NFATs (nuclear factor of activated T cells) are calcium-dependent transcription factors that are – under certain circumstances – responsible for regulating the expression of genes that drive the inflammatory process (such as RANTES) (63, 70).

In addition to ASIC1a, the big conductance (BK, MaxiK) calcium- and voltage-gated potassium channel $K_{Ca}1.1$ are also important in invasive FLS in RA (30, 71). BK channels were the major potassium channels in these cells, and there was a positive correlation between $K_{Ca}1.1$ channel-mediated current density and FLS invasiveness. Furthermore, treatment of FLS with the cytokines TNF-α and IL-1β in vitro recapitulated several features of arthritis at the transcriptomic level, including significant upregulation of KCNMA1, which codes for the pore-forming subunit of $K_{Ca}1.1$ channels (72). There was also a switch in the regulatory subunit composition of $K_{Ca}1.1$ channels from β1 to β3b in aggressive RA-FLS, making KCa1.1-β3b a highly attractive therapeutic target in RA (71, 73). While the specific molecular mechanisms by which $K_{Ca}1.1$ channels regulate FLS invasiveness remains to be understood, one possible scenario could be *via* β1 integrin expression and function.

Extracellular adenosine triphosphate (ATP) mediated autocrine and paracrine signaling *via* P2 purinergic receptors is known to be involved in pain generation in joint diseases (74). The ionotropic purinergic receptor P2X7 is a cation channel that appears to play a significant role in RA. Data shows that RA patients display elevated P2X7 mRNA expression in their synovial tissue. In the synovial tissues of RA patients, a positive correlation was found between expression levels of inflammatory markers (such as IL-1β, IL-6, and IL-8) and that of P2X7 receptor (75). The same study also demonstrated that pharmacological modulation of the P2X7 can reduce the secretion of inflammatory factors and thus quench inflammatory reactions (75).

All three pannexins are expressed in articular chondrocytes *in vivo* (74) and in chondrifying micromass cultures *in vitro* (76), indicating that their function is not only required in mature but also in developing chondrocytes. While in healthy chondrocytes, Panx3 activation reduces intracellular ATP and subsequent phosphorylation of CREB; in joint diseases, the ATP released through Panx3 activates P2 receptors, leading to ERK1/2 and MMP13 activation, allowing for the development of the aberrant hypertrophic chondrocyte

phenotype (74). In fact, in a rat temporomandibular joint osteoarthritis (TMJOA) model, damage to the articular cartilage was less severe in case of Panx3 silencing (77). Local inflammation and cartilage ECM degradation in the TMJOA model is likely mediated by ATP release *via* Panx3, which activates P2X7 (77). This mechanism is especially relevant because OA chondrocytes show a cellular phenotype resembling that of hypertrophic chondrocytes; thus, preventing this pathway may slow down OA progression.

2.4. Ion channels involved in inflammation and pain in bone

In addition to cartilage degeneration or damage and other intra-articular sequelae, several notable manifestations of OA involve the adjacent bone tissue. So much so, that in some of the cases, the possibility emerges that these can be regarded as preconditions of OA (78). Relevant symptoms include bone hyperplasia, subchondral bone sclerosis and lesions.

The chloride–proton antiporter ClC-3 appears to be a key player in abnormal extracellular matrix metabolism during OA pathogenesis (79). Mechanical stimulation induces the upregulation of ClC-3 expression; this can significantly increase the expression of osteogenic markers such as alkaline phosphatase (ALP), bone sialoprotein (BSP), and osteocalcin (OC), which is a hallmark of osteoblast differentiation. However, under pathological circumstances, such as OA, the same mechanism can result in subchondral bone sclerosis (79). The role of the ClC-3 chloride channel is further underpinned by the observation that it is activated by estradiol binding to the estrogen receptor alpha on MC3T3-E1 osteoblasts. Estrogen 17β-estradiol enhanced the expression of collagen I protein, activity of alkaline phosphatase activity, and mineralization. These effects were inhibited by chloride channel blockers (80).

Bisphosphonates (BPs) are already utilized as one of the first-line therapies in several bone diseases that are characterized by an imbalance between osteoblast and osteoclast activity.

In addition to targeting osteoclasts and thus reducing bone resorption, there has been an increasing interest in BPs for their osteoblast-activating properties (81). As the structural and functional integrity of subchondral bone are imperative for articular health, it is no surprise that BPs have been shown to have chondroprotective effects both *in vitro* and in OA animal models. In humans, BPs appear to reduce the need for knee replacement in OA-related observational studies (78). Evidence suggests that zoledronic acid (ZOL) activates the TRPV1 channel on MC3T3-E1 cells and bone marrow-derived osteoblasts. This mediates mineralization which acts against antiproliferative effects. Since osteoclasts lack this channel, the same mechanism is not functional (82). At the same time, ZOL has the potential to be utilized as a selective musculoskeletal ATP-sensitive K^+ channel blocker, which targets the weakly inward rectifier K^+ channels Kir6.1-SUR2B and Kir6.2-SUR2A. ZOL may keep overactive mutants of KCNJ9-ABCC9 genes under check. These mutants are responsible for the Cantú syndrome that involves musculoskeletal disorders such as bone fracture and bone frailty (81).

Pannexins are also expressed in bone cells. ATP released through pannexin 1 channels activates P2X4 and P27 receptors, triggers osteocyte apoptosis leading to macrophage recruitment, osteoclast activation and bone resorption, and also mediates the activation of the NLRP3 inflammasome (74). This pathway may have important implications for bone pathologies such as osteoporosis as targeted modulation of this signaling has the potential to prevent bone loss.

2.5. Ion channels involved in inflammation and pain in muscle and tendon

Many different ion channels have well-established functional roles in skeletal muscle disorders, as recently reviewed by Maggi *et al*. (83). $K_V1.1$ appears to play a key role in the development of a healthy musculoskeletal phenotype. It was recently established that a T268K switch (causing the functional impairment of specific residues in the voltage sensor domain of

the $K_V1.1$ tetramer) causes a distinctive phenotype with a primarily musculoskeletal presentation: a 9-year-old patient presented with rhabdomyolysis, lower limb stiffness, neuromyotonia, muscle hypertrophy, short stature, and skeletal deformities (84). The N255D mutation of the same channel protein resulted in neuromuscular tetanic hyperexcitability syndrome in a different patient (85). Since $K_V1.1$ plays a role in active magnesium reabsorption in the epithelium of the distal convoluted tubules (DCT) in the kidney, mutations in the KCNA1 gene have been commonly associated with hypomagnesemia (and the resulting tetanic episodes). However, the patient has always presented with normal serum and urinary magnesium values. These puzzling findings suggest that DCT epithelial cells not only set the ionic balance for Mg^{2+}, but also for Na^{+}, K^{+}, and Ca^{2+}.

Pathological periarticular muscle weakness is a common feature of OA. Growing evidence shows that pain, joint instability, maladaptive postures – all consequences of OA – inherently lead to decreased limb muscle strength and function (86). A major question that requires clarification is whether changes in the strength and tone of periarticular muscles are the cause or the consequence of joint degeneration. Regardless, it is likely that such muscle dysfunctions may lead to a further increase in cartilage deterioration (87). Inflammatory mediators and pro-inflammatory cytokines are upregulated in periarticular muscles of knee OA patients (88, 89). The idea of targeting the P2X7 purinoreceptor, a well-known ion channel in inflammatory processes, has already emerged in other inflammatory musculopathies (90), and it is reasonable to assume that this approach may soon be expanded to OA or RA patients. Notably, increased expression of P2X4 was shown on muscle macrophages in an animal model of activity-induced pain. Blocking these receptors in muscle appeared to prevent the development of hyperalgesia (91). It is therefore of pivotal importance to establish whether the inflammatory processes in knee OA patients also affect periarticular tissues, including skeletal muscle (92). Pannexin 1 expression was observed on the surface of myoblasts and it was

upregulated upon the induction of differentiation. Furthermore, Panx1$^{-/-}$ mice displayed impaired muscle regeneration after injury, especially in myoblast migration and fusion (93).

Compared to cartilage, muscle, and bone, much less is known regarding the involvement of ion channels and ionic currents in inflammation and pain in tendons. In fact, data regarding the pathophysiological mechanisms involved in tendon pain are generally lacking. The term tendinopathy describes a broad spectrum of chronic pain conditions and dysfunction of tendons. There are four theories for the etiology of tendinopathy: a mechanical theory, a vascular theory, an apoptosis theory, and a neural theory (94). The mechanical theory assumes that repetitive loading of the tendon causes microscopic degeneration, which results in scar tissue. The vascular theory describes tendon degeneration with secondary areas of focal vascular disruption. According to the apoptosis theory, hyper-physiological stress triggers programmed cell death *via* the activation of the stress-activated Jun N-terminal kinase (JNK), leading to degeneration of the tissue. Finally, the neural (neurogenic) theory proposes that tendinopathy is triggered by nerve ending-mediated mechanisms *via* the release of substance P and mast cell degranulation (94). In patients with pain symptoms from Jumper's knee affecting the patellar tendon, a significantly higher concentration of glutamate was detected compared to healthy tendons. Furthermore, NMDAR1 glutamate receptors were detected in the peripheral afferent nerves innervating the tendon (95). Similar results were obtained in case of chronic painful conditions of the Achilles and extensor carpi radialis brevis tendons, implicating the involvement of glutamatergic signaling in tendinitis, and highlighting possibilities for therapy (96). Glutamate, which is likewise produced by tenocytes, is involved in nociceptive signaling in persistent pain states; it also has a role in ECM metabolism and tenocyte proliferation and apoptosis (97).

Chronic pain in biceps tendinopathy, characterized by pain and weakness in the tendon of the long head of biceps brachii muscle, is presumed to arise from neurogenic inflammation,

central pain sensitization, excitatory nerve augmentation, inhibitory nerve loss, and/or dysregulation of supraspinal structures. Ion channels involved in the pain pathways include ASIC1b and 3, TRPV1 and 3, TRPA1, TRPM8, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ (94). In biopsies from patients with patellar tendinopathy, increased immunopositivity of NMDAR1, phospho-NMDAR1, substance P, and mGluR5 were found (98).

The management of tendinopathy includes pain relief using non-steroidal anti-inflammatory drugs (NSAIDs). In case NSAIDs prove to be unsuccessful in managing pain and inflammation, corticosteroid injections (GCI) may be administered (94). However, the increased expression of NMDAR1 glutamate receptor following GCI implicates potential excitotoxic tendon damage (95). A list of candidate ion channels and transporters that may be involved in mediating pain and inflammation in cartilage, synovium, bone, muscle, and tendon is summarized in *Table 1*.

3. Nociceptive signaling mediated by ion channels in the nervous system

The main neuroanatomical routes of nociceptive signaling have been well documented. Briefly, noxious stimuli elicit the activation of specialized peripheral terminals of primary sensory neurons (mainly Aδ and C fibers, but Aβ fibers may also be involved) resulting in electric impulses that subsequently propagate to the spinal dorsal horn *via* central axonal fibers. The central boutons constitute the presynaptic components of a complex neuronal network composed of functionally diverse populations of second-order excitatory and inhibitory interneurons and projecting neurons. The synapses between these cells play a pivotal role in modulating information prior to its further transmission (99, 100). Intriguingly, despite current knowledge regarding the major elements of pain-related pathways, a more detailed mechanistic understanding of nociception remains elusive. Thus, relatively little information is available about the molecular machinery responsible for the peripheral (owing to injury or inflammation,

increased sensitivity of the respective primary afferent fibers develops) and central sensitization (upon repeated stimuli, increased spinal neuronal excitability with diminished firing threshold occurs) in chronic pain states such as OA (101, 102). OA pain is driven by both nociceptive and neuropathic mechanisms. Central sensitization is likely to be involved in patients experiencing severe pain despite the less severe macroscopic joint damage (16).

Although many aspects of these phenomena have been discussed previously by others (103, 104), here we focus on a central element of the bigger picture: ion channels (*Table 2*). It is important to highlight that ion channels of the two regions (i.e., peripheral and central components) cannot be unambiguously separated due to significant overlapping types and features. For example, it should be noted that chondrocytes can play a direct role in inflammatory pain by producing mediators which can sensitize and activate peripheral nerves (23). These inflammatory mediators then affect a wide range of ion channels on neurons both at the periphery and in the CNS.

3.1. Ion channels involved in peripheral sensitization

Voltage-gated sodium ion channels (VGSCs-Na_v) are notably involved in nociceptive processing. Changes in Na^+ channel-mediated currents in pain states have been reported more than two decades ago (105, 106). VGSCs augment the activation of primary sensory neurons; moreover, a plethora of channel gene variants was identified in nociceptive processing (107). Specifically, TTX-sensitive $Na_v1.3$, $Na_v1.7$, and TTX-resistant $Na_v1.8$, $Na_v1.9$ channels have been extensively investigated. The role of $Na_v1.3$ channels in neuropathic pain was corroborated by targeted intraganglionic administration of virus-derived hairpin RNA, resulting in attenuated allodynia (108). Disruption of the SCN3A gene encoding the $Na_v1.3$ protein alleviated neuropathic pain (109, 110). $Na_v1.7$ is one of the most extensively studied VGSC in this context, showing substantial expression in small diameter peptidergic CGRP-

positive or non-peptidergic IB4-positive primary afferents fibers, acting as a pain modulator to aid the release of substance P from afferent terminals (111). Furthermore, upregulation of $Na_v1.7$ was detected in painful human neuromas (112), and its specific deletion from sensory cells abolished neuropathic pain (113). SCN9A gene (encoding $Na_v1.7$) modified with gain of function mutation(s) attenuated pain in diabetic neuropathy (114). Similarly, loss of function mutation in $Na_v1.7$ caused congenital insensitivity to pain disorder (CIP) (115). Selective deletion of $Na_v1.7$ promoted proencephalin and met-encephalin expression in dorsal root ganglion (DRG) cells; concurrently, naloxone-induced inhibition of opioid receptors also worsened analgesia in $Na_v1.7$ mutant mice and human CIP patients (113). Despite the fact that $Na_v1.8$ function was lost in damaged DRG neurons, the channel was mainly identified in uninjured cells (112). Blockade of $Na_v1.8$ contributed to hypoalgesia; moreover, its optogenetic silencing (i.e., *via* archaerhodopsin-3 proton pumps by optical activation) in DRG cells alleviated neuropathic pain. $Na_v1.9$ is preferentially expressed in IB4 positive non-peptidergic afferent fibers and DRG cell bodies. $Na_v1.9$ expression was downregulated upon nerve injury, owing to assumed loss of trophic glial cell line-derived neurotrophic factor (GDNF) support. In knockout animals, allodynia persisted following nerve damage, which questioned the role of $Na_v1.9$ in neuropathic pain (108). $Na_v1.8$, but not $Na_v1.9$, may be involved in monosodium urate-induced gout pain in a mouse model by increasing nerve excitability (116).

Voltage-gated K^+ (K_v) channels have also been studied in the context of pain regulation, albeit with a somewhat less promising therapeutic potential. Several types of delayed rectifier K_v channels have been cloned, established from homo- or heterotetrameric proteins by combining K_v1, K_v2 and K_v3 subtypes (117). $K_v1.2$ gene function was reduced in neuropathic pain states; siRNA silencing of $K_v1.2$ evoked mechanical and thermal hypersensitivity in rats (118). In contrast, $K_v1.3$, $K_v1.5$, and $K_v1.6$ channels displayed limited changes of expression in DRG cells upon nerve injury. Temporomandibular joint (TMJ) inflammation reduces the

expression of $K_v1.4$ subunits in the Aδ and C trigeminal ganglion neurons, which may contribute to trigeminal inflammatory allodynia (119). M-channel $K_v7.2$ emerged as a key determinant of firing accommodation (120). Knockout of $K_v7.2$ in DRG cells elicited hyperalgesia; additionally, peripheral nerve injury also robustly downregulated the channel (108, 117). Nerve injury also dampened whole-cell A-type currents in DRG cells, which implied the altered function of rapidly inactivating $K_v1.4$, $K_v3.4$ and K_v4 channels (121). Neuronal hyperexcitability, which is brought about in part by reduced A-type K^+ currents, may contribute to pain-related behavior in mice that accompany antigen-induced arthritis (122). Benzbromarone, a urate transporter inhibitor, evidently exerts its analgesic effects in rodent models of arthritis and gout *via* activating peripheral (and not central) voltage-gated KCNQ channels (123). All three types of Ca^{2+} activated K^+ channels (BK, IK, SK) are expressed in DRG nerve cells, and they are involved in pain phenotypes, partly by their functional coupling with TRPV1 or NMDARs (124).

Voltage-gated Ca^{2+} (Ca_v) channels (VGCCs) are well-known modulators of peripheral nerve sensitization (125). Specifically, the group of low-voltage activated T-type Ca^{2+} channels has been extensively studied with respect to their role in neuronal excitability and primary afferent transmitter release ($Ca_v3.1$, $Ca_v3.2$, $Ca_v3.3$). $Ca_v3.2$ and $Ca_v3.3$, but not $Ca_v3.1$, were expressed in DRG neurons. Although a mutation in $Ca_v3.2$ resulting in an enhanced pain phenotype in humans has not yet been described, the majority of the pain-related studies focused on this channel as opposed to $Ca_v3.1$ or $Ca_v3.3$ (108). $Ca_v3.2$ was enriched in central processes of nociceptive peptidergic CGRP and non-peptidergic IB4-positive DRG neurons making synapses in the superficial Rexed laminae of the spinal dorsal horn. Of note, abundant expression of $Ca_v3.2$ has been determined in several pain states such as nerve ligation or constriction injury, diabetic neuropathy, paclitaxel-induced peripheral neuropathy, and MIA-induced OA (126, 127). Intriguingly, augmentation of $Ca_v3.2$ expression in intact nerves also

led to neuropathy owing to the intermingling of healthy and injured fibers that secreted pro-inflammatory mediators regulating T-type Ca channels (128). Although $Ca_v2.2$ inhibition effectively suppressed arthritis-induced pain in sensory neurons involved in nociception in a mouse model, it also impaired recovery from induced arthritis (129).

Nonselective cation (mainly Na^+ and Ca^{2+}) channels termed transient receptor potential (TRP) channels have been principally characterized as molecular sensors of a wide range of stimuli (such as heat, chemical compounds, pH, osmolarity). Upon sensitization of TRPV1 *via* various pro-inflammatory mediators, protein-kinase A and C mediated phosphorylation occurs, leading to allodynia and hyperalgesia (130). Furthermore, nerve ligation increased the ratio of IB4 positive DRG cells expressing TRPV1, which ultimately evoked permanent thermal hyperalgesia (131). Overexpression of TRPV1 in DRG neurons has been reported in cancer-related chronic pain, in addition to capsaicin-potentiated TRPV1 currents (132). Following sciatic nerve injury, TRPV1 was over-activated in central fibers of primary afferents, participating in the release of neuropeptides such as CGRP and substance P to amplify neuropathic pain (133). Other TRP channels such as TRPA1 (subfamily A member 1) and TRPM8 (subfamily M-melastatin member 8) have been identified as markers of cold allodynia. Blockade of TRPA1 substantially reduced cold allodynia upon constriction injury. Disruption of TRPA1 also attenuated mechanical and cold allodynia in chemotherapy-induced neuropathic pain (134). TRPA1 channel activity also plays an important role in chronic arthritis-related pain behaviors in the mouse (135).

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels determine the H current, which can be enhanced upon nerve injury, thus promoting transmitter release of primary afferents (136). Of the four isoforms (HCN1-4), HCN2 was expressed mainly in small DRG sensory neurons, facilitating the firing frequency induced by nociceptive stimuli. HCN2 deletion of nociceptive neurons eventually resulted in neuropathic pain (137). Although HCN3

KO mice displayed increased neuronal excitability, the level of mechanical allodynia and thermal hyperalgesia obtained here was similar to that of wild-type animals; therefore, HCN3 had apparently no role in nociceptive processing (138).

3.2. Ion channels involved in central sensitization

As stated above, over-activated neurons of the spinal dorsal horn exhibit intensified nociceptive signals, which can result in maladaptive synaptic organization, in which traditionally known excitatory and inhibitory transmission are pathologically altered by genetic and environmental factors. (139). K_{Ca} channels are also involved in central pain processing (124). Nerve damage upregulates BK channel expression in second-order neurons, and activating these channels by an intrathecal application of activators such as NS-1619 reverses pain hypersensitivity (124). Inhibitors of K_{Ca} channels, conversely, antagonize the anti-nociceptive effects of muscarinic receptor agonists such as gabapentin (124).

Glial cells can also act as important participants of the central pain circuits by secreting a variety of mediators, including chemokines and cytokines, leading to chronic pain (140). Furthermore, a growing body of evidence supports the expression of non-selective ligand-gated purinergic P2X4 and P2X7 ion channels in microglial cells in their roles in immune system activation. After spinal nerve injury, ATP released from damaged fibers binds to P2X4 to release brain-derived neurotrophic factor (BDNF) *via* the P38 kinase cascade. Thereafter, BDNF *via* neuronal tyrosine kinase B receptor downregulates the K^+-Cl^- cotransporter 2 (KCC2) that shifts the reversal potential of the GABA A receptor, resulting in net hyperexcitation of the network (141). Interestingly, the underlying mechanism of microglial P2X4 signaling is thought to be sex-dependent; intrathecally administered ATP induces pain responses in male, but not in female animals. This has been explained by the predominant P2X4 expression in males, and also by the fact that microglia cells are presumably dispensable in

females, replaced with infiltrating adapting immune cells into the spinal cord (142). Additionally, this hypothesis was verified by the increase of T cell markers CD4, CD8 and CD3e in females, but not in males, upon nerve injury. P2X7 is also activated following nerve trauma, and its antagonists can ameliorate allodynia. P2X7 induces inflammasomal NOD-like receptor pyrin domain 3 (NLRP3) recruitment to release pro-inflammatory mediators and NO (102).

Upregulation and activation of pannexin channels have been associated with the mechanisms of both peripheral and central sensitization (143). Panx1 channels have been recently implicated as potential therapeutic targets for alleviating mechanical allodynia in animal models of inflammatory arthritis (144). In a MIA model of OA, P2X7 drives Panx1 channel activation. Panx1 function in the microglial cells in the spinal cord was increased in rats with mechanical allodynia, by mediating the release of the pro-inflammatory cytokine IL-1β. Probenecid, a clinically approved broad-spectrum Panx1 blocker, attenuated MIA-induced mechanical allodynia, without affecting acute nociception, making this a promising therapeutic approach for modulating joint pain (144). Panx1 expression is not restricted to microglial cells; it is also present in sensory ganglion cells (143). Global $Panx1^{-/-}$ mice did not develop allodynia; however, when Panx1 deletion was confined to sensory nerve cells, the onset of hypersensitivity was only slightly delayed (143). $Panx1^{-/-}$ mice are also resistant to chronic pain (145). A more recent study has documented that neuroinflammation caused by chronic constriction injury correlated with Panx1 activation in Schwann cells, which indicates that Panx1 channel blockage may reduce neuropathic pain (146). The above data suggest that targeting Panx1 by selective blockers in the PNS or CNS could be effective for pain relief.

4. Models for investigating mechanisms and the development of targeted therapeutics

The development of targeted pain therapeutics has traditionally relied upon the use of *in vitro* models for evaluating anti-inflammatory activity. This is then followed by employing the most appropriate preclinical, translational and clinical studies, and selecting models that are well suited to the mode of action of the therapeutic agent that is being developed. However, there are no appropriate *in vitro* models for OA pain and it is highly unlikely that *in vitro* systems will be able to replace animal models for the study of OA pain. Furthermore, the preclinical animal models that are currently available include significant limitations. In addition to spontaneously occurring animal models of OA, many experimental animal models have been developed to provide insights into mechanisms of OA pathogenesis and pain progression. Many of these animal models are also being used in drug development pipelines but the limitations of the currently available models are major impediments to the translation of research findings from bench to bedside (147).

5. Conclusions and perspectives

Pain in OA involves complex peripheral and central mechanisms, some of which are currently not well-understood. However, some pathways and mediators associated with OA pain are more fully mapped, such as nerve growth factor (NGF)/tropomyosin receptor kinase A (TrkA), calcitonin gene-related peptide (CGRP), C–C motif chemokine ligands 2 (CCL2)/chemokine receptor 2 (CCR2) and tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), the NOD-like receptor (NLR) family, pyrin domain-containing protein 3 (NLRP3) inflammasome, and the Wnt/β-catenin signaling pathway (16). A growing body of evidence suggests that ion channels in both musculoskeletal tissues and peripheral or central nerve pathways are involved in pain generation and transmission. The studies discussed in this review article highlight the coupling between joint degeneration, inflammation, and nociception pathways. However, while current knowledge regarding the role of ion channels

in peripheral and central pain pathways is convincing, the involvement and specific functions of ion channels in peripheral joint tissues such as the synovium, cartilage and subchondral bone are less clear.

The hypothesis that ion channels in tissues of the OA-affected synovial joint are prominently involved in pain generation and/or transmission is corroborated by the fact that ion channel inhibitors are already in clinical trials as candidate drugs for the management of OA pain. In a Phase 2 clinical study, a single intra-articular injection of a novel TRPV1 agonist CNTX-4975 treatment achieved dose-dependent improvement in knee OA pain until 24 weeks (148). A Phase 2 clinical study to evaluate the safety or efficacy of LY3526318, a TRPA1 antagonist, in patients with knee OA pain is currently ongoing (NCT05080660)[1]. Furthermore, there are neurotoxins in clinical development that have the potential to modulate ion channel function, impact on pain and alter the trajectory of the disease. One example is resiniferatoxin, which is a TRPV1 agonist currently in phase 2 clinical development for the management of OA pain[2]. Another example is PCRX-301, a thermosensitive hydrogel formulation of funapide, a preferential $Na_V1.7$ inhibitor (in development by Pacira Biosciences[3]), which is intended for the management of chronic pain as a lower extremity injectable nerve block for the post-surgical management of pain in a variety of orthopaedic contexts, including OA. To further evaluate the efficacy and long-term safety of these ion channel modulators for OA pain treatment, more comprehensive clinical trials are required.

As discussed, the mechanisms and mediators involved in OA nerve damage are not entirely clear. Nerve sensitization due to cartilage erosion is a major feature for pain transmission in OA patients, but bidirectional interactions between the immune and nervous

[1] https://clinicaltrials.gov/ct2/show/NCT05080660 (last accessed: 31 May 2023)
[2] https://www.empr.com/home/news/drugs-in-the-pipeline/resiniferatoxin-gets-breakthrough-therapy-status-for-knee-osteoarthritis-pain/ (last accessed: 31 May 2023)
[3] https://investor.pacira.com/news-releases/news-release-details/pacira-biosciences-acquire-flexion-therapeutics-further (last accessed: 31 May 2023)

systems are also recognized to be major contributors to chronic pain. The CNS and the immune system communicate using a variety of signaling molecules and interconnected signaling pathways. This communication is truly reciprocal: cells of the immune system communicate primarily by cytokine signaling *via* cytokine receptors but can also secrete and respond to neurotransmitters; whereas nerve cells typically communicate *via* neurotransmitters and neuropeptides, but can also secrete and respond to cytokines by expressing cytokines and/or cytokine receptors (149).

In addition to nociceptive pain, the prevalence of neuropathic pain in people with knee or hip OA is considerable (150). Neuropathic pain is unresponsive to commonly prescribed analgesics such as NSAIDs, necessitating the systemic use of other classes of drugs, such as opioids, to manage this type of pain (151). However, the ongoing adverse impacts of the opioid epidemic, especially in North America, highlight the need for the development of safer and more effective non-opioid drug alternatives.

Pannexins and their regulatory pathways are also exciting targets for pain therapy. A theory that has been developed to explain the self-sustaining mechanism of allodynia includes gap junctions and pannexins in glial cells and neurons, which would mediate increased rates of ATP release, as well as an increased sensitivity of purinergic receptors (143). Panx1 channels therefore could be candidate targets of therapy as an alternative to opioid analgesia. Given that clinically approved Panx1 blocking drugs such as probenecid are already available, there is a possibility of future development of even more selective and potent Panx1-targeted therapies for the treatment of joint pain conditions.

Big conductance (BK) Ca^{2+} and voltage-gated K^{+} channels (30) could also be exciting targets of pharmaceutical interventions in RA, especially since many RA patients do not respond to the plethora of biological drugs that have been developed for the treatment of refractory RA. Given that the β subunit composition of BK channels is different in minimally

versus highly invasive FLS (there is a switch from β1 to β3b in aggressive forms), BK channels with β3b subunit composition are highly attractive therapeutic targets in RA (71, 73). Due to the tissue-specific expression pattern of the β3b subunit (currently it has only been described in the testis), a selective inhibitor that only targets BK channels with this subunit and cannot cross the blood-testis barrier could be used to target RA-FLS without detrimental side effects.

Eliciting anti-nociceptive effects *via* selective activation of ATP-sensitive potassium channels (K(ATP)) expressed either in chondrocytes (152) or in the nervous system (153) would also be possible by slow-release hydrogen sulphide (H_2S) derivatives as these compounds are less toxic NSAID alternatives (154). H_2S has multiple anti-inflammatory mechanisms, and it reduced visceral pain in an ATP-sensitive potassium channel-dependent manner (155). Although the specific molecular mechanisms involved in the anti-nociceptive actions of H_2S releasing compounds in nociceptive, osteoarthritis, and neuropathic pain are not fully delineated, one possible pathway might be *via* potassium channels, such as the voltage-gated Kv7 and the ATP-sensitive potassium channels (156). However, the participation of these potassium channels in the possible anti-nociceptive effects of slow-releasing H_2S donors during inflammatory pain in OA has not been fully elucidated.

A better understanding of the molecular entities, including ion channels, contributing to the generation and transmission of OA pain, and how these components interact and function, may offer critical new insights into the development of more specific, effective, safer and personalized analgesic treatment.

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Author Contributions

CM, RT and AM made a substantial contribution to the concept and design of the article. All authors have drafted the article and revised it critically for important intellectual content. All authors have approved the final version to be published.

Conflicts of Interest

The authors declare no conflicts of interest.

Figures

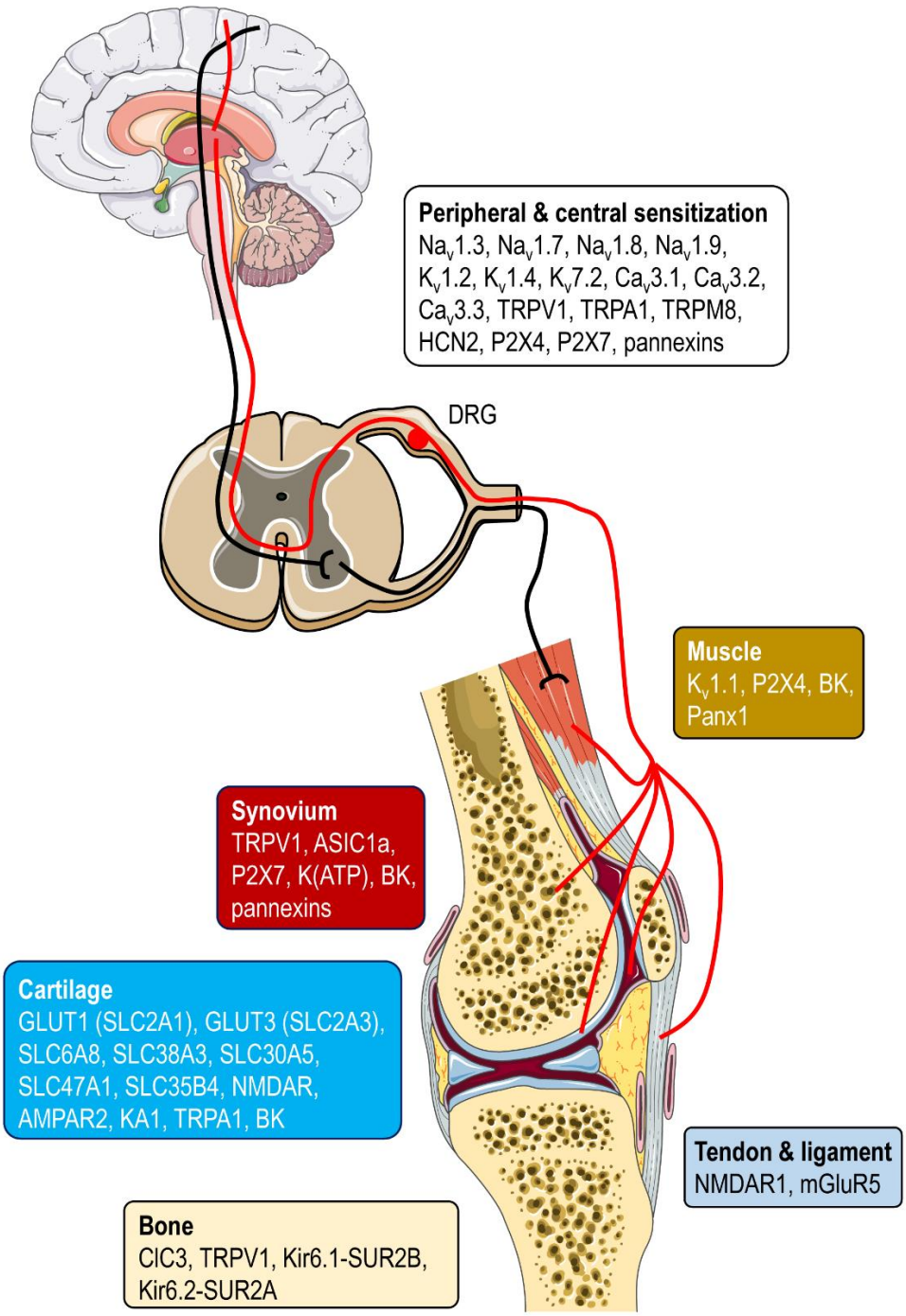

Figure 1. Spatial distribution of ion channels that are involved in the pathogenesis of OA pain in the brain–joint axis. The figure depicts the innervation of a typical synovial joint (in particular, the knee joint) and its constituent tissues (synovium, bone, tendon and ligament, muscle and cartilage) by afferent nociceptive C and Aδ fibers. (Note that OA-affected cartilage

and the subchondral bone may undergo neoinnervation.) The peripheral nociceptive fibers enter the central nervous system through the dorsal root ganglion (DRG), typically synapse in the dorsal laminae of the spinal cord, the ascend through the brain stem to the primary sensory cortex (precentral gyrus) *via* the thalamus, responsible for the conscious processing of pain stimuli. Ion channels mediating the pain pathway in the periphery and those involved in peripheral and central sensitization are shown. Red fibers, afferent nociceptive fibers. Black fibers, somatomotor efferent fibers.

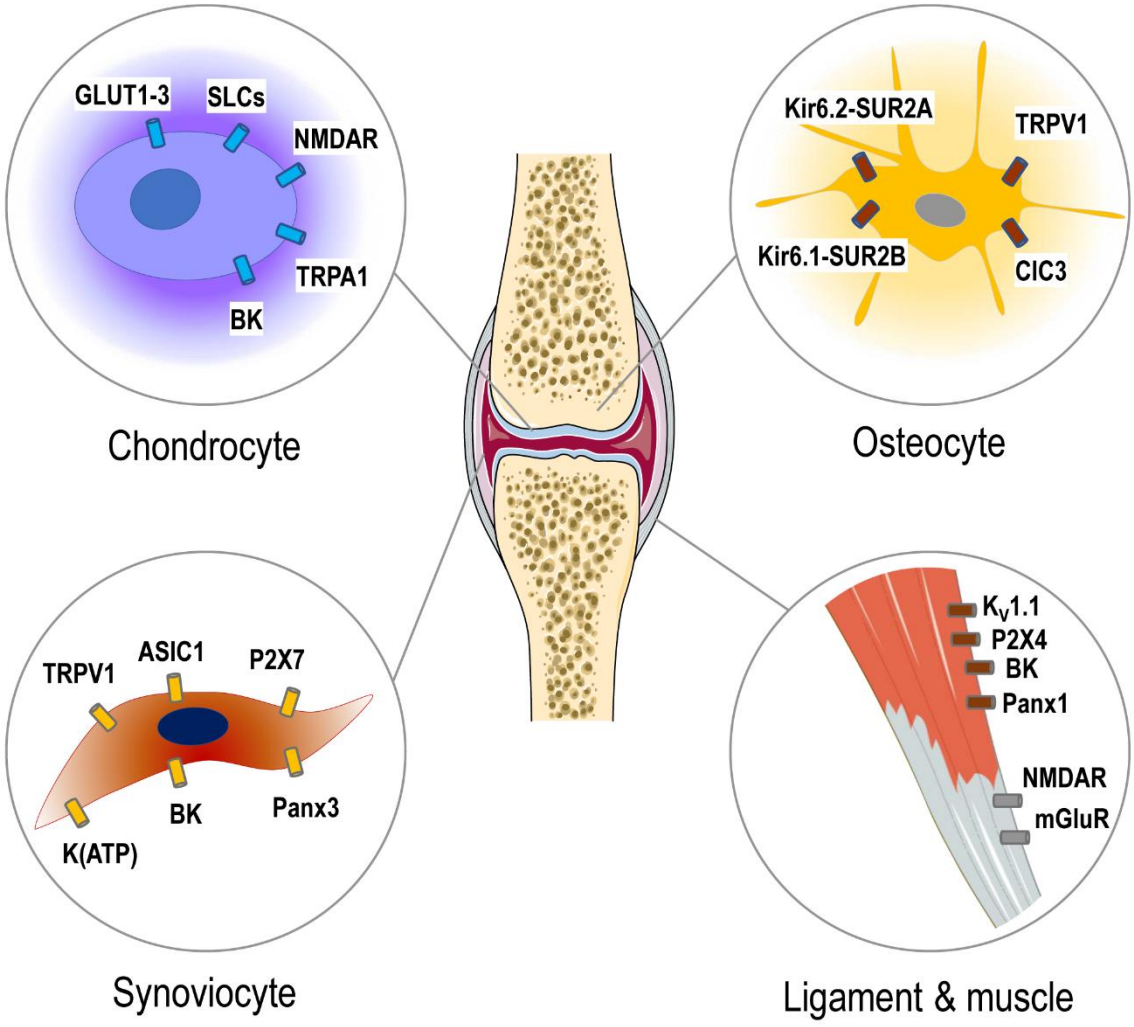

Figure 2. Major ion channels that are involved in joint nociception in chondrocytes, synoviocytes, osteocytes, tenocytes and muscle fibers are shown.

Table 1. Ion channels and transporters involved in inflammation and pain in peripheral joint tissues

Transporter name	Reference
Cartilage, chondrocytes	
GLUT1 (SLC2A1) facilitated glucose transporter 1	Fisch et al., 2018 (47)
GLUT3 (SLC2A3) facilitated glucose transporter 3	Fisch et al., 2018 (47)
SLC6A8 sodium- and chloride-dependent creatine transporter 1	Fisch et al., 2018 (47)
SLC38A3 sodium-coupled neutral amino acid transporter 3	Fisch et al., 2018 (47)
SLC30A5 zinc transporter 5	Fisch et al., 2018 (47)
SLC47A1 multidrug and toxin extrusion protein 1	Fisch et al., 2018 (47)
SLC35B4 UDP-xylose and UDP-N-acetylglucosamine transporter	Fisch et al., 2018 (47)
AMPAR2 α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor	Bonnet et al., 2015 (49)
KA1 kainate receptor	Bonnet et al., 2015 (49)
TRPA1 transient receptor potential ankyrin 1	Moilanen et al., 2015 (50)
$K_{Ca}1.1$ calcium- and voltage-gated potassium channel (BK)	Takacs et al., 2023 (30)
Panx1, Panx3 pannexins	Larranaga-Vera et al., 2021 (74); Xiao et al., 2023 (77)
K(ATP) ATP-sensitive K^+ channels	Mobasheri et al., 2007 (152)
Synovium, synoviocytes	
TRPV1 transient receptor potential cation channel subfamily V (vanilloid) member 1	Bok et al., 2018 (60)
ASIC1a acid-sensing ion channel 1a	Niu et al., 2020 (64); Zhang et al., 2020 (65)
P2X7 P2X purinergic receptor 7	Chen et al., 2018 (75)
$K_{Ca}1.1$ calcium- and voltage-gated potassium channel (BK)	Takacs et al., 2023 (30); Beeton, 2017 (71); Haidar et al., 2020 (72); Petho et al., 2016 (73)
Bone, osteocytes	
ClC-3 Chloride–proton antiporter 3	Lin et al., 2022 (79)
TRPV1 transient receptor potential cation channel subfamily V (vanilloid) member 1	Scala et al., 2019 (82)
Kir6.1-SUR2B ATP-sensitive K+ (KATP) channel	Scala et al., 2022 (81)
Kir6.2-SUR2A ATP-sensitive K+ (KATP) channel	Scala et al., 2022 (81)

Panx1 pannexin	Larranaga-Vera et al., 2021 (74)
Muscle	
$K_V1.1$ voltage-gated potassium ion channel	Imbrici et al., 2021 (84); Bianchi et al., 2020 (85)
P2X4 P2X purinergic receptor 4	Oliveira-Fusaro et al., 2020 (91)
Panx1 pannexin	Suarez-Berumen et al., 2021 (93)
Tendon	
NMDAR1 *N*-methyl-D-aspartate receptor 1	Alfredson et al., 2001 (95); Schizas et al., 2012 (98)
mGluR5 metabotropic glutamate receptor subtype 5	Schizas et al., 2012 (98)

Table 2. Ion channels involved in central and peripheral sensitization in chronic pain

Transporter name	Reference
$Na_v1.3$ voltage-gated sodium ion channel	Ye et al., 2020 (110)
$Na_v1.7$ voltage-gated sodium ion channel	Li et al., 2018 (111)
$Na_v1.8$ voltage-gated sodium ion channel	Hameed, 2019 (112)
$Na_v1.9$ voltage-gated sodium ion channel	Alles & Smith, 2021 (108)
$K_v1.2$ voltage-gated potassium ion channel	Zhang et al., 2021 (118)
$K_v1.4$ voltage-gated potassium ion channel	Takeda et al., 2008 (119)
$K_v7.2$ voltage-gated potassium ion channel	Smith, 2020 (117)
$Ca_v3.1$ voltage-gated calcium ion channel	Alles & Smith, 2021 (108)
$Ca_v3.2$ voltage-gated calcium ion channel	Shin et al., 2020 (127); Chen et al., 2018 (128)
$Ca_v3.3$ voltage-gated calcium ion channel	Alles & Smith, 2021 (108)
TRPV1 transient receptor potential cation channel subfamily V (vanilloid) member 1	Joseph et al., 2019 (130)
TRPA1 Transient receptor potential cation channel subfamily A (ankyrin) member 1	Naziroglu et al., 2017 (134); Horvath et al., 2016 (135)
TRPM8 Transient receptor potential cation channel subfamily M (melastatin) member 8	Naziroglu et al., 2017 (134)
HCN2 Hyperpolarization-activated cyclic nucleotide-gated channel	Dini et al., 2018 (137)
P2X4 P2X purinergic receptor 4	Duveau et al., 2020 (141); Kohno et al., 2021 (142)
P2X7	Totsch et al., 2017 (102)

P2X purinergic receptor 7	
K(ATP) ATP-sensitive K+ channels	Braga et al., 2020 (153)
Panx1 pannexins	Spray & Hanani, 2017 (143); Mousseau et al., 2018 (144); Weaver et al., 2017 (145); Wang et al., 2022 (146)

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