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Review

Excitatory amino acid glutamate: role in peripheral nociceptive transduction and inflammation in experimental and clinical osteoarthritis

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SUMMARY

Although a large proportion of patients with osteoarthritis (OA) show inflammation in their affected joints, the pathological role of inflammation in the development and progression of OA has yet to be clarified. Glutamate is considered an excitatory amino acid (EAA) neurotransmitter in the mammalian central nervous system (CNS). There are cellular membrane glutamate receptors and transporters for signal input modulation and termination as well as vesicular glutamate transporters (VGLUTs) for signal output through exocytotic release. Glutamate been shown to mediate intercellular communications in bone cells in a manner similar to synaptic transmission within the CNS. Glutamate-mediated events may also contribute to the pathogenesis and ongoing processes of peripheral nociceptive transduction and inflammation of experimental arthritis models as well as human arthritic conditions. This review will discuss the differential roles of glutamate signaling and blockade in peripheral neuronal and non-neuronal joint tissues, including bone remodeling systems and their potentials to impact OA-related inflammation and progression. This will serve to identify several potential targets to direct novel therapies for OA. Future studies will further elucidate the role of glutamate in the development and progression of OA, as well as its association with the clinical features of the disease.

Introduction

Role of excitatory amino acids in peripheral nociceptive transduction

Osteoarthritis (OA), 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. OA manifests as joint pain and loss of joint function and currently has no satisfying treatments. Although a large of proportion of patients with OA show inflammation in their affected joints, the pathological role of inflammation in OA development and progression has yet to be clarified. Glutamate is thought to be an excitatory amino acid (EAA) neurotransmitter in the mammalian central nervous system (CNS). Evidence that glutamatergic signaling is also functional in non-neuronal tissues outside the CNS such as the pancreas, skin, and bone is accumulating in the literature[1,2]. Glutamate may be one of the endogenous autocrine/paracrine factors that play a role in intercellular communications within the bone-related cells[3,4]. In bone, several possibilities of glutamate origin are conceivable. Both sympathetic and sensory nerve fibers innervate into bone, while glutamatergic innervation is distributed even in bone[5,6]. Involvement of EAA in peripheral nociceptive transduction has also been reported in animal models of acute arthritis[7]. In a kaolin/carrageenan-induced arthritis model in rats, the expected increase in synovial fluid (SF) glutamate levels was blocked by pretreatment with intra-articular lidocaine, which decrease neurotransmitter release from the peripheral neuronal endings in the joint in response to injury[8]. Indeed, glutamate may act as a more widespread “cytokine” rather than as a “neurotransmitter” and influence a variety of cellular activities in different tissues[1,2].

Glutamate is highly involved in both pain and metabolic pathways[9–12]. Beyond the physiological roles of glutamate in joint tissues, growing evidence in clinical and experimental

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models indicate that glutamate-mediated events may also contribute to the pathogenesis of human arthritic conditions[13]. Our group determined that glutamate and aspartate levels were significantly increased in the microdialysates of anterior cruciate ligament–transected (ACLT) knee joints in rats, suggesting a role of EAA in the acute and ongoing progressive processes of soft tissue injuries that impact early OA development[14]. Additionally, Alfredson *et al.*[15], reported an increase in glutamate levels in the tendon dialysate samples derived from in patients with painful chronic Achilles tendinosis. Franklin *et al.*[16] reported that glutamate and the glutaminergic system may play key roles in painful human supraspinatus tendon tears and importantly, previous studies have demonstrated significantly increased SF glutamate and aspartate levels in patients with active arthropathies[17,18].

Elevated levels of glutamate in SF dialysates have been shown to be relevant to increased swelling and sensitization to thermal hyperalgesia in experimental (inflammatory or injury-based) arthritis models[14]. In previous *in vitro* studies, local glutamate can bind and activate peripheral receptors on local osteocytes, chondrocytes, and synoviocytes to enhance local inflammation and pathologies[19–24]. Significant associations between EAA and inflammatory mediators have been demonstrated in the SF of patients with active arthropathies, including OA[18]. From clinical samples, SF glutamate levels correlated with increased SF levels of inflammatory mediators such as tumor necrosis factor-α (TNF-α), regulated on activation normal T-cell expressed and secreted (RANTES), and interleukin-8 (IL-8) in the SF of patients with active inflammatory arthropathies, such as RA, acute gout and symptomatic OA[18,19].

In our previous studies, injections of hyaluronic acid from weeks 8–12 in ACLT rat knees slowed the progression of OA related changes and was accompanied by decreased SF-microdialysate glutamate levels[25].

The excess glutamate released within inflamed tissues is likely derived from a variety of neuronal and non-neuronal sources. Among the sources investigated previously are nerves, lymphocytes, macrophages, synoviocytes, osteoblasts, osteoclasts and chondrocytes[8,13,19,26], mast cells[27], platelets[28], neutrophils[29], and fibroblasts or Schwann cells[30,31]. OA-associated pain may result at least in part from glutamate release from the axons innervating the inflamed region[32]. A vast number of potential mediators have been shown to be associated with both clinically and experimentally induced arthritic animals, also originating from multiple potential sources. Outlined below, we will address potential signaling systems in which glutamate may play a role as an extracellular signal mediator and clarify its role as an inflammatory mediator in the bones and joints of individuals with OA.

Expression and function of EAA glutamate receptors, glutamate transporters, and vesicular glutamate transporters (VGLUTs) in bone and joints

Glutamate receptors

The actions of extracellular glutamate are mediated by membranous receptors, which can be divided into ionotropic (iGluRs) and metabotropic (mGluRs) receptors, according to their differential intracellular signal transduction mechanisms and molecular homologies[33].

The former are further classified into *N*-methyl-D-aspartate (NMDA), DL-α-amino-3-hydroxy-5-methylisoxasole-4-propionate (AMPA), and kainate (KA) subtypes according to their sequential similarities and responsiveness to different agonists and antagonists[34], whereas the latter is further divided into three distinct subtypes with seven transmembrane domains, including group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3), and group III (mGluR4, mGluR6, mGluR7, and mGluR8), according to exogenous agonists and intracellular second messengers[35]. NMDA receptors are multimeric complexes that consist of NR1 subunits and one of four NR2 subunits (NR2A-D)[36]. The NMDA receptor subunit 1 (NR1) is considered an essential component of all functional NMDA receptors[37]. Increased phosphorylation of NR1 (p-NR1), which occurs via intracellular signaling pathways, has been recognized as an important mechanism contributing to the regulation of NMDA receptor function[38]. Various components of the glutamate receptors were recently detected in cartilage cells. For instance, rat costal chondrocytes express mRNA for NMDA and non-NMDA receptors[39]. Moreover, using immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR) techniques, the NMDAR1 and NMDAR2A subunits of NMDA receptors were both found to express in normal and OA chondrocytes[40]. Moreover, in mammalian bone, NMDA receptors are also expressed in osteoblasts and osteoclasts as revealed by RT-PCR, *in situ* hybridization, immunohistochemistry, and electrophysiology[41,42].

NMDA, AMPA, KA, and metabotropic glutamate receptor subtypes 1, 4, and 5 (mGlu1, mGlu4, and mGlu5) were shown to mediate pain in the arthritic joint[9,10]. Lawand *et al.*[43] showed that NMDA antagonists injected into an inflamed knee decreased blood flow and swelling in the periarticular regions, while Zhang *et al.*[11] demonstrated a role of NMDA receptors in the induction of arthritic joint pain. Glutamate and its receptor agonists were also reported to induce TNF-α production in synovial cells, which further upregulated chemokine and cytokine production in primary synoviocyte cultures from a patient with RA[18]. Flood *et al.* indicate that the activation of NMDA and KA glutamate receptors on human synoviocytes may lead to joint destruction by enhancing IL-6 expression[21]. NMDA glutamate receptor antagonists also decreased proliferation and inhibited IL-1β–induced increases in cyclooxygenase (COX)-2, IL-6, and matrix metalloproteinase 3 (MMP3) mRNA expression in rat chondrocytes[13].

Free radicals are intermediate products in cyclooxygenase (COX)-mediated prostaglandin (PG) synthesis. PG products produced in the COX pathway stimulated Ca^{2+}-dependent glutamate release in cultured astrocytes[44] and thus were shown to be involved in NMDA receptor-mediated glutamate excitotoxicity[45]. Our previous study in rats demonstrated that the selective COX-2 inhibitor parecoxib (Dynastat; Pfizer, USA) reduced OA model progression. The alleviation of synovitis and cartilage injury was associated with concomitantly decreased glutamate and aspartate levels in the knee joint dialysates after intra-articular parecoxib treatment[46].

Glutamate has also been shown to suppress the proliferation of mesenchymal stem cells[47], which have the potential to differentiate into chondrocytes. The differential expression and signaling of NMDA receptor subunits in chondrocytes may promote OA[24], by several contributory mechanisms including mediating inflammatory mediator responses and blocking chondrocyte development[13]. The above support that glutamate receptor activation could be a key regulator in peripheral pain[12], cytokine and MMP release, chondrocyte and synoviocyte proliferation[47,48], and immune reactions[49]. Conversely, glutamate receptor antagonists could potentially provide or complement novel therapies with multimodal activities against arthritis and OA symptoms.

Glutamate receptor activation has also been studied in bone physiology. Both ionotropic and metabotropic glutamate receptors are reportedly functional in osteoblasts[22] and osteoclasts[50], while antagonists to these receptors can modify bone cell phenotypes[42,50]. Functional NMDA receptors have been reported in several classes of osteocytes and bone cells, including rat and human osteoblasts and osteoclasts, MG-63 osteosarcoma cells, and bone marrow megakaryocytes[42,51]. Osteoblasts constitutively