

Neuropeptides and Articular Cartilage Pathology: Harmful or Helpful or Both?

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ABSTRACT

BACKGROUND: Osteoarthritis, a major cause of chronic disabling pain among older adults has long been considered a non-inflammatory “wear and tear” disease associated with aging that largely implicates the loss of articular cartilage of one or more freely moving joints such the knee and hip as a result of biomechanical mechanisms. Recent findings however, imply that in addition to mechanical disruption of an affected joint, its disabling features, including pain, inflammatory mechanisms, and changes in the synovium surrounding the joint play a pivotal role in this regard.

QUESTIONS: Do neuropeptides, small protein like molecules involved in neural transmission processes play a pivotal role in pathogenesis of osteoarthritis associated articular cartilage pathological changes, and can this information be applied clinically, to prevent or ameliorate the condition, and if so how?

METHODS: PUBMED, SCOPUS, and WEB OF SCIENCE articles related to the topic were searched and examined and reported in narrative form.

RESULTS: Neuropeptides are widespread neural modulators within the central and peripheral nervous system. Both excess as well as deficient neuropeptide production may prove harmful to articular cartilage, the tissue affected most clearly in osteoarthritis. Some neuropeptides appear trophic rather than catabolic, but very little definitive information prevails that can guide clinical practice safely and effectively.

CONCLUSION: More research to tease out a distinctive role for neuropeptides in the context of articular cartilage destruction and/or repair is strongly indicated.

Key words: Articular Cartilage; Inflammation; Neuropeptides; Osteoarthritis; Pain; Synovium

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INTRODUCTION

Osteoarthritis, a major cause of chronic disabling pain among older adults has long been considered a biomechanically-derived non-inflammatory form of arthritis associated with aging that largely involves the progressive destruction of the articular cartilage lining of one or more freely moving joints such the knee and hip joints^[1,2]. Recent findings however, imply that in addition to age and the impact of mechanical disruption of one or more increasingly vulnerable joints towards an end stage joint damage state^[3], its disabling features, including pain, may be due in part to the emergence of associated pathological inflammatory mechanisms that involve the synovial membrane lining the joint, along with those that can arise in other joint structures such as the bone, menisci, ligaments, tendons, and muscles^[1,2,4-6].

The current review examines what is known about neuropeptides, an array of substances that are present within the central and peripheral nervous systems and that influence behavior, and may be

influenced by stressful situations, such as in the face of a disabling disease such as osteoarthritis^[7]. Moreover, though not mentioned specifically in a recent review on osteoarthritis biology^[8], their influences on inflammatory mechanisms that have been reported in osteoarthritis may not only have a strong bearing on the spread of osteoarthritis-induced joint pain and articular cartilage destruction^[9], but may also be causative in nature^[2]. At the same time, some research points to the positive and protective trophic influence of some neuropeptides in the context of attempts to restore articular tissue homeostasis, rather than any sole destructive influence. These competing set of ideas, while not novel, appear very important to continue to examine and unravel temporally, especially if interventions to target neuropeptide release can favorably attenuate articular cartilage destruction mechanisms, while serving as a target for fostering reparative interventions in this regard, and the topic was hence duly chosen for examination in this report, given the lack of consensus or definitive information in this regard.

Significance

Osteoarthritis remains the most prevalent chronic disease disabler of older adults. Producing irreversible joint damage, and oftentimes intractable pain experiences, as well as centralization of pain, very few remedies for successfully ameliorating this common progressive disabling disease prevail despite years of research. Hence, all promising areas of research in this regard are thus of great interest to explore and pursue, especially by those who seek to advance successful health outcomes across the lifespan for those many aging adults at risk or suffering from various forms of osteoarthritis. To this end, the idea of examining the osteoarthritic disease cycle through a neurological lens, rather than solely through an age-associated biomechanical disease lens has been proposed for some time. In particular, one important aspect of current research that has been of special interest for more than 40 years^[10] is the diverse role of neuropeptides, a class of small proteins involved in neural transmission and secretory processes^[10] that may mediate joint pain and inflammation. Touted to be highly worthy of examination by Sutton *et al*^[11], Hoshino *et al*^[4], and Saito and Koshino^[2], among others^[7], this line of research which could open the doors to a better understanding of a role for neuropeptides in cartilage metabolism and biology if further pursued was presently reviewed.

Specific aim

The specific review aim was to examine the available data and to offer the reader a comprehensive synthesis of what we know, and suggestions on how this data on neuropeptides might be duly applied and advanced in the context of much needed efforts to develop effective treatments to ameliorate osteoarthritis.

METHODS

To achieve the overall aims of the review, and to answer the question of whether neuropeptides are clinically relevant in the context of osteoarthritic articular cartilage destruction and repair, all pertinent articles listed on **PUBMED, Scopus, and Web of Science 5 Data Bases** that appeared to inform about *neuropeptides* and *osteoarthritis* and/or *articular cartilage pathology* were sought and those of high relevance were examined specifically.

Since Grassel *et al*^[7,12] and Gatelholm *et al*^[13] have provided salient reviews on this topic, articles published in the last five years were those especially sought. The research material reviewed was not restricted to any particular animal model or explant, and both preclinical, as well as clinical data were considered relevant as the

literature is not voluminous or at all homogeneous in any respect. For the same reason, only a narrative overview of peer reviewed English language full length publications is provided herein, and articles not referring to neuropeptides and/or osteoarthritis in some way, as well as incomplete reports, were excluded..

RESULTS

Search results between January 1, 1980-June 1, 2020 show the following numbers of potential publication in response to a topic search (Table 1).

Table 1 Summary of numbers related studies on key data bases

	Pubmed	Web of science	Scopus
Neuropeptides + osteoarthritis	63	324	260
Neuropeptides + articular cartilage	17	90	77

Note: These data do not accurately reflect the topic information sought, including articles on anterior cruciate ligament injury, osteoarthritis model validation, hip dysplasia, nerve growth factor, conference abstracts, obesity, gait, nanotechnology.

General observations

A wide array of data tend to confirm that neuropeptides, a diverse class of signaling molecules^[10] located in unmyelinated free nerve endings in joints, plus the subchondral bone and synovia of freely moving joints, as well as in the central nervous system^[13,14] are inflammatory mediators and neurotransmitters^[15]. In this regard, several well-studied neuropeptides such as substance P, calcitonin gene-related peptide, vasoactive intestinal peptide and neuropeptide Y are deemed the major neuropeptides involved both in the generation of joint pain and inflammation, as well as reducing pain post-joint trauma. As well, neuropeptides serving as vasodilators^[15] reportedly potentiate a variety of chemotactic agents that can promote joint tissue inflammation. The interplay between these aforementioned neuropeptides and others and especially their influence on cytokines and their production, which appears to underpin the mechanisms whereby noxious mechanical/chemical stimuli are transduced, transmitted and modulated and finally perceived is thus of high interest to explore in the context of painful osteoarthritis symptomology and joint destruction processes.

In particular, important evidence indicates that there is a close interaction between the different neuropeptides located in various joint structures such as the synovium and articular cartilage and mensci that may influence the process of load adaptation and catabolic processes found in the context of joint injury, and inflammation, as well as osteoarthritis associated joint pain^[1,16]. Research also shows that many cells localized to the articular cartilage tissue itself express receptors for a variety of neuropeptide molecules, such as substance P, vasoactive intestinal peptide and neuropeptide Y. Given what we do know, the manner in which neuropeptides may conceptually impact articular cartilage viability in the context of osteoarthritis is depicted below in Figure 1 and assumes a role for the nervous system in this regard. While more research is needed to explore these interactive mechanisms, the direct influence of neuropeptide molecules inherent in articular cartilage must also be acknowledged in this respect.

Evidence based observations

Evidence supporting the interactions depicted in Figure 1, as well as observations that reveal neuropeptides can impact spinal cord dorsal root ganglion mechanisms associated with sensory transmission, thereby having the potential to induce an axonal reflex response in

injured tissue that may invoke further inflammatory substances to be released stems from several key publications^[1,2,5,11,14,17-19]. These researchers and others further reveal that osteoarthritis mononuclear cells such as T-cells and macrophages that can be found to infiltrate damaged or pathologically altered joint synovial tissues, can also heighten pro-inflammatory cytokine levels in the peripheral blood vessels as well as the synovial fluid in response to diverse neuropeptides, even if this feature is not a constant one. These inflammatory mediators including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) can also foster peripheral sensitization of joint nociceptors and the levels of nerve growth factors (e. g. NGF) found to trigger the expression of Transient Receptor Potential Vanilloid 1 (TRPV1) multistate ion channels in primary afferents that may have a bearing on the ability of the affected joint(s) to withstand stress, followed by subsequent cartilage breakdown, especially if protective neuromuscular reflexes are impaired for example, in some way. Moreover, in addition to a role in mediating the above mechanisms, neuropeptides have been found to lower the threshold of osteoarthritic joint nociceptors that contain substance P and other neuropeptide molecules^[1].

As outlined by Li *et al*^[17] substance P, a neuropeptide widely present in nerve fibers can impact the biology of bones and related tissues such as articular cartilage in various ways by binding to compatible receptors on their cell bodies. They can thereby potentially regulate bone and cartilage metabolism, the key tissues involved in osteoarthritis. The neuropeptide known as substance P can also be specifically recognized by both the immune and the nervous systems, and previous studies have shown that both bone cells and chondrocytes can synthesize and secrete these previously mentioned sensory neuropeptides, as well as expressing their receptors. As such, they can hypothetically be expected to have the ability to influence proliferation, differentiation, apoptosis, matrix synthesis, and the degradation of target cells through autocrine/paracrine mechanisms of action.

Li *et al*^[17] further indicate that substance P seems to be especially important for fostering cartilage health because it participates in mechanical transduction processes through the receptor known as Nk-R1. In this regard, it was shown that the addition of 1 $\mu\text{mol/L}$ of substance P to cultured chondrocytes or the application of 0.33 Hz of mechanical stimulation tended to cause cell membrane hyperpolarization, an observation strongly suggesting substance P might be a salient factor in the mechanical transmission processes that influence chondrocyte biology. In contrast, blocking the signaling processes of chondrocyte substance P production by a chemical antagonist of Nk-R1 inhibited the aforementioned chondrocyte responses to mechanical stimulation. Exercise related studies were also cited in this regard to support a role for substance P in regulating the physiological microenvironment of cartilage, and its metabolism, and hence its overall contribution to joint function.

Kawaria *et al*^[20] who investigated the local production of proinflammatory cytokines, pain-related sensory innervation of dorsal-root ganglia, and spinal changes in a rat model of induced hip osteoarthritis found pain-related pathologies due to the-induced hip osteoarthritis to originate from inflammation caused by cytokines, which led to progressive, chronic neuronal damage that may cause neuropathic pain. This group also measured the local production of TNF- α , immunoreactive (-ir) neurons for calcitonin gene-related peptide, and growth associated protein-43 in the dorsal root ganglion, as well as immunoreactive neurons for ionized-calcium-binding adaptor molecule-1 on post-osteoarthritis induction days 7, 14, 28, 42, and 56. For post-induction days 7-42, the arthritic rats were found to

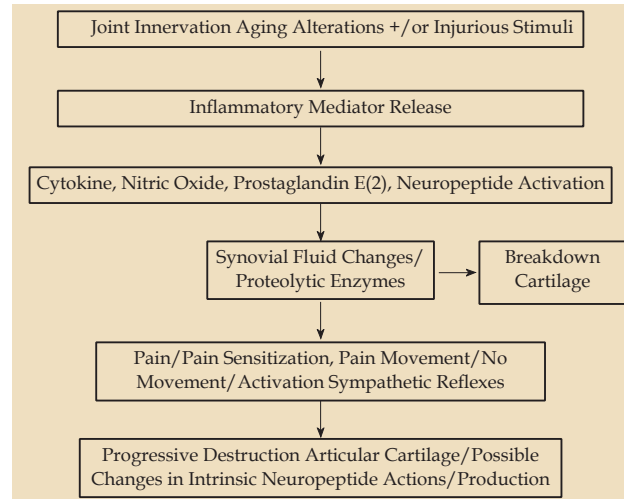


Figure 1 Hypothesized interactive mechanisms of multiple neuropeptide actions and articular cartilage pathology.

have a significantly elevated TNF- α concentration, as well as higher calcitonin gene-related peptide dorsal root ganglion expression than the sham group ($p < 0.01$), implying a role for calcitonin gene-related peptide in the inflammatory arthritic processes observed in the rat model of osteoarthritis.

In addition, Schou *et al*^[21] confirm that in addition to other neuropeptides, the neuropeptide, calcitonin gene-related peptide is widely distributed in nociceptive pathways in human peripheral as well as the central nervous system and that its receptors are also expressed in pain pathways. Performing a systematic literature search on PubMed, Embase and ClinicalTrials.gov for articles on calcitonin gene-related peptide and non-headache pain in humans, this group noted a positive association between measured levels of this neuropeptide and somatic, visceral, neuropathic and inflammatory pain in subjects with varying forms of musculoskeletal pain. However, a randomized clinical trial on a monoclonal antibody, which selectively binds to and inhibits the activity of calcitonin gene-related peptide (galcanezumab) in patients with osteoarthritis knee pain, failed to demonstrate any pain improvement when compared with the administration of a placebo.

In an early investigation by Buma *et al*^[22] who aimed to describe the normal distribution of calcitonin gene-related peptide and substance P containing fibres in the knee joint of the mouse to gain insight into possible innervation changes associated with joint degenerative processes, found a rich innervation of thin varicose calcitonin gene-related peptide and substance P immunoreactive fibres to be present in most peri- and intra-articular tissue components. The periosteum, synovial tissues, the joint capsule and the intra-articular fat tissues were especially richly innervated, with less intense innervation in the subchondral bone plates of the tibio-femoral joint and patella. Fibres were also found in the soft tissues between the patellar tendon and the femoral groove, and generally more calcitonin gene-related peptide than and substance P distribution levels were found. The collagenase-induced osteoarthrosis characterized by sclerosis of the subchondral bone, patellar dislocation, osteophyte formation, synovial proliferation and by severe cartilage abrasion, showed calcitonin gene-related peptide and substance P innervation was no longer detectable by immunolabelling with the antibodies, and that this might explain both osteoarthritis pain production and ensuing destruction of the joint.

As outlined in several key reviews^[7,12,16], the plausibility and possible clinical utility of the above findings appear justified given

the evidence that various resident cell types of the musculoskeletal system do appear to express receptors for sensory and sympathetic neurotransmitters and that these can mediate a variety of responses to peripheral neuronal stimuli that may implicate one or more neuropeptides. These include chondrocytes of different origin, which express distinct subtypes of adrenoceptors, plus receptors for vasoactive intestinal peptide, substance P, and calcitonin gene-related peptide. Some of these cell types also synthesize and secrete specific types of neuropeptides such as substance P and in turn these sensory and sympathetic neurotransmitters appear to be involved in the pathology of inflammatory diseases such as rheumatoid arthritis, and may play a role in the progression of degenerative joint disorders such as osteoarthritis. Indeed, it appears that sensory and sympathetic neurotransmitters do have the potential to exert crucial trophic effects that are critical for the maintenance of joint tissue and bone homeostasis, as well as also having a modulatory role in the context of articular cartilage, subchondral bone and synovial tissue properties, in addition to their classical neurological features. Thus attempts towards blocking these substances completely through artificial means may not prove favorable to the tissue in question, and may not take into account the possible changes that osteoarthritis might impose on cells such as macrophages^[23], which appear to change their mechanical responses.

Additional empirical findings

Early work by Kopp^[24] who studied the neuropeptide Y in the context of temporomandibular joint arthritis, not only found this neuropeptide to be present in high levels in the synovial fluid of painful arthritic temporomandibular joints, but that this pain-related mediator was also associated with restricted mandibular mobility as well as joint hyperalgesia. In the context of the interactions shown in Figure 1, this research group found that anterior open bite, a clinical sign of joint destruction was indeed associated with high levels of the neuropeptide as well as IL-1 β in the synovial fluid. IL-1 β , which, in turn, was related to radiographic signs of joint destruction.

Another endogenous neuropeptide, known as pituitary adenylate cyclase activating polypeptide secreted by non-neural cells, and also secreted by chondrocytes, appears to have a positive effect, rather than a negative effect on articular cartilage structural and metabolic processes. Even under conditions of inflammation, it appears to have the potential to inhibit the activity of matrix destroying enzymes under cellular stress. Moreover, the prospect that application of this neuropeptide can ameliorate articular cartilage destruction in joint diseases has been raised^[25].

However, obliterating pain that is due to persistent mechanical aberrations, and others, may not be effective if more mechanical trauma or stresses than normal are forthcoming, and no actual intrinsic cartilage repair mechanism can be initiated. Findings under controlled conditions in the laboratory, may also not replicate the human situation at all well, given that interactions between some neuropeptides may continue or be changed for the worse, medications the patient may already be taking may interfere with expected outcomes, and dosages that may be helpful or harmful are hard to ascertain, among other factors.

The exploration of this idea as well as other avenues for intervening to minimize articular cartilage damage in osteoarthritis does however seem a valid one as implied by findings of Perez-Garcia *et al*^[26]. This group focused on the role of the metalloproteinase ADAMTS known to play an important role in the pathogenesis of osteoarthritis, and its association with vasoactive intestinal peptide and corticotrophin-releasing factor, as immunoregulatory neuropeptides. Subjects'

synovial fluid was stimulated with pro-inflammatory mediators and treated with one of these named neuropeptides. Results showed that both neuropeptides decreased ADAMTS-4, -5, -7 and -12 expressions, aggrecanase activity, glycosaminoglycans, and cartilage oligomeric matrix protein degradation after stimulation with fibronectin fragments in the osteoarthritic synovial fluid. After stimulation with interleukin-1 β , additional favorable effects as far as cartilage degradation were noted. The findings appeared to implicate the neuropeptides examined in the pathology of osteoarthritis, but that their effects might be greater when a degradation loop has been established, an observation that may hold promise for efforts to ameliorate articular cartilage degradation and the ensuing process of possible overall joint degradation.

In addition to the above discourse and related conclusions, Zhao *et al*^[27] another group of researchers who examined the nature of cortistatin, a neuropeptide discovered over 20 years ago, and said to play a vital role in inflammatory reactions in the context of osteoarthritis, showed a deficiency of cortistatin led to an accelerated osteoarthritic-like phenotype, while exogenous cortistatin attenuated its development. Additionally, TNFR1- and TNFR2-knockout mice models indicated that TNF receptors might be involved in the protective role of cortistatin in osteoarthritis and that cortistatin inhibited activation of the NF- κ B signaling pathway in osteoarthritis. To this end the interaction between cortistatin and TNF- α receptors was investigated in several chondrocyte associated experiments using surgically induced and spontaneous osteoarthritic models.

Ghrelin, another recently discovered neuropeptide with anti-inflammatory actions, involved in osteoarthritis described by Qu *et al*^[28] appears additionally noteworthy. This group who collected human primary chondrocyte and cartilage samples from patients with osteoarthritis, and assessed the expression pattern of ghrelin in both human chondrocyte and cartilage samples stimulated with IL-1 β and TNF- α found ghrelin down-regulated the production of various inflammatory cytokines. It also inhibited chondrocyte apoptosis, decreased metalloproteinases levels, while maintaining the expression of critical matrix components, such as aggrecan and collagen 2. Moreover, the researchers showed that the suppression of the Akt signaling pathway and activation of NF- κ B signaling in chondrocytes during osteoarthritis development was antagonized by ghrelin administration. These results were hence taken as evidence to support the assessment of ghrelin as a potential therapeutic approach to treat degenerative cartilage diseases, including osteoarthritis. It was also concluded that ghrelin protects against osteoarthritis through the interplay of this neuropeptide with the Akt and NF- κ B signaling pathways.

Kang *et al*^[29] whose research, like that of Kopp^[24] focused on the direct effect of neuropeptide Y in the context of cartilage degradation, found this neuropeptide to be overexpressed in human osteoarthritic cartilage along with the increased expression of both the NPY1 receptor (NPY1R) and the NPY2 receptor (NPY2R). Stressors such as cold exposure resulted in the peripheral release of neuropeptide Y from sympathetic nerves, which in turn promoted upregulation of neuropeptide Y and the NPY2R in articular cartilage in vivo. Intra-articular administration of neuropeptide Y significantly promoted chondrocyte hypertrophy and cartilage matrix degradation, with a higher OARSI [functional] score than that of control mice, whereas inhibition of NPY2R but not NPY1R with its specific antagonist remarkably ameliorated neuropeptide Y-mediated effects. Along with related observations, the authors concluded that neuropeptide Y plays a crucial role in cartilage homeostasis. He *et al*^[30] who showed substance P is increased in the presence of osteoarthritis in rats also

showed cartilage improvements and reduced inflammation, including substance P levels in response to 2-aminoquinoline.

A further noteworthy study by Duarte *et al*^[31] has shown that this line of research is highly relevant given that they were able to show the spread of knee cartilage damage in response to lumbar spinal injuries inflicted on rats. Their findings that indicated neurogenic mechanisms may mediate the spread of substance P neurosegmentally were taken to tentatively explain how damage to a single joint might impact the cartilage homeostasis of distal joints.

Overview of clinical studies

In the realm of clinical observations, Saito and Koshino^[2] who examined the synovium of cases with knee osteoarthritis as far as neuropeptide distribution was concerned found the synovium to have an extensive somatic and autonomic nerve supply. Neuropeptides were abundant and included substance P, calcitonin gene related peptide immunoreactive free nerve endings in high numbers that varied in frequency depending on the knee joint compartmental site studied. It was concluded that these nerve endings, in particular those containing substance P may modulate osteoarthritis pain and inflammation. Neuropeptide Y immunoreactive nerve fibers were also found to be present. Accordingly, along with evidence that the substance P immunoreactive endings were found to encompass monocytes, it was hypothesized that these endings and others may function to transmit information to cells, such as mast cells, and monocytes, to invoke inflammatory process consequent to excess mechanical stimuli applied to synovial tissues containing these neuropeptides. As outlined in Figure 1, this response mechanism, in turn, has the potential effect of inducing synovial edema, joint fluid accumulation, plus the release of interleukin-1, tumor necrosis factors and other cytokines. In turn, these mediators may activate chondrocytes to secrete destructive collagenase and proteases.

In another clinical study, Xiao *et al*^[32] who aimed to explore the relationship between the distribution of neuropeptides, cancellous bone microstructure and joint pain in postmenopausal women with osteoarthritis and osteoporosis showed that neuropeptides play an important role in the pathogenesis of both conditions, by causing pain and negatively influencing the bone microstructure closely associated with articular cartilage. Jiang *et al*^[33] who examined 50 cases with knee osteoarthritis and ten trauma controls, specifically showed the osteoarthritis cases to have a lower vasoactive intestinal peptide concentration level in the synovial fluid and articular cartilage, and that these levels were correlated, indicating their impactful role in mediating progressive joint damage.

DISCUSSION

Although the question of whether inflammation co-exists with osteoarthritis, and whether neuropeptides are mediators of articular cartilage damage as is found in osteoarthritis, a topic of high potential relevance in ongoing efforts to minimize the onset and chronicity of the disease and its costly societal outcomes has been studied for more than 40 years, no universal conclusions on this topic have emerged to date. Indeed, not only are clinically derived data quite limited, as a whole, when compared to other topics in osteoarthritis research, but what is published in the realm of pre experimental studies is somewhat confusing at times and highly heterogeneous in terms of their diverse research approaches, questions, models, and conclusions. For example, Zhao *et al*^[27] who examined cortistatin, and its binding to TNF- α receptors implied this protected against osteoarthritis. As well, according to Grässel and Muscher^[7], pituitary

adenylate cyclase-activating polypeptide (PACAP) proved to be chondroprotective in their osteoarthritis related study. However, as discussed by Sutton *et al*^[11] other studies suggest that substance P, corticotropin-releasing factor, urocortin and vasoactive intestinal peptide may also be involved in osteoarthritis development.

Some of this confusion among published works is not only clearly attributable to the wide variation in study approaches, research questions, substrates, and measures, but other factors, such as the failure to clearly differentiate neurogenic from non-neurogenic sources of osteoarthritic pain and their associated responses^[34]. There is also a lack of agreement as to whether the synovium is innervated by sensory nerves that is not always accounted for^[35]. The underlying premise that osteoarthritis is not an inflammatory disease, may also explain the lack of focused research in this realm, while findings of a human inflammatory component in tissue from joint replacement cases may well be attributable to other causes, such as rheumatoid arthritis that is associated with osteoarthritis in its end-stages. As well, despite the possible importance of the nervous system as an entity for maintaining tissue homeostasis^[7,12], very few clinical studies have examined whether proposed changes in peripheral joint innervation do influence neuropeptide production as far as impacting degenerative alterations in those joint tissues said to possibly contribute to the development and progression of osteoarthritis^[36] and if so to what degree, and by what mechanisms^[35].

As suggested by Nissola *et al*^[37] however, the presence of any age-related degeneration of the proprioceptive, kinesthetic and vasoregulatory nerves and their interaction with neurogenic modulators that may underpin the primary pathogenic events seen in osteoarthritis should be studied further to more clearly to ascertain if in fact one or more of these degenerative processes predicts progressive joint tissue damage [unless very careful measures are taken to prevent this]. At the same time, the nature of any association of neuropeptides and inflammation, and under what conditions this association may foster peripheral sensitization and stimuli that may further amplify any inflammatory responses^[34] should be more thoroughly examined as implied by Sellam and Birnbaum^[38].

In this regard, it appears neuropeptides, serving as cellular messengers can potentially modulate and mediate the possible inflammatory cascades and destruction processes associated with osteoarthritis, but that precise details on these interactions are challenging to differentiate from possible favorable impacts of neuropeptides in experimental situations. While this may be due to the dual nature of neuropeptides, it may also arise owing to a general failure in this line of research to appreciate that the osteoarthritic process is one affecting the entire joint^[3,35], which is not always emulated well in acute inflammatory models and others. As well, examining single neuropeptides in isolation may clearly not capture the entire in situ set of interactive processes among neuropeptides and joint tissues such as cartilage, especially in aging joints faithfully. Similarly, the widespread study of only selected mechanisms and substrates that fail to clearly emulate either the multiple osteoarthritis human disease processes and/or human behaviors, to which humans and humans with diseased joints are subjected to over the course of time will undoubtedly continue to limit the ability to effectively interpret and translate the observations made in the laboratory to the bedside, unless addressed in a thoughtful and timely manner.

Thus, as discussed by Li *et al*^[17], while evidence does support the view that more emphasis on understanding the role of neural circuits and their substrates may be helpful in efforts to advance intervention opportunities eliminate or control articular damage (Table 2), much more carefully construed basic and longitudinal research studies,

Table 2 Randomly selected research examples of divergent findings concerning articular cartilage and neuropeptide interactions in osteoarthritis showing possible areas of promise for future study.

Research groups	Findings and Conclusions
Holmlund et al[44]	There is no significant correlation between clinical symptoms and signs, arthroscopic findings or use of analgesic/anti-inflammatory medication versus concentrations of peptides in the synovial fluid of temporomandibular arthritic joints
Kang et al[29]	Neuropeptide Y acts directly on articular cartilage homeostasis
Kim et al[45]	Bioactive peptides may help to inhibit osteoarthritis progression
Lisowska et al[46]	Substance P is associated with chronic osteoarthritis pain intensity
Nakajima et al[39]	Small hip joint neurons expressing calcitonin gene related peptide may foster hip joint pain
Obidat et al[47]	Nociceptive innervation of the mouse knee markedly declines after 26 weeks of age, before onset of spontaneous osteoarthritis. Late-stage surgically-induced disease is associated with striking plasticity of joint afferents in the knee medial compartment
Rein et al[48]	The synovium in trapeziometacarpal joint osteoarthritis produces several neuromediators causing a polymodal neurogenic inflammation. Neuropeptides may serve as biomarkers for osteoarthritis or as therapeutic targets
Saxler et al[49]	The upregulation of SP- and CGRP-positive neurons in response to arthritic stages suggests a mechanism involving neuropeptides in the maintenance of a painful degenerative joint disease and in mediating noxious peripheral stimuli
Sondergaard et al[50]	Salmon calcitonin appears to have strong positive effects on osteoarthritic histopathological changes after destabilization of the medial meniscus
Xiao et al[32]	Neuropeptides play an important role in osteoarthritis pathology

using advanced technologies and models deemed comparable to the human disease situation are surely warranted. Moreover, efforts to eliminate the situation where material from osteoarthritis cases generally represents only the result rather than the temporal changes that arise over the long term disease process are sorely needed in this respect^[35]. Attention to examining the precise role substance P and other key neuropeptides, in the process of osteoarthritic cartilage degeneration, and development, and especially their actions in the context of the sympathetic nervous system can surely also help to clarify the diverse role of neuropeptides in the pathogenesis of this disease, as well as any relevant clinical implications^[17,35].

In short, while the articles currently selected may not include every related research report, it is evident that very carefully construed further research in this realm not only to derive anti neuropeptide therapies, but to help establish if efforts to control any associated inflammatory damage from spreading, appear warranted. As well, efforts to uncover the manifold mechanisms underpinning osteoarthritis pathology that may implicate neuropeptides, such as obesity, as well as the varied roles of neuropeptides in inflammation resolution and possible cartilage repair, appear promising and are strongly indicated for advancing innovative as well as efficacious clinical practices^[17].

Additionally, since an increased substance P level has been found in the cerebrospinal fluid obtained from osteoarthritic patients, and immunohistochemistry has demonstrated an increase in substance P-immunoreactive nerve fibers in patients with this disease as discussed by Li *et al*^[17], studying this specific neuropeptide appears especially warranted. Alternatively, factors influencing the release of substance P by chondrocytes through mechanical stimulation or by other means that may affect the activity of various cell types in joints and periarticular tissues (including macrophages, bone cells, and pain fibers), as well as the structural changes associated with osteoarthritis as presented in Figure 1 might prove useful ideas to explore further.

Also recounted by Li *et al*^[17] more study to highlight the nature of the observed invasion of new blood vessels into the osteochondral junction of cartilage during osteoarthritis development, a finding that could involve sensory nerve fibers that grow into the diseased tissue alongside these vessels, and that then serve to convey substance P into the cartilage, should be studied further. On the other hand, simply applying substance P receptor antagonists in an effort to help reduce arthritis pain and swelling, and why this may increase rather than decrease the rate of adverse changes in osteoarthritis cartilage should be more closely analyzed, as should its potential anti-inflammatory

and regenerative properties^[39]. Moreover, since the absence of pain in the case of osteoarthritis-associated cartilage loss has been noted^[40], methods of periodically screening and assessing the presence of any unwanted neuropeptide products in vulnerable adults may help to effectively offset unwarranted articular cartilage damage and should be considered as a potentially plausible intervention approach^[40,41].

In the meantime, on account of all the overlapping features of osteoarthritis pathology, and as proposed by Hoshino *et al*^[4], efforts to classify and categorize the varied neuropeptide influences on articular tissues are likely to be extremely valuable. The role of age, and genetics and how these factors impact neuropeptide production and distribution in the context of joint maintenance and disease, as well as pain production, an understudied area, also warrants attention^[42]. In addition, more research to elucidate the possible highly salient role of the innervated capillary network of a joint in mediating joint destruction is recommended^[35]. Clinically, studies that can provide further insight into what triggers the presence and impact of harmful neuropeptide activity in relation to articular cartilage, and what is helpful in mobilizing their possible trophic and pain and inflammatory relieving effects have also been advocated^[43]. In the meantime, several lines of evidence that might be further pursued to a possible good effect are outlined below as well as Table 2.

CONCLUSION

Substance P, calcitonin gene-related peptide, vasoactive intestinal peptide and neuropeptide Y are the major peptides that appear involved both in the generation of pain and osteoarticular damage, as well as in possibly reducing pain and fostering repair post-joint trauma. There is also a close interaction between the different components of a joint that may be impacted by osteoarthritis and the aforementioned neuropeptides plus others, such as neurovascular neuropeptides^[51] that may cross-talk interactively in efforts to adapt to impact load responses and catabolic factors released in response to injury and persistent inflammation^[13,32,52]. They may also modulate bone regeneration, and bone remodeling, as well as impacting articular cartilage homeostasis, in addition to their classic neurological actions. As such, they might also play a highly significant role in the pathogenesis of degenerative joint disorders, such as osteoarthritis^[12], as well as in reparative approaches that might be harnessed to counteract cartilage collagen degradation and structural disintegration of affected joint cartilage^[53-55].

However, to be useful clinically, much more research that can

examine the very complex molecular and cellular interactions that exist between the immune and nervous system that may be involved in the development of osteoarthritis, and subsequent attempts to regain joint homeostasis, which is very hard to discern at present, appears warranted^[54]. In light of their possible co-existing catabolic and anabolic properties that may be emerge impactfully at different times and differentially under different environmental, pain, joint loading forces and mechanisms, plus genetic conditions, phenotypic dispositions, and overall health and disability status to regulate chondrocyte metabolism and inflammation^[55], systematic investigations in this realm seem especially warranted as well. Since not all cases with osteoarthritis may experience pain, but may still incur joint damage, efforts to routinely screen for changes in neuropeptide levels and peptidase enzymes known to degrade these molecules^[41] and that may be present in the synovium or surrounding vasculature at various stages of osteoarthritis pathology may be helpful in ameliorating the extent of future disablement and should be encouraged^[40].

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