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Osteoarthritis pain mechanisms: basic studies in animal models

R.-X. Zhang †, K. Ren ‡, R. Dubner ‡*

† Center for Integrative Medicine, School of Medicine, University of Maryland, Baltimore, MD 21201, USA
‡ Department of Neural and Pain Sciences, Dental School, University of Maryland, Baltimore, MD 21201, USA

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SUMMARY

Objective: Osteoarthritis (OA) is a complex and painful disease of the whole joint. At present there are no satisfying agents for treating OA. To promote OA research and improved treatment, this review summarizes current preclinical evidence on the development of OA.
Methods: Preclinical OA research was searched and key findings are summarized and commented.
Results: Mechanisms of OA-associated pain have been studied in rodent knee OA models produced by intra-knee injection of the chondrocyte glycolytic inhibitor mono-iodoacetate (MIA), surgery, or spontaneous development in some species. These models are clinically relevant in terms of histological damage and functional changes, and are used to study mechanisms underlying mechanical, thermal, ambulatory, body weight supporting-evoked, and ongoing OA pain. Recent peripheral, spinal, and supraspinal biochemical and electrophysiological studies in these models suggest that peripheral pro-inflammatory mediators and neuropeptides sensitize knee nociceptors. Spinal cytokines and neuropeptides promote OA pain, and peripheral and spinal cannabinoids inhibit OA pain respectively through cannabinoid-1 (CB1) and CB1/CB2 receptors. TRPV1 and metalloproteinases contribute and supraspinal descending facilitation of 5-hydroxytryptamine (5-HT)/5-HT 3 receptors may also contribute to OA pain. Conditioned place preference tests demonstrate that OA pain induces aversive behaviors, suggesting the involvement of brain. During OA, brain functional connectivity is enhanced, but at present it is unclear how this change is related to OA pain.
Conclusion: Animal studies demonstrate that peripheral and central sensitization contributes to OA pain, involving inflammatory cytokines, neuropeptides, and a variety of chemical mediators. Interestingly, brainstem descending facilitation of 5-HT/5-HT3 receptors plays a role OA pain.

Introduction

Osteoarthritis (OA), is a complex disease of the whole joint, is characterized by structural degradation of the articular cartilage, peri-articular bone, synovial joint lining, and adjacent supporting connective tissue elements. It manifests as joint pain and loss of joint function. There are currently no satisfying treatments to this disease. The current standard of care is to manage and alleviate symptoms[1], but despite treatment with conventional analgesic drugs most individuals with OA continue to experience pain[2]. A recent study demonstrated that subjects with chronic back pain and complex regional pain syndrome had significantly less bilateral hippocampal volume compared to controls, while those with OA did not[3]. This suggests that OA-induced pain might be related to unique mechanisms and this has attracted researchers' attention in recent years. Since the most common joints affected by OA are large weight-bearing joints such as hip and knee[4], intra-knee injection of the chondrocyte glycolytic inhibitor mono-iodoacetate (MIA)-, surgically induced, and spontaneous knee OA models have been used to investigate mechanisms of OA-induced pain[5–8].

In the MIA model, histological examination shows chondrocyte degeneration/necrosis at days 1–7 post-MIA, increased osteoclasts and osteoblasts in subchondral bone by day 7, focal fragmentation and collapse of bony trabeculae with fibrosis by day 28, and large areas of bone remodeling by day 56[9,10]. *In vivo* microCT-arthrography clearly detects cartilage degeneration in the injected knee[11].

Tramadol, celecoxib, and diclofenac improve movement-induced pain behavior evaluated with compressive hind limb grip force in the MIA model[12], and subcutaneous morphine and gabapentin significantly decrease the mechanical and thermal sensitivity and ambulation-evoked pain[13]. MIA-induced articular cartilage loss, progressive subchondral bone lesions, and the efficacy of clinical analgesics in inhibiting MIA-induced pain indicate that this model is clinically relevant and will continue to be useful

* Address correspondence and reprint requests to: R. Dubner, Department of Neural and Pain Sciences, University of Maryland Dental School, 650 W. Baltimore St., Room 8251, Dental-8 South, Baltimore, MD 21201, USA. Tel: 1-410-706-0860; Fax: 1-410-706-0865.
E-mail addresses: rzhan001@umaryland.edu (R.-X. Zhang), kren@umaryland.edu (K. Ren), RDubner@umaryland.edu (R. Dubner).

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for the development of better therapeutic strategies and better understanding of the mechanisms of chronic OA pain.

Surgery-induced medial meniscal tear (MMT), partial medial meniscectomy (PMM), destabilization of the medial meniscus (DMM), and anterior cruciate ligament transection (ACLT) have been used to induce knee OA[14,15]. The MMT results in a progressive cartilage lesion[16]. The MMT plus ACLT-induced OA model presents bone and cartilage remodeling, infiltration of immune cells into joint tissues, and pain[17]. The ACLT + PMM model showed no hind-limb difference in gait analysis or mechanical allodynia over a period of a month[18], making it analogous to patients who show radiological changes but no pain. PMM in female C57BL/6 mice produces progressive degenerative joint damage and OA-related pain[19]. The DMM-induced OA model displays a time-dependent cartilage lesion between 2 and 12 weeks, including cartilage surface fibrillations, loss of superficial cartilage and ulceration of subchondral bone, and produces pain assessed 12 weeks after surgery[7]. In that model, opioid receptor antagonists led to pain onset 4 weeks earlier than in vehicle-treated animals, and opioid receptors increased in the peripheral nerves that innervate the joint in naloxone-responsive mice[7], suggesting that endogenous opioids might inhibit early-stage OA pain.

Guinea pigs, particularly the Dunkin-Hartley strain, STR/1N, STR/ort, and C57 black mice, and several transgenic and genetically altered strains of mice develop characteristics of arthritic joints, but rats rarely develop them spontaneously (for a review, see D'Souza *et al.*, 2011). In Duncan-Hartley guinea pigs, age-dependent cartilage degeneration can be assessed by T(1ρ) magnetic resonance imaging (MRI) from 3 to 9 months[20].

All three model types have been used to investigate the mechanisms of OA pain, which has been assessed with various methods, including mechanical, thermal, ambulatory, and body weight supporting-evoked methods (for a review, see D'Souza *et al.*, 2011). MIA has been reported to be differentially potent: weight-bearing ≥ von Frey filaments > running wheel[21]. Von Frey filaments are a set of calibrated filaments used to perpendicularly stimulate skin. Recently, the MIA model has also been used to study ongoing pain assessed with conditioned place preference [22,23]. Additionally, it has been reported that biglycan (BGN) and fibromodulin (FMOD) play roles in regulating chondrogenesis and extracellular matrix turnover[24] and that doubly deficient BGN/FMOD mice develop premature temporomandibular joint OA[25]. Peripheral, spinal, and supraspinal l mechanisms (Fig. 1) have recently been discovered using these rodent models.

Peripheral mechanisms

Cytokines

Synovitis is highly correlated to OA patients' pain[26,27] and plays an important role in such pain[2,28]. An intra-articular MIA injection significantly increased tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in the knee synovium and capsule between days 1 and 28 post-MIA; the levels of TNF-α and IL-6 peaked at day 4. The injection also induced mechanical allodynia of the ipsilateral hind paw, which was significantly mitigated by local application of nonsteroidal anti-inflammatory drugs (NSAIDs)[29,30]. A TNF-α injection into the normal knee joint caused significant and persistent sensitization of nociceptive sensory fibers to mechanical stimuli that was abolished by co-administration of etanercept, a TNF-α inhibitor; and TNF-α induced excitation of isolated dorsal root ganglion (DRG) neurons with C-fiber axons[31] and increased mechanosensitivity and peripheral receptive fields of DRG neurons[32].

An electrophysiological study demonstrated increased spontaneous activity in C-mechanosensitive fibers and increased mechanical sensitivity in A-mechanosensitive fibers of the knee in MIA-treated rats[33]. Furthermore, knee primary afferents, thinly myelinated type III and unmyelinated type IV fibers, showed significant mechanosensitivity in response to normal and noxious joint rotation as compared to saline controls in an MIA model[34,35]. This was reduced by local NSAID application[35]. Collectively, these studies indicate that increased TNF-α sensitizes primary afferents to facilitate joint pain and that NSAIDs can alleviate OA-induced pain by inhibiting TNF-α expression.

IL-6 increased intracellular calcium in cultured DRG neurons in calcium-imaging studies. Additionally, glycoprotein 130, to which the IL-6/IL-6R complex binds, was found in almost all DRG neurons cultured from adult rats. This suggests functional IL-6 receptors in

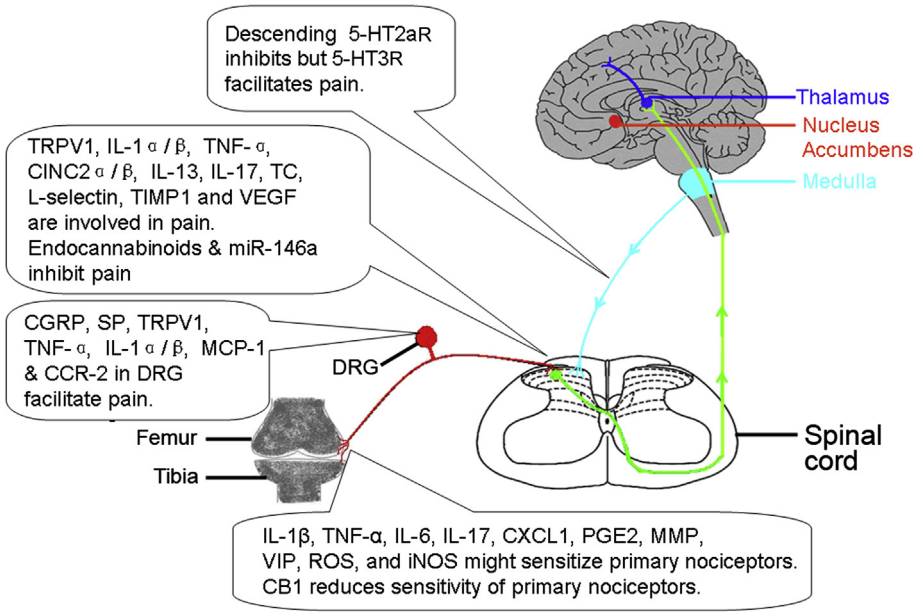

Fig. 1. Diagram illustrating mechanisms of OA-induced pain. Bioactive chemicals are involved in OA-induced pain at peripheral, spinal and supraspinal levels. IL-13, interleukin-13; IL-17, interleukin-17; IL-1α/β, interleukin alpha/beta.

DRG neurons[36]. Moreover, an intra-articular IL-6 (20 ng/joint) injection into the normal knee joint increased C-fiber response to noxious outward rotation, and co-administration of IL-6 and its soluble receptor significantly increased response to innocuous outward rotation[37]. These studies indicate that IL-6 sensitizes primary nociceptors in the knee.

IL-1β expression was higher between days 120 and 180 in cartilage, menisci, synovia, and subchondral bone in OA-prone Hartley than in OA-resistant Strain 13 guinea pigs. This parallels the fact that OA develops faster in Hartley than in Strain 13 animals[38]. It has been shown that an IL-1β application increases DRG neuron mechanosensitivity[32]. IL-1β treatment for 5–6 days increased the excitability of medium- and small-diameter isolectin B(4) (IB(4))-positive DRG neurons through its receptor, IL-1RI[39]. Further, in an electrophysiological study, the mechanical threshold required to initiate afferent firing was significantly lower in aged (9–12 months) guinea pigs than in young (12–14 weeks) ones[40]. Thus, IL-1β might sensitize primary nociceptors in the knee.

Other pro-inflammatory cytokines such as IL-7, IL-17, and IL-18 have also been implicated in synovitis, subchondral bone damage, and cartilage homeostasis alteration in spontaneous or surgically induced OA animal models and in transgenic mice primed to develop OA[41]. Of these, IL-17 plays a critical role in nociception during antigen-induced arthritis[42]. Local IL-17 is significantly higher in a mouse model of arthritis, and treatment with the antibody against IL-17 inhibits hypernociception. Intra-articular IL-17 also induced nociception and upregulation of TNF-α, IL-1β, keratinocyte-derived chemokine (KC/CXCL1), prostaglandin E2 (PGE2), matrix metalloproteinases-9 (MMP-9) activity, and cyclooxygenase-2 (COX-2) in synovial membranes[42]. Consistent with these data, treatment with the non-specific MMP inhibitor doxycycline, the COX inhibitor indomethacin, the anti-TNF antibody infliximab, or an IL-1 receptor antagonist inhibited IL-17-induced hypernociception[42]. Intra-articular IL-17 also elicited slow-developing, long-lasting sensitization of nociceptive C fibers of the joint to mechanical stimuli[43]. IL-17A receptors were found in most rat DRG neurons, and IL-17 enhanced excitability of cultured DRG neurons[43]. Therefore, although the role of IL-17 in OA pathophysiology is not entirely established, IL-17 appears to play a role in OA-induced pain.

In accordance with studies in animals, synovial tissue from patients with all grades of OA show inflammatory cell infiltration and cytokine production[44]. Human OA synovial cell cultures demonstrated that macrophages produce TNF-α and IL-1[45]. When DRG from adult rats were co-cultured with normal or knee OA synovial the mRNA levels of substance P (SP), neurokinin/tachykinin receptors (NK1, NK2), neuropeptide Y receptors (NPYR1, NPYR2), the calcium channel α2δ1, and inflammation mediators such as COX2, IL-6, and interferon β2 were clearly elevated in these DRG compared to control synovia[46]. This suggests that human OA synovium-produced cytokines sensitize primary sensory neurons and that blocking peripheral cytokine activity might alleviate OA pain. Indeed, clinical researchers have been investigating a novel IL-1 inhibitor for OA treatment[47]. It was showed that human monoclonal antibody to IL-1RI, AMG 108, showed greater improvements in pain than placebo. Although some studies showed no statistically significant difference of Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score between IL-1 receptor antagonist, orthokine, and placebo groups, some data demonstrated that orthokine considerably improved clinical signs and symptoms of knee OA patients[47].

Neuropeptides

Inflamed synovia also produce vasoactive intestinal peptide (VIP) to cause pain[48]. Local application of VIP to normal knees significantly increased afferent firing in response to normal rotation and hyper-rotation that was blocked by pre-administration of the VIP receptor antagonist VIP (6–28), and VIP (6–28) significantly reduced afferent firing in MIA-injected rats[49]. Behaviorally, VIP induced hind limb incapacity and mechanical allodynia in the hind paw, while VIP (6–28) diminished these symptoms[50]. This suggests that VIP sensitizes primary knee joint nociceptors during knee OA.

Cannabinoids

Studies show that activation of peripheral cannabinoid-1 (CB1) receptors with local application of the CB1 receptor agonist arachidonyl-2-chloroethylamide (ACEA) reduces mechanosensitivity of afferent nerve fibers in control and OA knee joints. The CB1 receptor antagonist AM251 significantly increased mechanosensitivity in the OA joint but not in controls. This indicates that MIA-induced OA activates endogenous CB1 receptors in the knee that in turn decrease the excitability of afferent fibers. Further, a transient receptor potential vanilloid 1 (TRPV1) ion channel antagonist significantly reduced the efficacy of ACEA, which suggests that TRPV1 is involved in CB1 receptor-mediated anti-nociception[34].

A local injection of the endocannabinoid hydrolysis inhibitor URB597, which blocks anandamide catabolism, significantly inhibited afferent nerve activity in MIA-treated joints but had no effect on saline-treated joints. Inhibition was prevented by CB1 but not CB2 antagonist pretreatment. Behaviorally, URB597 produced CB1- but not CB2-dependent analgesia. Similarly, URB597 inhibited afferent nerve activity in the aged guinea pig knee OA animal model but not in the young guinea pig[40]. Those data confirm that peripheral CB1 activation can relieve OA-induced pain.

CB2 and TRPV1 receptor co-localization in synoviocytes of sham- and MIA-treated rats suggest that CB2 receptors play a role in pain[51]. Local application of the CB2 receptor agonist GW405833 significantly reduced joint afferent firing rate in control knees but potentiated firing in OA knee joint mechanoreceptors. This illustrates the paradoxical effects of CB2 in healthy and pathological joints. GW405833 also increases calcitonin gene-related peptide (CGRP) release via a TRPV1 channel-dependent mechanism to facilitate pain[51]. These studies show that CB1 and CB2 have different effects on OA-associated pain.

MMPs

MMPs are zinc-dependent endopeptidases that play an important role in OA pathogenesis[52]. Recent studies demonstrate the analgesic efficacy of MMP inhibitors in OA pain. A non-selective and equipotent MMP − 2, 8, 9, 12, and 13 inhibitor reduced osteochondral vascularity, chondropathy, and weight-bearing asymmetry in a rat knee OA model induced by transecting the medial collateral ligament and cutting the full thickness of the meniscus of the knee[53]. A specific MMP-13 inhibitor not only significantly reduced MIA-induced cartilage damage but also improved weight bearing in an MIA-injected hind limb[54]. Additionally, the protease inhibitor MG132 reduces pain and reverses cartilage MMP-3 upregulation[55]. These studies indicate that protease inhibitors such as selective MMP inhibitors produce both chondroprotection and analgesia during OA.

Other bioactive agents

In the MIA-induced knee OA model, ABC294640[56], a selective inhibitor of sphingosine kinase-2; AS1892802[57,58], which rarely penetrates the central nervous tissue and is a selective Rho kinase inhibitor; and MEN16132[59], a kinin B(2) receptor antagonist, significantly improved weight bearing of the injected limb and alleviated cartilage degradation. Systemic co-administration of the

selective positive allosteric modulator NS-9283 enhanced the analgesic potency of the nicotinic acetylcholine receptor (nAChR) α4β2 agonist ABT-594 5-fold[60]. These studies indicate that sphingosine kinase-2, Rho kinase, kinin B(2) receptors, and nAChR are involved in OA pain. Rebamipide, a free radical scavenger[61], significantly inhibited MIA-induced pain and cartilage degeneration by decreasing MMP-13, IL-1β, hypoxia-inducible factor-2alpha (HIF-2α), inducible NO synthase (iNOS), and nitrotyrosine expression in OA cartilage and by increasing tissue inhibitor expression of MMP-1 and MMP-3[62]. This suggests that reactive oxygen species (ROS) are involved in OA pain.

In the ACLT-induced knee OA model, nitrite levels in joint exudates and iNOS in synovia were significantly increased. Systemic pretreatment with the non-selective iNOS inhibitor L-N(G)-nitroarginine methyl ester or the selective iNOS inhibitor 1,400W reduced joint pain[63], showing that NO release is associated with such pain. Zoledronate inhibition of osteoclasts prevented cartilage loss and pain in MIA and MMT models[64], which suggests osteoclast involvement in OA pain.

In the Dunkin-Hartley guinea pig model of spontaneous OA, long-term (1 month) administration of AZ12606133, a selective cathepsin K inhibitor, significantly reduced mechanosensitivity in response to both noxious and non-noxious joint movement[65].

Additionally, PGEs are involved in OA pain (for a review, see D'Souza *et al.*, 2011). Nerve growth factor, sodium channels, angiogenesis inhibitors, and hyaluronic acid are also involved in OA pain[66]. One study of Affymetrix Gene Chip expression arrays and articular chondrocytes from an ACLT rat model showed 1,619 differentially expressed genes[67], which suggests that more bioactive agents are involved in OA pain. The aforementioned bioactive chemicals might work in concert to produce OA pain.

Spinal mechanisms

The report of variable links between injury, pain, and spreading pain in OA patients indicates that spinal and supraspinal processing of painful inputs are altered during OA.

Glia cells/cytokines

In the MIA-induced OA model, microglia show significant hyperactivity between days 7–28, while reactive astrocytosis were seen at day 28, the late stage of OA. Nimesulide, a COX inhibitor, and minocycline attenuated pain behavior assessed with weight bearing, mechanical hind paw allodynia, and microglia and astrocyte activation in the ipsilateral spinal cord[68]. MIA-injected rats displayed reduced hind limb grip force 1, 2, and 3 weeks post-MIA, gradual increase of phospho-extracellular signal-regulated kinase 1/2 (ERK1/2) in neurons with a significant increase 3 weeks post-MIA, and rapid increase of phosphorylation of p38 mitogen-activated protein kinases in microglia and neurons that peaked a week post-MIA. Intrathecal injection of the mitogen-activated protein kinase 1 inhibitor PD98059 blocked hind-limb grip force reduction and pERK1/2 induction in MIA-OA rats[69]. These studies demonstrate that spinal glia are involved in OA-induced pain.

MIA injection at 5 weeks resulted in significant protein increases in the lumbar spinal dorsal horn of multiple pro-inflammatory cytokines and chemokines such as IL-1α/β, chemokine (C–C motif) ligand 5, cytokine-induced neutrophil chemoattractant 2α/β (CINC 2α/β), IL-13, IL-17, thymus chemokines (TC), TNF-α, L-selectin, tissue inhibitor of metalloproteinases-1 (TIMP-1), and vascular endothelial growth factor (VEGF). It decreased the protein levels of IL-4 and IL-10, fractalkine, an acute spinal injury pain-associated chemokine, and granulocyte–macrophage colony-stimulating factor in the spinal dorsal horn. Consistent with the protein data, TNF-α mRNA was increased in the spinal dorsal horn at week 5 but not at week 2. In DRG, TNF-α and IL-1α/β mRNA increased in MIA, ACLT, and DMM models[70].

MicroRNAs-146a (miR-146a) mRNA from the lumbar DRG and the spinal dorsal horn of MIA-injected rats significantly decreased 2 and 4 weeks post-MIA compared to sham controls, and human astrocytes transfected with miR-146a significantly decreased mRNA expression of TNF-α, COX-2, iNOS, IL-6, IL8, and TRPV1[71]. Most of these bioactive chemicals are involved in pain, so a miR-146a decrease during OA might facilitate pain.

A recent study in mice demonstrated that monocyte chemoattractant protein (MCP)-1 (CCL2) and its high-affinity receptor, chemokine (C–C motif) receptor 2 (CCR2), significantly increased in L3-5 DRG neurons at 8 weeks and returned to base levels at 16 weeks post-DMM surgery. Movement-provoked pain behaviors appeared at 8 weeks and were maintained for at least 16 weeks. A systemic CCR2 receptor antagonist administered to wild type DMM mice 9 weeks after surgery reversed movement-evoked pain. After DMM, CCR2-null mice showed an absence of movement-evoked pain behaviors, and rapid recovery from mechanical allodynia occurred at 4 weeks in both wild and CCR2-null mice. CCR2-null mice also had less DRG infiltration by macrophages that express numerous algogenic molecules that contribute to pain. These data indicate that DRG neuronal MCP-1 and its receptor, CCR2, participate in the development of movement-evoked pain and the maintenance of mechanical allodynia[72].

Cannabinoids

Endocannabinoids show adaptive changes in the spinal cord during the development of MIA-induced OA. MIA injection into the knee not only decreased weight-bearing force and induced mechanical allodynia in the ipsilateral hind paw but also significantly facilitated spinal wide dynamic range (WDR) neuron response to innocuous and noxious mechanical stimulation. The relationship between weight-bearing force and WDR neuron response to hind paw stimulation were significantly correlated on day 28 after the MIA injection. Further, anandamide and 2-arachidonoyl glycerol (2-AG) as well as N-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and diacylglycerol lipase alpha (DAGLα), which synthesize anandamide and 2-AG, respectively, increased in spinal cords of MIA-treated rats. Spinal administration of CB1 (<10 μg/50 μl) and CB2 (0.001–0.1 μg/50 μl) receptor antagonists significantly facilitated innocuous and noxious mechanically evoked responses of WDR neurons in MIA-treated but not saline-injected rats. Administration of the endocannabinoid hydrolysis inhibitor URB597 significantly inhibited mechanically evoked WDR neuron response in MIA-treated rats compared to saline-treated rats[73]. These data demonstrate that spinal endocannabinoids adaptively dampen nociceptive transmission through CB1 and CB2 receptors.

TRPV1

An early investigation showed that the protein levels of CGRP and TRPV1 was higher in primary neurons innervating the knee in MIA-induced OA rat model than in those of control animals[74]. In more recent studies, spinal TRPV1 activities were enhanced[75], and a TRPV1 antagonist alleviated hind limb grip force impairment in an MIA OA model[75,76]. A TRPV1 antagonist inhibited OA-enhanced glutamate and CGRP release in the spinal cord[75] and spinal WDR and nociceptive specific (NS) neuron response to 300-g von Frey stimulation of the MIA-OA knee joint[76]. Moreover, mechanical allodynia was relieved in MIA-induced OA rat pretreated with the TRPV1 agonist capsaicin[77], likely due to dysfunction of nociceptive fibers, and a systemic TRPV1 antagonist effectively blocked thermal hypersensitivity in MIA-

induced OA model[23]. These studies confirm that spinal TRPV1 is involved in OA-related ambulatory, mechanical, and thermal pain.

Although systemic administration of the TRPV1 receptor antagonist A-889425 reduced enhanced spontaneous firing of WDR neurons in OA rats[76], the systemic TRPV1 antagonist AMG9810 did not block ongoing pain assessed with conditioned place preference [23]. Inconsistency between the electrophysiological and behavioral studies might be the result of the difference in MIA dosage (3 vs 4.8 mg). The assessed ongoing pain behavior involves supraspinal mechanisms. It is possible that the TRPV1 antagonist does not modulate activity of supraspinal neurons such as those of nucleus accumbens which is associated with OA-induced spontaneous pain[78]. Furthermore, a TRPA1 antagonist did not reduce the spontaneous activity of spinal WDR neurons in OA rats[79], nor did a systemic or intra-articular TRPA1 antagonist block weight asymmetry and ongoing pain. These data suggest that MIA-induced ongoing pain is independent of TRPV1 and TRPA1 activation[23].

Additionally, since surgery-induced OA and spontaneously-developed OA models also mimic some characteristics of human OA, the involvement of TRPV1 in those models-induced pain warrant further investigation.

Neuropeptides

Intra-articular MIA induced significant mRNA expression of SP and CGRP in DRG neurons, and significant increase of SP and CGRP-immunoreactivity in synovia, periosteum, and subchondral bone on day 21 post-MIA compared to control[55]. Notably, the hip joint capsule and soft tissue in patients with painful OA also show SP and CGRP upregulation[80]. Another study showed CGRP and SP upregulation starting on days 7 and 28, respectively, and dynorphin (1–32) downregulation on day 14 in the spinal cord[19,81]. At day 35 post-MIA, DRG CGRP and SP declined while galanin, neuropeptide Y and downstream regulatory element antagonist modulator increased[70]. Double labeling showed that intra-articular MIA induced CGRP upregulation in DRG neurons that innervate the knee on day 31[82]. These studies indicate that OA pain can be modulated by spinal neuropeptides. Celecoxib improved gait parameters such as swing speed and swing phase duration, relieved mechanical allodynia, and decreased spinal CGRP but not SP in MIA-induced OA rat model[19]. Eugenol, the main constituent of clove oil, improved dynamic gait parameters (swing speed, swing phase duration and duty cycle) and mechanical allodynia of the affected limb. Concomitantly, it decreased spinal SP and CGRP and increased spinal dynorphin in MIA-induced OA rat model[83]. Furthermore, intrathecal injection of the peptide antagonist CGRP (8–37) mitigated MIA-induced mechanical allodynia[84]. These studies suggest that chemicals that reverse spinal neuropeptide regulation alleviate OA pain.

Neuropathic components of OA pain

Because of alterations in the central nervous system and the peripheral nerves that innervate the joints, OA-associated pain gradually develops the characteristics of neuropathic pain[85]. Previous studies show that activation of a marker of nerve injury, transcript factor (ATF-3) in the DRG, significantly increased between days 8 and 14 post-MIA and that NSAID analgesia peaked on day 14 and decreased in efficacy thereafter[85]. This indicates a transition at days 8–14 from early inflammatory pain to late-stage inflammatory-neuropathic pain. Another study also showed that ATF3-immunoreactive growth-associated protein-43-immunoreactive DRG neurons significantly increased in the ipsilateral DRG 14 days after the injection[29]. Additionally, an MIA injection resulted in a significant reduction in intra-epidermal nerve fiber density of the plantar hind paw post-MIA between days 7 and 14[86]. Microgliosis, a characteristic of neuropathic pain[87], also occurs in the spinal cord 7–21 days post-MIA[29,86]. Taken together, these data suggest that neuronal damage occurs during the development of OA, which might explain why OA pain treatment and management is unsuccessful. It is not known whether surgery-induced and spontaneously-developed OA models show any characteristics of nerve injury, which warrant further investigation.

Supraspinal mechanisms

A study showed that electroacupuncture (EA) significantly improved MIA-induced hind limb incapacity within 7 days of the MIA injection; the effect was prevented by 5-hydroxytryptamine (5-HT) 2A/C antagonism. This suggests that EA activates descending inhibitory 5-HT to inhibit early-stage OA-pain[88].

Studies in rats demonstrate that, compared to a saline injection, spinal WDR neurons show stimulus intensity-dependent and significantly enhanced response to mechanical stimulation 15–19 days after an intra-articular MIA injection. Response to thermal stimulation was also higher in MIA- than in saline-injected rats, although this response was not statistically significant. Further, spinal 5-HT3 antagonism significantly decreased the neuron response induced by tactile mechanical stimulus and produced greater inhibition of response to 45°C thermal stimulation in MIA rats than in control. These data indicate tactile mechanical and noxious thermal stimulation-evoked responses are facilitated by spinal 5-HT3 receptors. Since spinal 5-HT is supraspinally produced, descending 5-HT/5-HT3 receptor facilitation might contribute to OA-induced pain. Moreover, the α2δ-1 subunit of voltage-gated calcium channels (VGCCs) increased in ipsilateral DRG in MIA rats compared to control. Consistent with these results, pregabalin inhibited spinal neuronal responses evoked by noxious electrical stimulation and innocuous and noxious natural stimulation in MIA but not in saline control rats. This inhibition was prevented by 5-HT3 receptor antagonism. Therefore, descending 5-HT/5-HT3 facilitation coupled with upregulation of α2δ-1 VGCC subunit expression might contribute to OA pain[89].

Recently, a study in the MIA model demonstrated that intra-articular lidocaine induces conditioned place preference for the lidocaine-paired compartment, indicating OA pain has an aversive component[23]. This suggests that OA pain also involves supraspinal mechanisms. Although clinical imaging studies demonstrate brain changes during chronic pain, image studies have not been performed in OA pain animal models until recently. In an MMT animal model, increased functional connectivity was observed 3–5 weeks post MMT in nucleus accumbens- and ventral posterior lateral thalamus-based functional connectivity analyses. Those changes were attenuated by sustained treatment with a broad-spectrum MMP inhibitor or acute treatment with celecoxib[90]. In humans, gray matter decreases in the anterior cingulate cortex (ACC), dorsolateral prefrontal cortex, amygdala, and brainstem were reversed when patients feel no hip OA-induced pain after hip replacement surgery. This suggests that brain changes are related to the pain[91]. Additionally, it has been reported that nucleus accumbens activity is associated with spontaneous OA-induced pain[78] and facilitates nociception[92]. Whether this increased functional connectivity during OA contributes to chronic pain requires further investigation.

Conclusion

OA is the most common form of joint disease. Recent studies on OA-induced pain demonstrate that peripheral pro-inflammatory mediators and neuropeptides can sensitize knee nociceptors (Fig. 1). The pro-nociceptive plasticity of spinal cytokines and neuropeptides promotes OA-associated pain. Peripheral and spinal

cannabinoids might respectively inhibit OA pain through CB1 and CB1/CB2 receptors. TRPV1 contributes to OA pain (Fig. 1). MMP-13 inhibitor reduces MIA-induced cartilage damage and indirectly alleviates pain[54]. Other bioactive chemicals such as sphingosine kinase-2, Rho kinase, kinin B(2) receptors, ROS, iNOS, cathepsin K, PGEs, nerve growth factor, and sodium channels are all involved in OA pain, and supraspinal descending facilitation of 5-HT/5-HT3 receptors might contribute to OA pain. During OA, brain functional connectivity is enhanced, but how connectivity changes are related to OA pain remains elusive.

Author contributions

Ruixin Zhang contributed to drafting, revisions and final approval of the article. Ke Ren and Ronald Dubner contributed to revisions and final approval of the article.

Conflict of interest

The authors declare no conflicts of interest.

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