Peripheral antinociceptive effects of endogenous ligands (endomorphin-1, 2-arachidonoyl glycerol and kynurenic acid) in a joint pain model

Summary of Ph.D. Thesis

László Mécs M.D.

University of Szeged
Faculty of Medicine
Department of Physiology

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1. Introduction

The pain:

The pain „an unpleasant sensory and emotional experience associated with actual or potential damage, or described in terms of such damage". Pain is a complex perceptual experience that, in addition to conveying sensory information such as location, type, and intensity of a stimulus, has profound affective and cognitive features. The experience of pain is the final product of a complex information-processing network. Whether or not a particular stimulus will be perceived as painful depends not only on the nature of the stimulus, but also on the context within which it is experienced, memories, emotions and so on.

Musculoskeletal disorders are a major cause of morbidity both in the community and in the workplace. They affect all age groups and frequently cause disability, impairments, and handicaps. Arthritis affected 43 million U.S. adults and is the leading cause of disability in the United States. These patients are affected by musculoskeletal signs or symptoms, including limitation of motion and pain of the joint**.**

Several information is available on the innervations of joints. The joint nerves contain Aβ-, Aδ- and C-fibers. Corpuscular endings of Aβ-fibers were identified in the ligaments and in the fibrous capsule. Free nerve endings were referred in all structures of the joint except the normal cartilage. Pain was elicited when noxious mechanical, thermal and chemical stimuli were applied to the fibrous structures such as ligaments and fibrous capsule. While most fibers in the Aβ-fiber range show responses to innocuous movements of the joints, a large number of Aδ- and C-fibers show thresholds in the noxious range. One group of mainly C-fibers is so-called "silent nociceptors" because these neurons do not respond even to noxious mechanical stimuli of the normal joint. They begin to respond to mechanical stimulation during inflammation of the joint.

Joint pain can be caused by many types of injuries or conditions. The most frequent types of the joint pain are the rheumatoid arthritis and the osteoarthritis. Further causes: inflammation of the bursa, injury, including fracture, sprains, infectious diseases, septic arthritis, osteomyelitis, gout, tendinitis and so on.

A diversity of chemical mediators that are produced or released locally following tissue injury or inflammation can activate peripheral sensory nerve endings. Under normal physiological conditions, nociceptive signals are produced by intense stimulation of primary afferent sensory Aδ and C nerve fiber terminals by chemicals, thermal and mechanical stimuli.

The first relay in pain pathways activated by Aδ- and C-nociceptors is the spinal dorsal horn and, as such, this represents an important site for the modulation of the pain signal.

Pain is a dynamic phenomenon resulting from the activity of both excitatory and inhibitory endogenous modulation systems. It is well known that a multitude of substances and receptors are involved in the nociceptive system, some of them increase, and others inhibit the pain sensation both peripherally and centrally. Virtually no ligands or receptors are to be found that have not been investigated in this respect. These substances, which include neurotransmitters, neuromodulators, hormones, cytokines, etc., can modify the activity of nerves involved in the pain pathways. One of the physiological functions of the endogenous system is to tonically regulate nociceptive transmission; therefore the ratio of the pronociceptive and antinociceptive ligands determines the pain sensitivity. The balance between these actions ensures effective modulation of acute pain, while during chronic pain the pronociceptive effects appear to prevail. Therefore, the organism can express very effective antinociception in different circumstances, and during such situations the levels of various endogenous ligands change. The endogenous ligands can produce their effects at both peripherally and centrally. The endogenous antinociceptive ligands may have potentially advantageous features: their synthesizing and breakdown enzymes (or the mechanism of their excretion) are available in the body; thus, in general they have short half-lives and they may have lower toxicity. On the other hand, most of the endogenous ligands exhibit lower specificity and affinity for their receptors as compared with exogenous drugs, and/or they exert their effects at several types of receptors at different parts of the body. Therefore, the net effect depends on the localization of the ligands/receptors, and on which receptors and where they will be influenced by a ligand.

Analgesic therapies: Analgesic therapies for acute and chronic pain conditions currently rely on three major classes of drugs: nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and a group of drugs with diverse pharmacological actions collectively known as adjuvants (antidepressants, anticonvulsants, local anesthetics, α_2-adrenoceptor agonists). The systemic administration of both NSAIDs and opioids exhibit a variety of adverse actions (nausea, vomiting, gastric ulcer, kidney failure, liver failure respiratory depression, cough suppression, etc.) and many chronic pain states, particularly those involving nerve injury, are not adequately controlled by these agents. With adjuvants, it is often necessary to titrate the dosage until adequate pain relief or intolerable side effects develop. Unfortunately, the latter outcome often occurs, and the degree of pain relief that results is only partial. An alternative

important approach to pain control is to apply drugs locally to the peripheral site of origin of the pain. This can be attained by the topical application of a cream, lotion, gel, aerosol, or patch to skin or by injections directly into the joints. These application methods allow of a higher local concentration of the drug at the site of initiation of the pain and lower or negligible systemic drug levels producing fewer or no adverse drug effects. Other potential advantages of localized applications are the lack of drug interactions, the lack of need to titrate doses to tolerability, and importantly, the ease of use. Both acute and chronic pain conditions are likely to be amenable to this approach, but to date; there are only a limited number of topical therapies available for the relief of somatic pain.

Local anesthetics: A local anesthetic is a drug that causes reversible local anesthesia and a loss of nociception. When it is used on specific nerve pathways (nerve block), effects such as analgesia (loss of pain sensation) and paralysis (loss of muscle power) can be achieved. Clinical local anesthetics belong to one of two classes: aminoamide and aminoester local anesthetics. Synthetic local anesthetics are structurally related to cocaine. All nerve fibers are sensitive to local anesthetics, but generally, those with a smaller diameter tend to be more sensitive than larger fibers. All local anesthetics are membrane stabilizing drugs; they reversibly decrease the rate of depolarization and repolarization of excitable membranes.

The **NSAIDs** are among the most widely used of all therapeutic classes of drugs applied both systemically and topically. Their effects are due to inhibition of the cyclooxygenase (COX) enzyme that converts arachidonic acid liberated from the phospholipid membrane by phospholipases to prostanoids such as prostaglandins. Two forms of COX are well characterized, a constitutive form (COX1) that is normally expressed in tissues such as stomach and kidney and plays a physiological role in maintaining tissue integrity, and a form that is induced by inflammatory mediators (COX2) and plays a significant role in pain and inflammation. Systemic administration of NSAIDs is associated with several side effects, including nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headache, and drowsiness. NSAIDs may also cause fluid retention, leading to edema. The most serious side effects are kidney - and liver failure, ulcers and prolonged bleeding after an injury or surgery. An additional strategy to try to minimize adverse effects has been the development of topical formulations of NSAIDs.

Use of **corticosteroids** in the treatment of muscle and joint inflammatory reactions (including pain) is becoming increasingly popular. The mechanism of corticosteroid action includes a

reduction of the inflammatory reaction by limiting the capillary dilatation and permeability of the vascular structures. These compounds restrict the accumulation of polymorphonuclear leukocytes and macrophages and they also inhibit the release of destructive enzymes that attack the injury debris and destroy normal tissue indiscriminately. Additionally, corticosteroids may inhibit the release of arachidonic acid from phospholipids, thereby reducing the formation of prostaglandins, which contribute to the inflammatory process. Thus, intra-articular steroid injections caused a significantly greater reduction in pain and tenderness than placebo in osteoarthritis. On the other hand, some authors' experience shows, that intra-articular glucocorticoids promote the increase the destruction of articular cartilage in the joint. Therefore, and considering the other side effects, the steroids are not ideal drugs for articular pain therapy by themselves.

Opioids: Morphine, the main alkaloid of opium, is utilized for the treatment of severe pain, and is the gold standard, which all analgesics are compared to. Early efforts to understand the endogenous targets of opiate drugs led to the identification of receptor sites. Binding studies suggested four main classes of opioid receptors, named μ- δ-, κ-, and opioid receptor-like (ORL1) receptors. Opioid receptors comprise a subfamily of structurally homologous GPRs. Activation of these receptors inhibits the formation of cAMP, close voltage-gated Ca^{2+}-channels and opens inwardly rectifying potassium channels. The net effect of these cellular actions is to reduce neuronal excitability and neurotransmitter release. The central effects of opioids on pain transmission by actions within the dorsal horn of the spinal cord and at brainstem and other supraspinal sites have been recognized for some time. It is known that opioid receptors are also present on the peripheral terminals of thinly myelinated and unmyelinated cutaneous sensory fibers. Dorsal root ganglia contain mRNA for opioid receptors, and when synthesized, these receptors are transported both centrally and peripherally. Systemic administration of opioids has many side effects (respiratory depression, nausea, vomiting, constipation).

There are a large number of behavioral studies that have examined peripheral antinociceptive effects of exogenous opioids, and these effects have been demonstrated primarily using models of inflammation. Several clinical data have shown the efficacy of intra-articular morphine in wide dose-ranges.

Endogenous opioids: Opioid receptors and their endogenous ligands are widely distributed in the organism, thus both central and peripheral activation of this system might lead effective

antinociception. A high dose of naloxone (opioid antagonist) produces hyperalgesia, suggesting a significant role of endogenously released opioids in the development of normal pain sensitivity. The endogenous opioid ligands can also induce antinociception at peripheral levels. During inflammation of the peripheral tissues leukocytes are the important source of the endogenous opioid peptides, and β-endorphin, methionine-enkephalin, dynorphins and endomorphins are produced and released by these cells. However, only a few of them were investigated at peripheral level.

β-endorphin binds with high affinity to both μ- and δ-opioid receptors. The only data about its peripheral administration showed that β-endorphin caused a short-lasting decrease in the mechanical hyperalgesia in Freund's adjuvant induced inflammatory model.

Nociceptin has been reported to be an active ligand at multiple sites of nociceptive transmission, ranging from peripheral nociceptors to nociceptive centers in the brain. ORL-1 receptors can be found peripherally as well, and their activation can lead to peripheral antinociception.

Endomorphins: More than 10 years ago, a new group of μ-opioid receptor agonists was discovered and named endomorphins (EMs) by Zadina et al. EMs are real endogenous opioid neurotransmitters/modulators, although their synthesis has not been clarified. Some data suggest that EMs can be synthesized from dipeptides and not from a large propeptide. The distribution of the EMs along the nociceptive pathway implicates them as particularly important for the modulation of pain. They interact specifically and with high affinity with μ-opioid receptors, and they possess partial rather than full agonist properties at μ-opioid receptors. EM1 and EM2 produce their effects through different subtypes of μ-opioid receptors, EM1 affecting predominantly the μ_2-opioid receptors, while EM2 the μ_1-opioid receptors. A huge amount of data proved the antinociceptive potential of these tetrapeptides at both spinal and supraspinal levels, but only a few studies supported the beneficial effects of EMs at peripheral level. Some data suggest the role of EM1 in the control of inflammatory processes at joint level.

Glutamate is a major excitatory amino acid neurotransmitter acting on metabotropic and ionotropic glutamate receptors. Within the dorsal spinal cord, both ionotropic glutamate receptors [N-methyl-d-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainic acid (KA)] and metabotropic glutamate receptors are involved

in nociceptive signaling and central sensitization in conditions of chronic pain. Several data suggest the efficacy for ketamine (NMDA receptor antagonists) in treatment of many chronic pain disorders.

Kynurenic acid: Degradation of the essential amino acid tryptophan along the kynurenine pathway yields several neuroactive intermediates, including kynurenic acid. This is found both centrally and peripherally in low concentrations (10-150 nM). It has been detected in synovial fluid collected from knee joint of rheumatoid arthritic patients, and it inhibited the proliferation of synoviocytes in vitro. KYNA acts as an antiexcitotoxic and anticonvulsant, and it may influence important neurophysiologic and neuropathologic processes. KYNA at high, non-physiological concentrations is a broad-spectrum antagonist of ionotropic excitatory amino acid receptors, acting at the glycine receptors (GlyRs; half-maximal inhibitory concentration: IC_{50}~20 μM) and the N-methyl-D-aspartate recognition sites (IC_{50}~200 μM) of the NMDA receptor complex. In higher concentrations (0.1-1 mM), it also antagonizes the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and kainate receptors, and KYNA is a potent noncompetitive antagonist of α7 nicotinic acetylcholine receptors (alpha7 nAChRs) (IC_{50}~7 μM) too. Thus, direct support for its physiological role in glutamatergic and cholinergic neurotransmission has been reported. A recent study has shown that GPR35, a previously orphan GPR, functions as a receptor for the KYNA. KYNA elicits calcium mobilization and IP3 production in a GPR35-dependent manner, and it also induces the internalization of this receptor. Our group investigated the antinociceptive potency of KYNA at a spinal level in an inflammatory pain model. The intrathecal infusion of KYNA alone resulted in a dose-dependent increase in heat pain latency on both the normal and the inflamed sides, but it also caused motor impairments at higher doses.

Cannabinoids (CBs) are a distinct class of psychoactive compounds, which produce a wide array of effects on specific receptors (CB1. and CB2). Cannabinoid receptors are among the most abundant GPRs. The CB1 receptor is widely distributed in the CNS and in the periphery, and it preferentially presents on axons and their terminals. CB2 receptors are expressed predominantly peripherally, where they are localized extensively to cells of the immune system, but it can be found on the peripheral nerve terminals as well.

Systemic, spinal, and supraspinal administration of cannabinoids produce analgesia in a variety of nociceptive test systems and several data suggest the antinociceptive potential of peripherally acting cannabinoid agonist drugs.

Endocannabinoid system. In the beginning the 1990s discovered a new class of fatty acid derivatives, i.e. the endogenous cannabinoid ligands, that serve naturally to modulate pain.
A feature that distinguishes lipid endocannabinoids from many other neuromodulators is that they are not synthesized in advance and stored in vesicles. Rather, their precursors exist in cell membranes (lipids) and are cleaved by specific enzymes on demand, and endocannabinoids release generally postsynaptically, and they act presynaptically. The first endocannabinoid identified was arachidonoyl-ethanolamine (anandamide), and the second one was 2-arachidonoyl-glycerol (2-AG). Other putative endogenous ligands of cannabinoid receptors are palmityl-ethanolamide (PEA) and virodhamine (O-arachidonoyl-ethanolamine). Several endogenous lipoamino acids were detected in a variety of tissues in the rat, i.e. N-arachidonoyl-glycine (NAGly), N-arachidonoyl-alanine, N-arachidonoyl-serine, N-arachidonoyl-taurine and N-arachidonoyl-GABA, N-oleyl-ethanolamide (OEA), N-arachidonoyl-dopamine, oleamide, N-oleoyl-dopamine and N-palmitoyl-glycine are also fatty acid derivates, and they have also been identified as endogenous lipids. The peripheral action of the endocannabinoids may possibly be extremely important, because low doses of these endogenous ligands may reduce pain without dysphoric side-effects, and without the abused potential typical of centrally acting cannabimimetic drugs.

2-Arachidonoyl-glycerol (2-AG): This 2-acyl-glycerol ester is the most abundant endogenous cannabinoid, and its concentration in the brain is 50-500 fold higher than that of anandamide, and it has also been identified peripherally. It is formed from arachidonic acid-containing phospholipids through increased phospholipid metabolism, such as enhanced inositol phospholipid turnover, in various tissues and cells upon stimulation. It is a short-lived ligand, being rapidly inactivated mainly by the enzyme monoglyceride lipase (MAGL), but it might also be metabolized by FAAH. 2-AG is a full agonist for CB1 and CB2 receptors with no direct binding to the TRPV1 receptor. It is also a substrate for cyclooxygenase-2 (COX-2), and 2-AG is capable of suppressing elevation of COX-2 expression by activating the CB1 receptors. A few studies have investigated the antinociceptive potency of 2-AG at peripheral level. These reports have shown that 2-AG administered intraplantar inhibited both neuropathic allodynia and formalin-induced pain behavior effectively by the activation of CB2 and/or CB1 receptors.

2. Aim of the studies

Earlier studies proved that endomorphin-1, kynurenic acid and 2-arachidonoyl-glycerol can produce antinociceptive effects at central and/or peripheral levels. The goal of the thesis was to determine the antinociceptive potency of these ligands and their interactions in carrageenan-induced inflammatory arthritis rat model. Therefore, the main objectives of the thesis were:

1. **to determine the dose-dependent and time-course effects of intra-articularly administered EM1,**
2. **to determine the dose-dependent and time-course effects of intra-articularly administered KYNA,**
3. **to determine dose-dependent and time-course effects of intra-articularly administered 2-AG,**
4. **to examine the interaction of EM-1 and KYNA,**
5. **to examine the interaction of EM1 with 2-AG.**

3. Methods

Male adult Wistar rats were housed in groups of 5-6 per cage, with free access to food and water, and with a natural light/dark cycle. We used two cohorts of the animals. The first cohort was used for the investigation of the effects and interaction of EM1 and KYNA. The second cohort was applied for the experiments with EM1 and 2-AG. The weight in the two cohorts did not differ significantly.

The following drugs were administered: λ-carrageenan, endomorphin-1 (EM1), kynurenic acid (KYNA), naltrexone (NTX) and 2-arachidonoyl-glicerol (2-AG)

Inflammation was produced by injecting carrageenan (300 µg/20µl) into the tibiotarsal joint of the right hind leg. To determine the changes in the size of the inflamed joint, we measured the antero-posterior and medio-lateral diameter of the paw at the level of ankle joint with a digital caliper.

The threshold for withdrawal from mechanical stimulation to the plantar aspect of the hindpaws was determined with logarithmic series of calibrated von Frey monofilaments. Prior to baseline testing, each rat was habituated to a testing box with a wire-mesh grid floor for at least 15 min. Von Frey filaments were applied in ascending order using a single, steady 1-2 s

application perpendicularly through the grid floor to the plantar surface of the right hindpaw of each rat until a paw withdrawal occurred.

Experimental protocol: After baseline determination of joint diameter and mechanical paw withdrawal threshold (pre-carrageenan baseline value at -180 min), carrageenan was injected. These measurements were obtained again three hours after carrageenan injection (post-carrageenan baseline values at 0 min).

Treatments: 1st series: EM-1 (30, 100 and 200 µg), KYNA (30, 100, 200 and 400 µg), their combinations in a fixed-dose ratio: EM-1 and KYNA 1:1 (30-30, 100-100 and 200-200 µg) were given into the inflamed joint (20 µl), and mechanical sensitivity was defined at 10, 20, 30, 45, 60, 75 min after the drug administrations. To reveal the role of the opioid receptor activation by EM1, a group of animals was pretreated with naltrexone (a well-known antagonist on µ-opioid receptors; 4 mg/kg subcutaneously) 20 min before 200 µg endomorphin-1 administration.

2nd series: EM-1 (100, 200 and 300 µg) and 2-AG (30, 100, 200 µg, the highest dose possible in this volume) and EM-1 and 2-AG ratio 10:1 (100-10, 200-20 and 300-30 µg) were given into the inflamed joint (20 µl), and mechanical sensitivity was defined at 10, 20, 30, 45, 60, 75, 90 and 105 min after the drug administrations. At the end of the experiment the joint diameters were measured again.

4. Results

Joint edema

Three hours after the injection of carrageenan into the right ankle, there was a significant ($p<0.01$) increase in joint cross-section area compared with preinjection control levels This conspicuous increase in joint size was a result of edema formation, confirming that carrageenan treatment resulted in an inflammatory reaction. None of the treatments influenced the degree of edema.

Mechanosensitivity

1st series: EM1 produced dose-dependent antinociceptive effect, which developed gradually, and it reached its maximum between 30 and 45 min. 30 µg EM1 was ineffective,

while 200 µg caused a prolonged effect, leading to nearly perfect relief of allodynia. Naltrexone pretreatment reversed the antinociceptive effects of EM1.

KYNA by itself also caused a dose-dependent antiallodynic effect, which developed at 30 min after the injection. Only the highest dose produced a prolonged antinociception and almost total relief of allodynia.

Regarding the interaction of these ligands, coadministration of 30-30 µg EM1 and KYNA did not produce any antiallodynic effect. As regards the coadministration of 100-100 µg, ANOVA revealed significant effects of treatment. KYNA and EM1 200-200 µg EM + KYNA produced longer-lasting antinociception compared to the single treatments. The dose–response curve of the cocktail is between the EM1 and KYNA lines. The data analysis revealed additive interaction between the two ligands.

2nd series: EM1 produced again a dose-dependent antinociceptive effect, which developed gradually, and it reached its maximum between 45 and 60 min.

2-AG by itself caused a dose-dependent antiallodynic effect, which also developed slowly. The highest dose produced a prolonged antinociception, and its potency was higher compared with EM1.

As regards the interaction of 2-AG and EM1, coadministration of 10 µg 2-AG with 100 µg EM1 did not show significant differences compared to the single treatments. As regards the 20-200 µg 2-AG + EM1 combination, it produced an increased antinociception compared to vehicle, 2-AG and EM1. Similarly, 30-300 µg 2-AG + EM1 also caused long-lasting and more effective antinociception compared to the single treatments.

The dose - response curve of the cocktail became more steeply compared to the EM1 and 2-AG lines. This suggest, that lower doses produce additive interactions, while the largest dose combination produced synergism.

5. Discussion

Suffering from pain is a major medical, social and economic burden worldwide, however, the ideal solution for effective pain-relief remains elusive. The systemic administration of both NSAIDs and opioids exhibit a variety of adverse actions (nausea, vomiting, gastric ulcer, kidney and liver failure respiratory depression, cough suppression, etc.).

An alternative important approach to pain control is to apply drugs locally to the peripheral site of origin of the pain. Previous studies indicate that opioids can produce an additive or synergistic interactions with cannabinoids or NMDA antagonists primarily at central level. These studies led us to the question whether similar interaction may exert between EM1 and KYNA and between EM1 and 2-AG at peripheral level.

Locally released opioid peptides at the site of injury are known to inhibit the inflammatory response and to reduce the pain associated with it. Only a few studies supported the beneficial effects of EM1 at peripheral level. EM1 can also decrease the joint inflammation and this effect may contribute to its antinociceptive potency.

Kynurenic acid is an endogenous excitatory amino acid antagonist with preferential activity at the NMDA receptors, and is also a non-competitive antagonist at alpha7 nicotinic receptor. Our earlier data have revealed that intrathecally administered KYNA produced antinociception but the effective dose caused motor impairment as well. Only a few studies suggest the role of kynurenic acid in the periphery.

The use of cannabinoids for the management of a wide range of painful disorders has been well documented at spinal, supraspinal, and peripheral levels. Peripheral nerve fibers express CB1 and CB2 receptors and their activation can inhibit pain sensation, and the peripheral immune cell CB2 receptor stimulation may down-regulate inflammation by suppressing the release of inflammatory mediators. Thus, topically applied cannabinoids have provided effective analgesia in different pain models, and this effect is mediated by CB1 and CB2 activation. Only few data have proved the antinociceptive potency of 2-AG. Systemic administration to mice 2-AG has caused antinociception in acute pain tests, intraplantar injected 2-AG has decreased pain behavior in a dose-dependent manner in the late phase of formalin test in rats.

This thesis showed that the intra-articularly administered EM1, KYNA and 2-AG dose-dependently decreased the mechanical allodynia without effect on the edema. Mechanical threshold did not change on the non-injected side, suggesting that the intra-articularly injected endogenous ligands do not produce systemic effects in these doses.

The coadministration of EM1 and KYNA, or EM1 and 2-AG produced additive interactions, however, the dose-response curve of the EM1 + 2-AG combination was steeper compared to the single treatments, suggesting a synergistic effect at higher dose ranges.

6. General conclusions:

1. **We proved the antinociceptive potency of EM1 at joint level in an inflammatory pain model.**
2. **We firstly demonstrated the antiallodynic potency of intra-articularly administered KYNA without any side-effects.**
3. **We showed the antinociceptive effect of 2-AG at peripheral level.**
4. **We have found that the combination of EM1 with KYNA produced additive antinociceptive interaction.**
5. **Further, the coadministration of EM1 and 2-AG yielded additive interaction as well.**

We wish to draw the attention to the rapidly evolving recognition that the endogenous ligands may exert effects on several receptors and/or systems. Furthermore, the combination of these endogenous ligands may provide a new and beneficial combination for pain therapy with potentially fewer side effects at joint level.

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List of publications related to the thesis

Full papers related to the thesis

Mecs L, Tuboly G, Nagy E, Benedek G, Horvath G. Peripheral antinociceptive effects of endomorphin-1 and kynurenic acid in the rat inflamed joint model. *Anesth. Analg*. 109:1297-1304, 2009. (Impact factor: 3.083)

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(Impact factor: 1.936)

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Mecs L., Benedek G., Horvath G. Az izületi fájdalom csökkentése lokálisan alkalmazott endogén ligandokkal. *A Magyarországi Fájdalom Társaság Tudományos Ülése.* Kecskemét, Október 12-13. 2007. (Abstract)

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