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

Ph.D. Thesis

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1 Introduction

1.1 The pain

The World Health Organization has defined pain (1979) as „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.

We distinguish acute and chronic pain, furthermore nociceptive and neuropathic pain. Nociceptive pain is initiated by stimulation of nociceptors, and may be classified according to the mode of noxious stimulation; the most common categories being "thermal" (heat or cold), "mechanical" (crushing, tearing, etc.) and "chemical". Nociceptive pain may also be divided into "superficial somatic" and "deep", and deep pain into "deep somatic" and "visceral". Neuropathic pain is a type of pain which is caused by damage to or dysfunction of the nervous system.

Within the nociceptive pain the inflammatory type is very frequent. It is precipitated by an insult to the integrity of tissues at a cellular level. This can happen with penetration wounds, burns, extreme cold, fractures, autoimmune conditions, excessive stretching, infections and vasoconstriction. Multiple chemicals mediate the inflammatory process. There are chemicals that act directly and those that act as precursors for other more direct acting substances. There are vascular components, fibroblastic components and tissue cell components. Blood vessels carry circulating precursor that are released into the area of injury and are enzymatically activated. Mast cells release histamines and 5-hydroxytryptamine (5HT). Macrophages activate fibroblasts, which in turn release interleukins (IL) and tumour necrosis factor (TNF). Cyclooxygenases activate prostaglandins and leukotrienes. All of these substances contribute to the inflammatory process.

1.2 Pain in the joint

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 (Theis et al., 2007). These patients are affected by musculoskeletal signs or symptoms, including limitation of motion and pain of the joint**.**

Most information is available on the innervation 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 identified in all structures of the joint except the normal cartilage. From all joint structures including ligaments, fibrous capsule, adipose tissue, meniscus, periosteum and synovial layer, but not cartilage, conscious sensations can be evoked. In awake humans direct stimulation of fibrous structures with innocuous mechanical stimuli evoked pressure sensations. Pain was elicited when noxious mechanical, thermal and chemical stimuli were applied to the fibrous structures such as ligaments and fibrous capsule. No pain was elicited by stimulation of cartilage, and stimulation of normal synovial tissue rarely evoked pain. Recordings from joint afferents revealed different fiber types in joint nerves concerning their mechanosensitivity. 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 (rotation of the joint against the resistance of the tissue and intense local pressure). A large group of mainly C-fibers are so-called silent nociceptors because they do not respond even to noxious mechanical stimuli of the normal joint. They begin to respond to mechanical stimulation during inflammation of the joint (Schaible et al., 2009).

Joint pain can be caused by many types of injuries or conditions. No matter what causes it, joint pain can be very bothersome. The most frequent types of the joint pain are the rheumatoid arthritis and the osteoarthritis.

Rheumatoid arthritis is an autoimmune disorder that causes stiffness and pain in the joints. It is an ongoing, progressive disease that affects the joints of the body with episodes of painful inflammation. Rheumatoid arthritis causes destructions of joints and it also affects other organs of the body (e.g. kidney, heart) leading to disability, and in severe

cases, life threatening complications (Moreland and Curtis, 2009; van Zonneveld et al., 2010; Wood, 2009).

Osteoarthritis involves growth of bone spurs and degeneration of cartilage at a joint. It is very common in adults older than 45 and can cause joint pain. Osteoarthritis is also known as degenerative arthritis, which is a group of mechanical abnormalities involving degradation of joints, including articular cartilage and subchondral bone. Symptoms may include joint pain, tenderness, stiffness, locking, and sometimes an effusion. A variety of causes may initiate processes leading to loss of cartilage. When bone surfaces become less well protected by cartilage, bone may be exposed and damaged (Hayes et al., 1990; van Jonbergen et al., 2010; Williams et al., 2010).

Joint pain may also be caused by inflammation of the bursae. The bursae are fluid-filled sacs that cushion and pad bony prominences, allowing muscles and tendons to move freely over the bone. Further causes: injury, including fracture, sprains, infectious diseases, septic arthritis, osteomyelitis, gout, tendinitis and so on (Harrington and Schneider, 2006; Tasto and Elias, 2007).

1.3 Pain signals and pathways

A diversity of chemical mediators that are produced or released locally following tissue injury or inflammation can activate peripheral sensory nerve endings. These can directly activate the sensory nerve [H^+, Adenosine-triphosphate (ATP), glutamate, 5-HT, histamine, bradykinin], sensitize the nerve ending to the action of other stimuli [e.g., prostaglandins and prostacyclin, cytokines such as ILs (IL-1β, IL-2, IL-6, IL-8), tumor necrosis factor-α], or exert regulatory effects on the sensory neuron, adjacent inflammatory cells, and sympathetic nerves [e.g., bradykinin, tachykinins, nerve growth factor (NGF)] (Germolec et al., 2010; Serhan, 2006).

Some agents that activate sensory neurons do so by acting directly on ion channels (e.g. H^+ via acid-sensitive ion channels; heat and cold stimuli via transient receptor potential (TRP) channels; ATP via purinoreceptor (P2X); glutamate via ionotropic glutamate receptors), whereas other agents sensitize sensory neurons by acting on G-protein-coupled metabotropic receptors (GPR) to alter intracellular messengers [cyclic adenosine monophosphate (cAMP), Ca^{2+}, inositol triphosphate (IP3)], and activate protein

kinases [protein kinase A (PKA), protein kinase C (PKC)] that then phosphorylate ion channels and modulate their function (Coutaux et al., 2005; Dray, 1995; Grubb, 2004; Kidd et al., 2004; Kohli and Levy, 2009).

Sensory neurons can be divided into subgroups based on anatomical (fiber size, degree of myelination, postsynaptic connections in the spinal cord), histochemical (presence of peptides and other neurotransmitters, ion channels and receptors, growth factors), and physiological (responsiveness to sensory modalities, conduction velocity) properties (Caterina and Julius, 1999; Lawson, 1996; Snider and McMahon, 1998). 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 (Kidd et al., 2004; Millan, 1999; Treede et al., 1992; Wall and Melzack, 1999). Nociceptive signals are transmitted to the superficial layers of the dorsal spinal cord where they undergo substantial modulation by local mechanisms, as well as by projections from supraspinal structures, which can provide both inhibitory and facilitatory influences; further transmission to brainstem and thalamic sites, and subsequently to the cerebral cortex, then occurs (Basbaum and Fields, 1984; Besson and Chaouch, 1987; Millan, 1999).

1.4 Endogenous antinociception system

The first relay in pain pathways activated by Aδ- and C-nociceptors is the spinal dorsal horn (SDH) and, as such, this represents an important site for the modulation of the pain signal. The activation of several pathways is involved in the production of analgesia including pathways that project from the amygdala, hypothalamus (arcuate nucleus: ARC, and lateral area of anterior hypothalamus: LAAH), the somatosensory cortex and the anterior cingulated cortex (ACC) to the midbrain periaqueductal grey matter (PAG) (Millan, 2002; Pilcher et al., 1988; Walker et al., 1999). ACC and amygdala are particularly related to the affective component of pain and ACC is also implicated in cognitive processing of pain (Fields, 2004; Ji and Neugebauer, 2008; Neugebauer et al., 2004; Rainville et al., 1997). The hypothalamus is known to be one of the key structures involved in pain modulation and transmission (Dafny et al., 1996), and the hypothalamic fibers containing opioid neurons terminate in PAG (Pilcher et al., 1988). The LAAH has

the capacity to differentially modulate components of the pain signal, i.e. activation of this nucleus inhibits the responses to unmyelinated C-fiber activation, while did not change the activity of Aδ fibers (Simpson et al., 2008). The overall effect of this would be to safeguard sensory-discriminative information that could direct motivational behaviors and, at the same time, filter out those components of the pain signal that are less relevant to emergency situations. PAG represents the mechanisms whereby cortical and other inputs act to control the nociceptive "gate" in the dorsal horn of the spinal cord. PAG projects rostrally to the medial thalamus and orbital frontal cortex, and also interacts with several brainstem structures to modulate nociception including the rostroventral medulla (RVM) (Jensen and Yaksh, 1989; Sandkuhler, 1996; Smith et al., 1988; Zhao et al., 2007). The thalamus contributes to the emotional component of pain and in particular, the intralaminar parafascicular nucleus receives nociceptive information from the spinal cord by both the spinothalamic and spinopontothalamic tracts and its output is to the ACC. RVM including the nucleus raphe magnus (NRM) is considered an important source of descending control of spinal nociceptive neurons (Fields and Basbaum, 1999). RVM is the principal relay in the integration of ascending nociceptive inputs with descending outputs from rostral sites (Fields et al., 1999), as well as the major source of bulbospinal projections that terminate in laminas I, II and V of the SDH. Descending control of spinal nociception, which originates from locus ceruleus (LC), is another major determinant of pain sensitivity in different behavioral and emotional states (Jensen et al., 1989; Pertovaara et al., 1996). These descending modulations are exerted by three main neurochemicals: noradrenergic, serotonergic and opioidergic systems (Millan, 2002).

Significant advances in understanding pain signaling mechanisms and the pathophysiology of pain have occurred in the past decades. This has involved an appreciation of the diversity of the agents and the mechanisms that can modulate the pain signal in peripheral and central compartments, as well as an understanding of the neurobiological changes that can occur in chronic pain states involving inflammation and nerve injury.

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 (Furst, 1999; Sandkuhler, 1996).

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.

Sensory nerve endings also express a number of receptors for neurotransmitters that can modify pain transmission. Many of these receptors were characterized initially in the dorsal spinal cord (Yaksh et al., 1998), but some receptors that are synthesized in the cell body of dorsal root ganglia cells and transported centrally to reside presynaptically on primary afferent neurons also are transported peripherally (Coggeshall and Carlton, 1997). Therefore one important possibility for the antinociception might be effects at peripheral level. Selective activation of peripheral receptors has the important advantage of providing effective analgesia without side effects typically associated with centrally acting drugs. Yet, in clinical practice most pain treatment strategies are based on systemic administration of conventional centrally penetrating substances.

1.5 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 somatic sites or by injections directly into the joints. These application methods allow for 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. Their actions may be on the inflammatory response itself (decreased production of inflammatory mediators, block of action of inflammatory mediators) or on sensory neurons (altered impulse generation through actions on up-regulated sodium channels, actions at specific receptors on the sensory neuron to attenuate activation of that neuron). 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.

1.5.1 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. Local anesthetics vary in their pharmacological properties and they are used in various techniques of local anesthesia such as: topical anesthesia (surface), infiltration, intra-articular, plexus block, epidural block, spinal anesthesia. 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 (like nociceptors). Local anesthetic drugs act mainly by inhibiting sodium influx through sodium-specific ion channels in the neuronal cell membrane, in particular the voltage-gated sodium channels. When the influx of sodium is interrupted, an action potential cannot arise and signal conduction is inhibited. The receptor site is thought to be located at the cytoplasmic (inner) portion of the sodium channel. Local anesthetic drugs bind more readily to sodium channels in activated state, thus onset of neuronal blockade is faster in neurons that are rapidly firing. This is referred to as state dependent blockade (Catterall, 2002).

Lidocaine, the first amino amide-type local anesthetic, was synthesized under the name xylocaine by Swedish chemist Nils Löfgren in 1943. Lidocaine is a common local anesthetic and antiarrhythmic drug. The efficacy profile of lidocaine as a local anesthetic is characterized by a rapid onset of action and intermediate duration of efficacy. Therefore, lidocaine is suitable for infiltration, block and surface anesthesia and it can use for intra-articular analgesia as well (Fitch and Kuhn, 2008; Maldini et al., 2010). Longer-acting substances such as bupivacaine are sometimes given preference for spinal and peridural anesthesia. For surface anesthesia several formulations are available that can be used e.g. for endoscopies, before intubations.

1.5.2 Nonsteroidal Anti-Inflammatory Drugs

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 (Marnett and Kalgutkar, 1999).

Systemic administration of NSAIDs is associated with several side effects. The most common side effects are 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 failure, liver failure, ulcers and prolonged bleeding after an injury or surgery.

These agents have been understood for many years to act peripherally to reduce the production of prostaglandins that sensitize nerve endings at the site of injury (Vane, 1971). Therefore, an additional strategy to try to minimize adverse effects has been the development of topical formulations of NSAIDs, as this can minimize plasma concentrations of drugs and lead to fewer adverse effects at sites remote from the area of application. Bioavailability and plasma concentrations following topical application are 5 to 15% of those achieved by systemic delivery (Heyneman et al., 2000). As an intra-articular injection of a NSAID (tenoxicam) not only improves the inflammation in a joint but also works to protect the ligament and respective tissue from further deterioration. Intra-articular injection of tenoxicam provides rapid pain relief in the patients with acute flare-up of knee osteoarthritis and helps to prevent effusion (Oztuna et al., 2007; Papathanassiou, 1994; Unlu et al., 2006).

1.5.3 Corticosteroids

Use of corticosteroids in the treatment of muscle and joint inflammatory reactions (including pain) is becoming increasingly popular. First popularized by Janet Travell, muscle injections are remarkably effective adjunct to pharmacologic and physical therapies. Joint injections, while technically more difficult to perform, can also be of great benefit in the patient's recovery. 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 (Doan and Massarotti, 2005). Thus, intra-articular steroid injections caused a significantly greater reduction in pain and tenderness than placebo in osteoarthritis (Hepper et al., 2009). On the other hand, some authors' experience shows, that intra-articular glucocorticoids promote the increase the destruction of articular cartilage in the joint (Behrens et al., 1975). Therefore, and considering the other side effects, the steroids are not ideal drugs for articular pain therapy by themselves.

1.5.4 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 (Dhawan et al., 1996; Jordan et al., 2000; Lambert, 2008). 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 (Papathanassiou, 1994). Dorsal root ganglia contain mRNA for opioid receptors, and when synthesized, these receptors are transported both centrally and peripherally (Coggeshall et al., 1997; Hassan et al., 1993; Maekawa et al., 1994; Minami et al., 1995; Stein et al., 1990c).

Systemic administration of opioids (e.g. morphine, fentanyl) has many side effects. The most dangerous is respiratory depression. Common side effects are nausea, vomiting, cough suppression. Morphine also has an effect on the muscle of the bowel and urinary tract, causing the sphincter to contract and reduce the peristalsis, resulting in a delayed

emptying of the stomach, constipation, and may also lead to urinary retention. Morphine can also cause histamine release, which causes itching of the skin and it has little direct effect on the heart or blood pressure.

A number of studies have addressed the issue of whether peripheral opioid mechanisms are of significance in a clinical setting. Typically, the application of conventional opioid receptor agonists in small, systemically inactive doses directly into injured peripheral tissues, or the administration of opioids with limited access to the CNS has been used efficiently in animal and human studies (Labuz et al., 2007; Stein et al., 1989; Stein et al., 1993; Stein, 1993; Watterson et al., 2004). Thus, centrally penetrating μ-δ-, κ-, receptor agonists, when administered systemically, produce a considerable part of antinociception through peripheral opioid receptors (Labuz et al., 2007). 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 (Stein, 1993; Watterson et al., 2004). μ-opioid receptor agonists are generally the most potent at producing peripheral analgesia, with δ- and κ-opioid receptor agonists being less active. It was found that the effects of morphine injected intra-articularly were mediated by peripheral opioid receptors, because the analgesic effect could be reversed by the intra-articular injection of naloxone. Several clinical data have shown the efficacy of intra-articular morphine in wide dose-ranges (Gupta et al., 2001; Stein, 1993). The majority of studies report significant effects by at least one pain measure (visual analog scale, numerical scales). Intra-articular effects of morphine, produced a definite reduction in postoperative pain intensity compared with placebo, and this was seen during all postoperative phases (Gupta et al., 2001). Effects were reversible by naloxone, similar in magnitude to conventional local anesthetics, and lasted up to 48 h after injection. Others studies in which pain intensity were more pronounced (anterior cruciate ligament repair) and in which there was preexisting inflammation (e.g. chronic arthritis). The intra-articular injection of morphine (1-3 mg) produced a long-lasting analgesia (up to 6 days) (Katz et al., 2010; Likar et al., 1997; Rosseland et al., 1999; Sloan and Babul, 2006; Stein et al., 1999). Therefore, morphine and other opioids have been injected in the vicinity of practically every peripheral nerve and many joints to induce pain relieve (Kalso et al., 1997; Likar et al., 1997).

1.5.4.1 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 (Akil et al., 1984; Bach, 1997; Basbaum et al., 1984; Bodnar and Klein, 2004; Bodnar, 2008; Horvath, 2000; Menetrey and Basbaum, 1987; Palkovits, 2000; Pan et al., 2008; Rittner et al., 2008; Stein, 1993; Vaccarino et al., 2000) A high dose of naloxone (opioid antagonist) produces hyperalgesia, suggesting a significant role of endogenously released opioids in the development of normal pain sensitivity (Boschi et al., 1983). 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 (Labuz et al., 2006; Mousa et al., 2002; Rittner et al., 2008). However, only a few of them were investigated at peripheral level.

1.5.4.1.1 β-Endorphin

Since the discovery and characterization of the proopiomelanocortin (POMC) - derived β-endorphin (31 amino acids) as an opioid peptide in 1976, the opinion has been widely held that this peptide has a role in the control of pain and it is a key component of the endogenous antinociceptive system attenuating the stress- and inflammation-induced hyperalgesia (Akil et al., 1984; Basbaum et al., 1984; Loh et al., 1976; Rossier et al., 1977; Stein et al., 1990b; Sun et al., 2003).

It binds with high affinity to both μ- and δ-opioid receptors (Akil et al., 1984). 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, while it did not influence the normal mechanical sensitivity (Labuz et al., 2006; Stein et al., 1990b). The effect of β-endorphin was antagonized by μ- and δ- but not by κ-opioid receptor antagonists.

1.5.4.1.2 Nociceptin

Shortly after the cloning of the three known opioid receptors, a member of this family was identified, the ORL-1 receptors, which was found not to bind any of the known natural or synthetic opioid ligands (Reinscheid et al., 2000). In 1995, the natural ligand for this receptor was isolated and named orphanin FQ or nociceptin (Reinscheid et al., 1995). It is a 17-amino acid peptide, the amino terminus of which displays a striking similarity to the known mammalian opioid peptides. It is derived from pronociceptin, and it is widely distributed in central structures involved in sensory, emotional and cognitive processing, and in the periphery including the immune cells (Lambert, 2008; Reinscheid et al., 2000). 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 (Lambert, 2008; Obara et al., 2005), while other data suggest that nociceptin has pain-inducing effects (McDougall and Larson, 2006) Thus, Obara et al. showed that intraplantar (IPL) administration of nociceptin (0.5-40 µg) significantly attenuated mechanical (von Frey) and thermal (cold water) allodynia in rats, and the observed effect was dose-dependent (Obara et al., 2005). In contrast, hindlimb weight bearing and von Frey hair algesiometry were measured before and following a single injection of nociceptin (in the knee joint of rats; 18 µg) (McDougall et al., 2006). Compared to saline-treated controls, nociceptin caused a conspicuous shift in hindlimb weight bearing in favour of the contralateral non-injected leg. Similarly, paw withdrawal threshold and latency were significantly reduced following nociceptin administration indicative of secondary hyperalgesia.

1.5.4.1.3 Endomorphins

More than 10 years ago, a new group of µ-opioid receptor agonists was discovered and named endomorphins (EMs) by Zadina et al. (Zadina et al., 1997). Endomorphin-1 (EM1): Tyr-Pro-Trp-Phe-NH_2 and endomorphin-2 (EM2): Tyr-Pro-Phe-Phe-NH_2 differs from conventional endogenous opioid receptor ligands in their N-terminal sequence, peptide length and C-terminal amidation. EMs are real endogenous opioid neurotransmitters/modulators, although their synthesis has not been clarified. However,

several studies have identified EMs in the different parts of the organism, and their metabolizing enzymes have also been shown. Furthermore, some data suggest that EMs can be synthesized from dipeptides and not from a large propeptide (Ronai et al., 2006). The distribution of the EMs along the nociceptive pathway implicates them as particularly important for the modulation of pain (Horvath, 2000; Zadina et al., 1997). They interact specifically and with high affinity with μ-opioid receptors (Horvath, 2000; Zadina et al., 1997), and they possess partial rather than full agonist properties at μ-opioid receptors (Sim et al., 1998). 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 (Sakurada et al., 2000). A huge amount of data proved the antinociceptive potential of these tetrapeptides at both spinal and supraspinal levels (Horvath, 2000), but only a few studies supported the beneficial effects of EMs at peripheral level (Labuz et al., 2006; Obara et al., 2004).

Some data suggest the role of EM1 in the control of inflammatory processes at joint level (Barin and McDougall, 2003; Li et al., 2005; McDougall et al., 2003; McDougall et al., 2004; Straub et al., 2008).

1.5.5 The role of Glutamate Receptors in the pain/antinociception

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 (Coderre et al., 1993; Dickenson et al., 1997; Fields et al., 1994). Both the systemic and spinal administration of multiple classes of glutamate receptor antagonists have been observed to produce analgesia in a variety of persistent pain models, and although their potential for development as a novel class of analgesics has been considered, this may be hampered by the presence of adverse motor and other effects (Coderre et al., 1993; Fisher et al., 2000). Accordingly, NMDA receptor antagonists such as ketamine and memantine can be used for the treatment of pain patients with these disorders (Correll et al., 2004; Sinis et al., 2007). Although data on this indication in the literature is limited, several case reports and case series suggest efficacy for ketamine in treatment of many

chronic pain disorders, including peripheral neuropathy, chronic post-traumatic neuropathic pain, postherpetic neuralgia, spinal cord injury pain, neuropathic pain associated with multiple sclerosis and Guillain–Barré syndrome, orofacial pain, complex regional pain syndrome, phantom limb pain, and fibromyalgia (Hocking and Cousins, 2003).

It has been appreciated that multiple glutamate receptors are also expressed on peripheral nerve terminals, and these may contribute to peripheral nociceptive signaling. Ionotropic and metabotropic glutamate receptors are present on membranes of unmyelinated peripheral axons and axon terminals in the skin (Carlton et al., 1995; Zhou et al., 2001), and peripheral inflammation increases the proportions of both unmyelinated and myelinated nerves expressing ionotropic glutamate receptors (Carlton and Coggeshall, 1999). Local injections of NMDA and non-NMDA glutamate receptor agonists to the rat hindpaw or knee joint cavity (Jackson et al., 1995; Lawand et al., 1997; Zhou et al., 1996) enhance pain behaviors generating hyperalgesia and allodynia. In a study of acute postoperative pain, ketamine enhanced local anesthetic and analgesic effects of bupivacaine by a peripheral mechanism (Tverskoy et al., 1996). In a thermal injury model in healthy volunteers, peripheral injection of ketamine produced a long-lasting reduction in hyperalgesia (Warncke et al., 1997). Ketamine also produces local anesthetic actions, blocks voltage-sensitive Ca^{2+} channels, alters cholinergic and monoaminergic actions and interacts with opioid mechanisms, and these actions also may contribute to its analgesic profile (Hirota and Lambert, 1996; Meller, 1996; Sawynok and Reid, 2002).

1.5.5.1 Kynurenic acid

Degradation of the essential amino acid tryptophan along the kynurenine pathway yields several neuroactive intermediates, including kynurenic acid (KYNA; 4-oxo-1H-quinoline-2-carboxylic acid) (Moroni et al., 1988; Schwarcz and Pellicciari, 2002; Vecsei and Beal, 1991). This is found both centrally and peripherally in low concentrations (10-150 nM) (Moroni et al., 1988; Nemeth et al., 2005; Pawlak et al., 2000; Schwarcz et al., 2002; Turski and Schwarcz, 1988; Urbanska et al., 2000). It has been detected in synovial fluid collected from knee joint of rheumatoid arthritic patients, and it inhibited the proliferation of synoviocytes in vitro (Parada-Turska et al., 2006). 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 (Carpenedo et al., 2001; Ganong et al., 1983; Stone, 1993). 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 (Hilmas et al., 2001; Stone, 1993; Stone, 2000). Thus, direct support for its physiological role in glutamatergic and cholinergic neurotransmission has been reported (Carpenedo et al., 2001; Nemeth et al., 2005; Schwarcz et al., 2002). A recent study has shown that GPR35, a previously orphan GPR, functions as a receptor for the KYNA (Wang et al., 2006). 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 (Kekesi et al., 2002). 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.

1.5.6 Cannabinoids

Cannabinoids (CBs) (e.g. Δ^9-tetrahydrocannabinol) are a distinct class of psychoactive compounds, which produce a wide array of effects on specific receptors (CB1. and CB2) (Calignano et al., 1998; Martin et al., 1999; Hohmann, 2002). 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 (Guindon and Hohmann, 2007b; Szabo, 2008). Both CB1 and CB2 receptors primarily signal through the inhibitory GPR proteins (Gi/o), however, under certain conditions and with certain agonists, coupling via Gs and Gq/11 has also been demonstrated (Mackie, 2008; Pertwee, 2001). Stimulation of CB1 receptors leads to the inhibition of adenylate cyclase (AC), the inhibition of certain voltage-gated calcium channels, and the activation of G protein-linked

inwardly rectifying potassium channels and these effects are associated with depression of neuronal excitability and transmitter release. The complexity of the actions of CB2 agonists on neuronal and non-neuronal cells and their signalling properties are only beginning to be explored. Activation of CB2 receptors inhibits AC and in contrast to CB1 receptors, CB2 receptors do not couple to ion channels, but both receptors can activate the mitogen-activated protein kinase (MAPK) signaling cascade (Howlett et al., 2004).

Systemic, spinal, and supraspinal administration of cannabinoids produce analgesia in a variety of nociceptive test systems, and the potential for development of cannabinoids as an alternative class of analgesics is being considered (Lever and Rice, 2007; Rice et al., 2002; Richardson et al., 1998a; Richardson et al., 1998b). Several data suggest the antinociceptive potential of peripherally acting cannabinoid agonist drugs (Agarwal et al., 2007; Dogrul et al., 2003; Yesilyurt et al., 2003). Dorsal root ganglia cells that express neuropeptide markers found in nociceptive primary afferents contain mRNA for CB_1 cannabinoid receptors (Hohmann et al., 1999), and these receptors are transported both centrally (Guindon et al., 2007b; Hohmann, 2002; Walker and Hohmann, 2005) and peripherally (Hohmann et al., 1999). In behavioral experiments, the peripheral administration of agents selective for CB_1 receptors produces a local analgesia in the formalin test (Calignano et al., 1998), the carrageenan hyperalgesia model (Richardson et al., 1998b), and the partial nerve injury model (Hohmann, 2002; Hohmann and Suplita, 2006). Interestingly, coadministration of agonists for both CB_1 and CB_2 receptors produced a dramatically potentiated analgesia (Calignano et al., 1998). Cannabinoids can reduce the production and release of proinflammatory signaling molecules and enhance the release of antiinflammatory cytokines, moreover, CB2 receptor activation may stimulate the local release of endorphins from cells such as keratinocytes (Ibrahim et al., 2005; Walter and Stella, 2004). The peripheral actions of CB_1 receptor agonists are attributed to an effect on the sensory nerve terminal itself to inhibit release of calcitonin gene-related peptide (Richardson et al., 1998b) or inhibit sensitizing effects of NGF (Rice et al., 2002). Local analgesic actions of directly and indirectly acting agonists for CB_2 receptors, that are expressed on mast cells and inhibit mast cell function, also have been demonstrated (Calignano et al., 1998; Malan et al., 2002), and CB_2 receptor mechanisms may play a particularly prominent role in inflammatory pain (Rice et al., 2002).

1.5.6.1 Endocannabinoid System and Related Fatty Acid Derivatives

In the early 1990s, Mechoulam's group opened the door to a new class of fatty acid derivatives, i.e. the endogenous cannabinoid ligands, that serve naturally to modulate pain (Devane et al., 1992; Martin et al., 1999). 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 (Walker et al., 2005). The first endocannabinoid identified was arachidonoyl-ethanolamine (anandamide: AEA), 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) - a derivative of anandamide (Di Marzo et al., 1998; Porter et al., 2002; Walker et al., 2002) 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 (De Petrocellis et al., 2004; Devane et al., 1992; Huang et al., 2001; Huang et al., 2002; Walker et al., 2002; Walker et al., 2005). 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 (Huang et al., 2002). All of these ligands constitute a family of ubiquitous endogenous lipids present in varying levels throughout the body, and several of them produce their effects through modulation of CB receptors, while other receptor activation/inhibition has also been suggested.

Considerable progress has been made in understanding the physiological functions of the endocannabinoids, and their corresponding potential pathological implications. The peripheral action 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. However, local administration of CB1 and CB2 antagonists by themselves failed to induce hyperalgesia, suggesting that the endocannabinoids do not act tonically in the periphery to dampen sensitivity to pain (Guindon et al., 2007a). From the above mentioned ligands only five were studied as an antinociceptive ligand at peripheral level until now.

1.5.6.1.1 N-Arachidonoyl-ethanolamine (Anandamide; AEA)

Anandamide, the first identified and best-studied endocannabinoid, can be found both centrally and peripherally (Calignano et al., 1998; Devane et al., 1992; Walker et al., 2002). It is principally formed from glycerophospholipid by two successive enzymatic reactions: N-acylation of phosphatidyl-ethanolamine to generate N-acylphosphatidyl-ethanolamine (NAPE) by Ca^{2+}-dependent N-acyltransferase, and release of AEA from NAPE by a phosphodiesterase of the phospholipase D type (NAPE-PLD) (Okamoto et al., 2007). It has been hypothesized that AEA could be recycled by the cell to form new endocannabinoid molecules and released into the extracellular space (Placzek et al., 2008). AEA is extremely short-lived, being rapidly inactivated by the enzymes fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996). AEA binds to both CB1 and CB2 receptors, behaving as a partial agonist, but it also activates transient receptor potential vanilloid 1 (TRPV1) receptors, and it has CB receptor-independent G protein-coupled antinociceptive potency through the activation of the GPR55 (Di Marzo et al., 2000; Pertwee, 2007; Ryberg et al., 2007). Furthermore, AEA may directly affect the GlyRs and functionally antagonizes the transient receptor potential melastatin 8 (TRPM8) receptor-mediated responses (De Petrocellis et al., 2007; Hejazi et al., 2006; Lozovaya et al., 2005). Furthermore, AEA targets potassium channels, T-type calcium channels, and gap junctions. It is a substrate for COX2 giving rise to amino acid conjugates of the prostaglandins, and induces the expression of COX2 enzyme as well (Chemin et al., 2001; Chen et al., 2005; Maingret et al., 2001).

Some data proved the action of AEA at peripheral level. Local administration of AEA significantly decreases the formalin-induced pain behavior but not the paw edema (Calignano et al., 1998; Guindon et al., 2006a; Guindon et al., 2006b). Thus, AEA (50 µg) decreases the first phase of formalin-induced pain behavior in mice (Calignano et al., 1998), while it was effective (1 ng-5 µg) in both phases in rats (Guindon et al., 2006a). Furthermore, it also inhibits (10 ng) the TRPV1 receptor activation-induced drop in hot plate latency by activation CB1 receptors in rat (Almasi et al., 2008). It is supposed that anandamide may activate cannabinoid CB1 receptors located on capsaicin-sensitive primary afferents, resulting in the decreased responsiveness of these afferents to noxious stimuli. However, others have shown that locally administered anandamide in high doses (350 - 1000 µg) activates nociceptors in normal and arthritic rat by stimulating TRPV1

receptors on primary sensory neurons, suggesting a pain-inducing potential of anandamide at this level (Gauldie et al., 2001).

1.5.6.1.2 N-Arachidonoyl-glycine (NAGly)

N-Arachidonoyl-glycine (NAGly) was first synthesized as a structural analog of the AEA. It is expressed within the CNS, particularly high levels within the spinal cord, but it can be detected in the skin as well (Burstein, 1999; Huang et al., 2001; Rimmerman et al., 2008). NAGly is formed via oxidation of AEA and by conjugation of glycine with arachidonic acid by arachidonyl-coenzyme A, and being rapidly inactivated by FAAH (Burstein, 1999; Huang et al., 2001). While the pharmacology of NAGly is still poorly understood, several targets for NAGly are emerging. NAGly has no affinity for the CB1 and TRPV1 receptors, although it can activate CB2 binding sites (Devane et al., 1992; Huang et al., 2001; Sheskin et al., 1997; Sipe et al., 2005). It is also a substrate for COX2 giving rise to amino acid conjugates of the prostaglandins, and it inhibits activation of COX2 and 5-lipoxygenase enzymes (Burstein, 1999; Prusakiewicz et al., 2002). Thus, it has a complex effect on prostaglandin synthesis, and a role for COX2 cannot be excluded in its effects (Burstein et al., 2007). Furthermore, NAGly inhibits FAAH, and it is a substrate for this enzyme, therefore, NAGly can regulate the levels of AEA in tissues (Grazia Cascio et al., 2004; Huang et al., 2001). NAGly is a ligand for the orphan receptors GPR18, and it activates this receptor in a pertussis toxin sensitive manner (Kohno et al., 2006). NAGly has also been shown to stimulate another orphan receptor GPR92, which is highly expressed in DRG and colocalized with TRPV1 receptors and has been postulated to play a role in sensory perception (Oh et al., 2008). In addition, NAGly inhibits the glycine transporter GLYT2, but it can also influence the GlyRs, and these systems could also mediate some of the analgesic effects of NAGly (Wiles et al., 2006; Yang et al., 2009). Consistent with its high levels in skin, NAGly (50 µg) produces analgesia administered peripherally in the second phase of formalin test in rats, and it also has anti-inflammatory activity (Burstein et al., 2007; Huang et al., 2001).

1.5.6.1.3 N-Palmitoyl-ethanolamide (PEA)

Although the subfamily of arachidonoyl amides has received considerable attention, much less is known about the presence and activity of their saturated counterparts (Rimmerman et al., 2008). The most studied member of the saturated acyl amides is N-palmitoyl-ethanolamide (Di Marzo et al., 1998; Walker et al., 2002). PEA, found in neural and non-neural tissues, inhibits mast-cell activation and reduces inflammatory responses by a mechanism that may involve binding to CB2 receptors (Calignano et al., 1998; Martin et al., 1999). However, since PEA does not produce an effective activation of cannabinoid receptors, it is generally classified as a cannabimimetic compound. Furthermore, PEA is an agonist at the peroxisome proliferators-activated receptor α (PPARα), and at the orphan receptor GPR55 (Lo Verme et al., 2005; Ryberg et al., 2007). An "entourage" effect on anandamide-mediated action may be due to the PEA-induced inhibition of FAAH that leads to an increase of tissue levels of AEA (Costa et al., 2008). Some results suggest its antinociceptive properties at peripheral level (Calignano et al., 1998; Calignano et al., 2001; Lo Verme et al., 2006). Local administration of PEA (0.1-50 μg) did not modify the capsaicin-induced pain behavior, but produced antinociception in the both phases of formalin test that was blocked by a CB2 receptor-selective antagonist, but the role of PPARα receptor activation has also been proved in this respect (Calignano et al., 1998; Calignano et al., 2001; Lo Verme et al., 2006). It is noteworthy that local coadministration of PEA together with exogenous anandamide produced a synergistic analgesic effect in both phases of the formalin test through a mechanism that involves both CB1 and CB2 receptor subtypes (Calignano et al., 1998; Calignano et al., 2001).

1.5.6.1.4 N-Oleoyl-ethanolamide (OEA)

OEA is a derivative of oleic acid (monounsaturated omega-9 fatty acid) and it is an endogenous regulator of food intake, and may have some potential as an anti-obesity drug, however few studies investigated its effects on sensory neurons as well (Fu et al., 2003; Hansen and Artmann, 2008). It does not bind to CB1 and CB2 receptors, but it is an endogenous agonist of TRPV1 and PPARα (Ahern, 2003; Almasi et al., 2008; Fu et al., 2003; Lo Verme et al., 2006; Wang et al., 2005a). Only a few inconsistent results suggest its role in the pain. Local administration of OEA (0.1-50 μg) did not modify the capsaicin-induced pain behavior, but produced antinociception in the first phase of formalin test that

was blocked by CB1 and CB2 receptor-selective antagonists (Calignano et al., 2001). Its IPL administration (0.16-1.6 µg) does not change the acute heat-pain latency, but reverses the thermal hyperalgesia after TRPV1 receptor activation and it inhibits the first phase of formalin-induced pain behavior in mice and rats (Almasi et al., 2008; Calignano et al., 2001). In contrast, other study found that the local administration of OEA in high doses induced nocifensive behavior (10-50 µg) in mice, which could not be observed in TRPV1 KO animals (Lo Verme et al., 2006).

1.5.6.1.5 2-Arachidonoyl-glycerol (2-AG)

The second endocannabinoid identified was 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 (Agarwal et al., 2007; Kondo et al., 1998). 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 (Bisogno, 2008; Cravatt et al., 1996; Dinh et al., 2002; Saario and Laitinen, 2007; Sugiura et al., 2006). 2-AG is a full agonist for CB1 and CB2 receptors with no direct binding to the TRPV1 receptor (Mechoulam et al., 1995; Mechoulam et al., 1996; Pertwee, 2001; Sugiura et al., 2006). 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 (Bleakman et al., 2006; Kozak et al., 2000; Zhang and Chen, 2008). A few studies have investigated the antinociceptive potency of 2-AG at peripheral level. These reports have shown that 2-AG administered IPL inhibited both neuropathic allodynia and formalin-induced pain behavior effectively by the activation of CB2 and/or CB1 receptors (Romero-Sandoval et al., 2008; Zhang et al., 2003)

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

3.1 Animals

After institutional ethical approval had been obtained (Institutional Animal Care Committee of the Faculty of Medicine at the University of Szeged), male adult Wistar rats (Charles River strain, Bioplan, Budapest, Hungary) were housed in groups of 5-6 per cage, with free access to food and water, and with a natural light/dark cycle. Animal suffering and the number of animals per group were kept at a minimum. 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 (1^{st} cohort: 247 ± 2.2 g; 2^{nd} cohort: 244 ± 2.0 g).

3.2 Drugs

The following drugs were administered: λ-carrageenan, endomorphin-1 (EM1), kynurenic acid (KYNA), naltrexone (NTX) and 2-arachidonoyl-glicerol (2-AG) (Sigma-Aldrich Ltd., Budapest, Hungary). Carrageenan, EM1 and NTX were dissolved in physiological saline, 2-AG purchased as a solution, while KYNA was dissolved in 0.1 M NaOH. The excess NAOH was back-titrated with 0.1 M HCl to a neutral pH and the volume was adjusted with physiological saline. The solutions were prepared freshly on the day of experiment. Physiological saline was used as control. We also tried acetonitrile solution (solvent for 2-AG) as well (four animals), and it did not differ from the saline-treated control group.

3.3 Carrageenan-induced inflammation

Inflammation was produced by injecting carrageenan (300 µg/20µl) into the tibiotarsal joint of the right hind leg as was described earlier (Peter-Szabo et al., 2007). Thus, all treatments were given to gently restrained conscious animals, using a 27-gauge needle, without anaesthesia so as to exclude any drug interaction. These injections did not elicit any sign of major distress.

To determine the changes in the size of the inflamed joint, we measured the anteroposterior and mediolateral diameter of the paw at the level of ankle joint with a digital caliper. The cross section area was calculated with the formula $a \times b \times \pi$, where a and b are the radius in the two aspects.

3.4 Behavioral nociceptive testing

The threshold for withdrawal from mechanical stimulation to the plantar aspect of the hindpaws was determined with logarithmic series of calibrated von Frey monofilaments (SenseLab – Aesthesiometer, Somedic, Sweden). Prior to baseline testing, each was habituated to a testing box with a wire-mesh grid floor for at least 15 min. Von Frey filaments (bending force ranging from 0.064-110 g) 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 (Wei et al., 1998). The lowest force producing a withdrawal response was considered the threshold. Only robust and immediate withdrawal responses from the stimulus were considered.

3.5 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).

3.5.1 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.

3.6 Statistical analysis

Data are presented as means ± SEM. Paw withdrawal latencies on the inflamed side were transformed to % maximum possible effect (%MPE) by using the following formula:
%MPE = ([observed force – post-carrageenan baseline force]/[pre-carrageenan baseline force – post-carrageenan baseline force]) × 100
Therefore, 100% MPE means perfect relief of allodynia (equivalent to pre-carrageenan baseline value; which is generally close to the maximum value: 110 g), while 0 % MPE means that the observed force is equivalent to the post-carrageenan baseline value. Thus, these two baseline values are not shown in the figures.

Data transformations in the 1st series:

As treatments generally produced their effects between 30 and 60 min, their mean values on the inflamed side were used for dose–effect curves and linear regression analysis (a common method for the determination of dose-response effects in *in vivo* studies). The 50% effective dose (ED_{50}) was defined as the dose that yielded 50% MPE. As a lower level of the effect might also be important for therapeutic practice, we also determined ED_{30}, which means about 10 times increase in the pain threshold compared to post-carrageenan baseline value. The ED_{30} and ED_{50} values with 95% confidence intervals (CI) were calculated by linear regression.

Data transformations in the 2nd series:

The area under the curve (AUC) values were obtained by calculating the area of the %MPE values between 20-90 min. The mean AUC values were used for dose–effect curves and linear regression analysis. AUC 7000 value would mean the complete relieve of the hyperalgesia (100% MPE) during the whole period. As regards the AUC values after saline treatment, we observed almost no effects (about 29 ± 17.2 in the AUC). The 50% effective dose (ED_{50}) would mean the dose that yielded 50% MPE for the whole period (3500 AUC). However, the AUC values were much lower for these ligands by themselves, therefore, we determined the 27 % of ED (1900; which means about 63 times increase in the withdrawal threshold compared to the control group) for the AUC by linear regression.

Data sets were examined by one-way and two-way analyses of variance. The significance of differences between experimental and control values was calculated using the Tukey-Kramer test for post hoc comparison (*p* value <0.05 was considered significant). Statistics were performed by STATISTICA (Statistica Inc., Tulsa, Oklahoma, USA) and GraphPad Prism (GraphPad software Inc. La Jolla, California, USA) softwares.

4 Results

4.1 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 (first series: from 46 ± 0.7 mm2 to 82 ± 0.9 mm^2; second series: from 46 ± 0.3 mm^2 to 75 ± 1.0 mm^2). 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 (data are not shown).

4.2 Mechanosensitivity

Basal mechanical withdrawal threshold was around 100 - 110 g, i.e. about 90% of the animals did not withdraw their paws at the cut-off value. Carrageenan caused a significant decrease in paw withdrawal threshold on the inflamed side (in the 1st and 2nd series: 0.36 ± 0.054 g; 0.29 ± 0.033 g, respectively), but it did not have a significant influence on the noninflamed side. None of the treatments changed the mechanosensitivity on the normal side; therefore, results were analyzed only on the inflamed paws.

1st series:

EM1 produced dose-dependent antinociceptive effect, which developed gradually, and it reached its maximum between 30 and 45 min (Fig. 1). ANOVA with repeated measurements showed significant effects of treatment ($F_{3,30}$=6.9, p<0.005), time ($F_{5,150}$=8.5, p<0.001), and interaction ($F_{15,150}$=5.4, p<0.001). Thus, **30 µg EM1 was ineffective, while 200 µg caused a prolonged effect, which was about 80% MPE at 30th and 45th min,** leading to nearly perfect relief of allodynia. **The ED_{30} and ED_{50} values were 112 µg (CI: 80-146) and 167 µg (CI: 135–220), respectively. NTX pretreatment alone did not influence the pain threshold** (data are not shown), but prevented the anti-allodynic effect of EM1 (200 µg) (Fig. 1).

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 (Fig. 2). ANOVA proved significant

effects of treatment ($F_{4,40}$=16.1, p<0.001), time ($F_{5,200}$=10.5, p<0.001), and interaction ($F_{20,200}$=3.9, p<0.001). Its potency was lower compared with EM, i.e. the ED_{30} and ED_{50} values were 204 µg (CI: 160–251) and 330 µg (CI: 280-407), respectively.

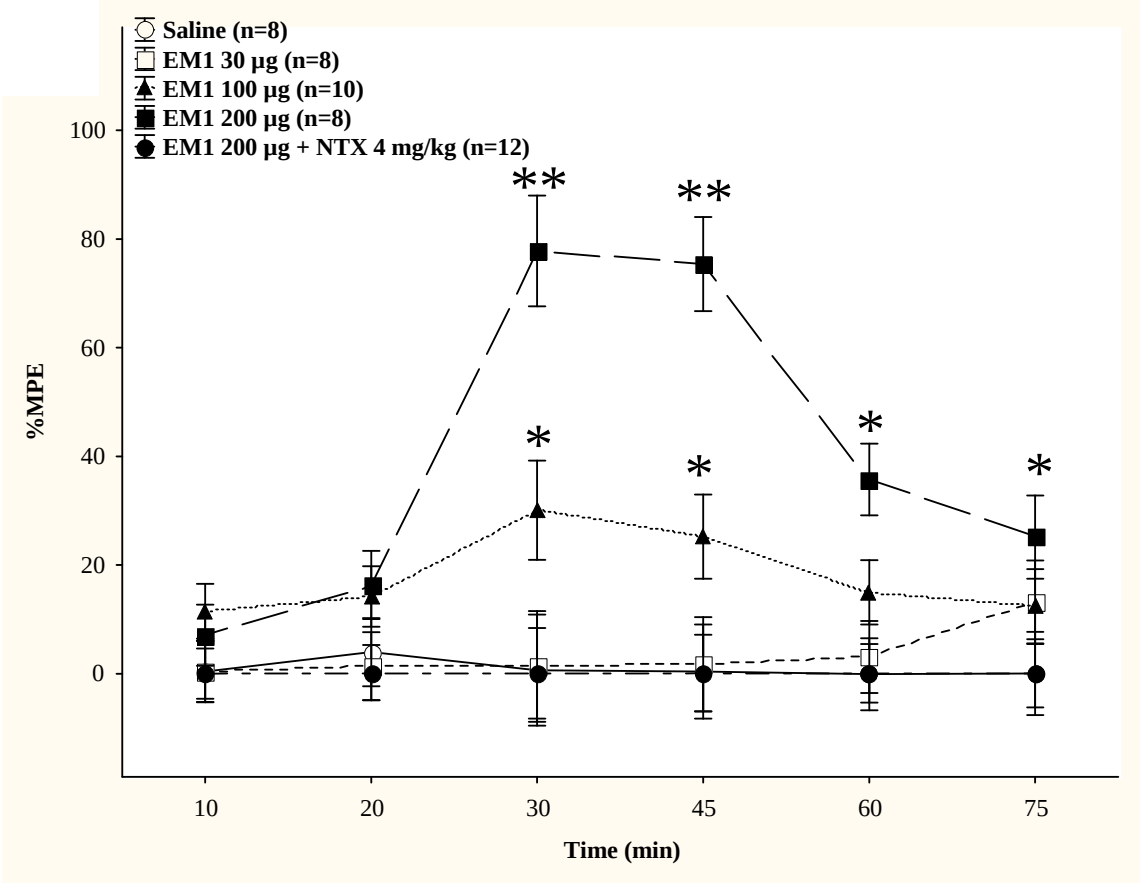

Figure 1.

Time course of the effects of EM1 (30, 100 and 200 µg) and 200 µg EM1 after NTX pretreatment on the mechanical pain threshold on the inflamed side. Each point denotes the mean ± SEM of the results. Symbols ***** and ****** indicate significant (p<0.05; p<0.001; respectively) differences as compared with the vehicle-treated group.

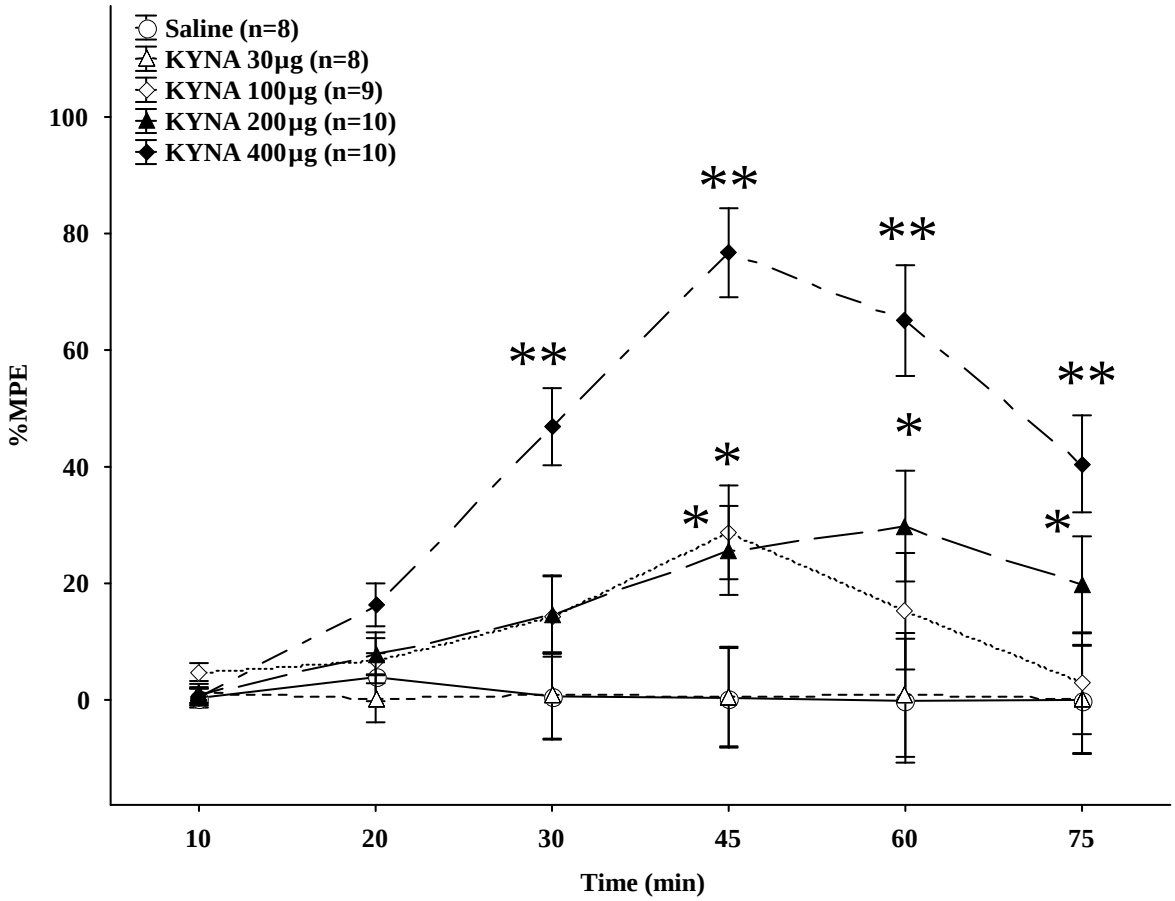

Figure 2.

Time course of the effects of KYNA (30, 100, 200 and 400 µg) on the mechanical pain threshold on the inflamed side. Each point denotes the mean ± SEM of the results. Symbols *****and ****** indicate significant (p<0.05; p<0.001; respectively) differences as compared with the vehicle-treated group.

Regarding the interaction of these ligands, coadministration of 30-30 µg EM1 and KYNA did not produce any antiallodynic effect (Fig. 3). **As regards the coadministration of 100-100 µg, ANOVA revealed significant effects of treatment** ($F_{3,34}$=4.2, p<0.05), time ($F_{5,170}$=9.4, ***p*<0.001**), and interaction ($F_{15,170}$=2.4, p<0.005). Post hoc comparison revealed that this combination produced an increased antinociception at some time points compared to vehicle, **KYNA and EM1** (Fig. 4). **200-200 µg EM + KYNA produced longer-lasting antinociception compared to the single treatments**

(Fig. 5). ANOVA showed significant effects of treatment ($F_{3,31}$=11.6, p<0.001), time ($F_{5,155}$=14.0, p<0.001), and interaction ($F_{15,155}$=4.4, ***p*<0.001**).

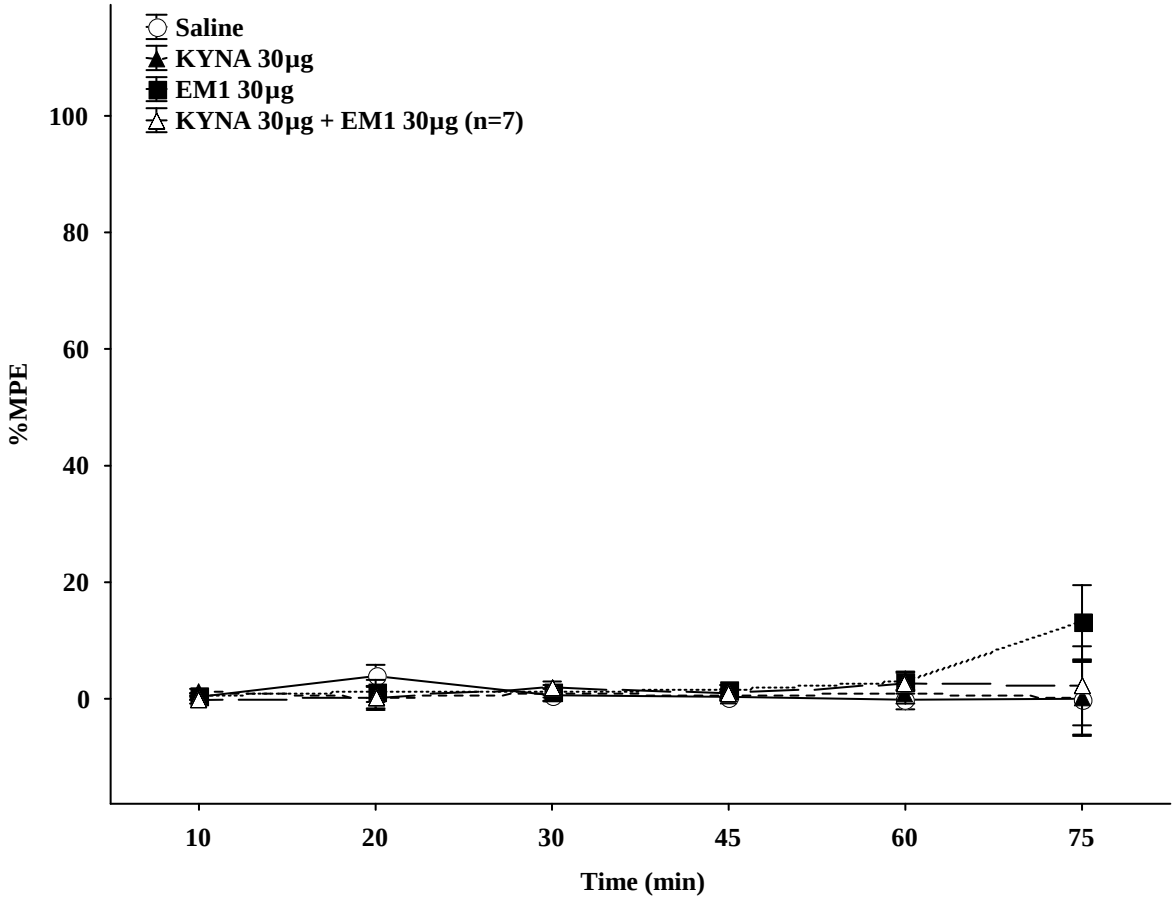

Figure 3.

Time course of the antinociceptive effects EM1 and KYNA 30-30 µg.

Each point denotes the mean ± SEM of the results.

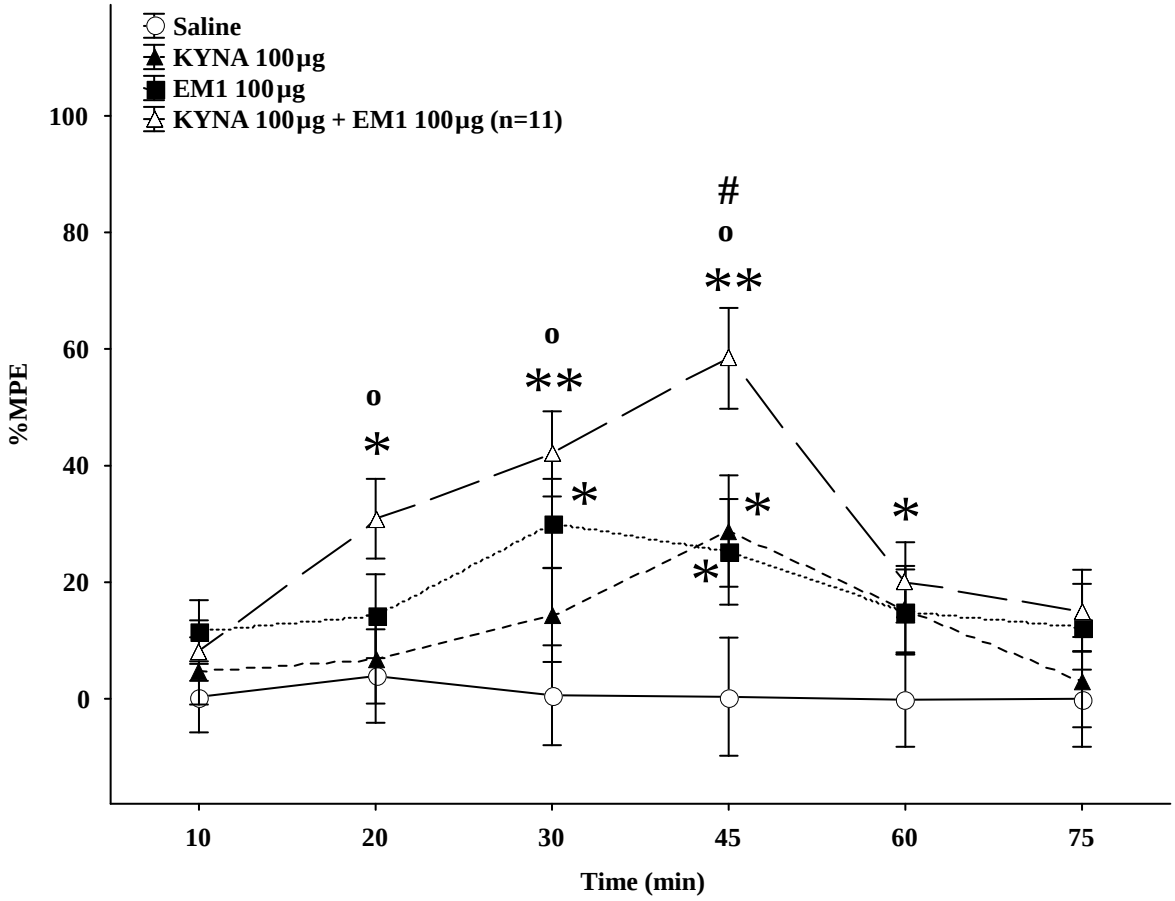

Figure 4.

Time course of the antinociceptive effects EM1 and KYNA 100-100 µg.

Each point denotes the mean ± SEM of the results. Symbols *****and ****** indicate significant (p<0.05; p<0.001; respectively) differences compared to the vehicle-treated group. # denotes a significant difference (p<0.05) from EM1 treated groups. **o** denotes a significant difference (p<0.05) from KYNA treated groups.

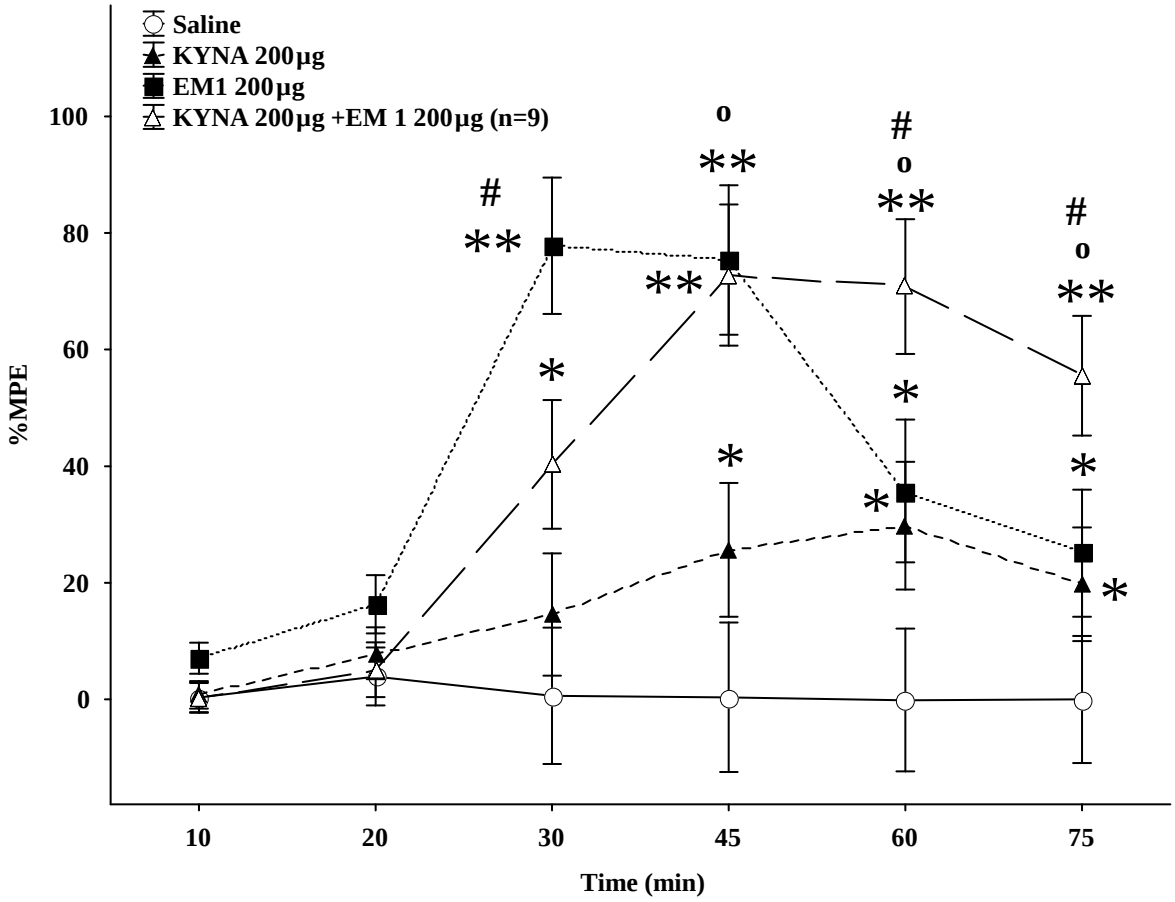

Figure 5.

Time course of the antinociceptive effects of different doses of EM1 and KYNA 200-200 µg. Each point denotes the mean ± SEM of the results. Symbols ***** and ****** indicate significant (p<0.05; p<0.001; respectively) differences compared to the vehicle-treated group. # denotes a significant difference (p<0.05) from EM1 treated groups. **o** denotes a significant difference (p<0.05) from KYNA treated groups.

As the ratio of the ED_{50} values of EM/KYNA was about 2, the doses of the combinations were calculated in this proportion (Tallarida et al., 1989). **The dose–response curve of the cocktail is between the EM1 and KYNA lines** (Fig. 6). **The ED_{30} and ED_{50} values were 141 µg [CI: 83–182] and 231 µg [CI: 190-293], respectively, which did not differ significantly from the theoretically additive values (ED_{30} and ED_{50} 145 µg [CI: 68–237] and 220 µg [CI: 144-230], respectively)** (Tallarida et al., 1997).

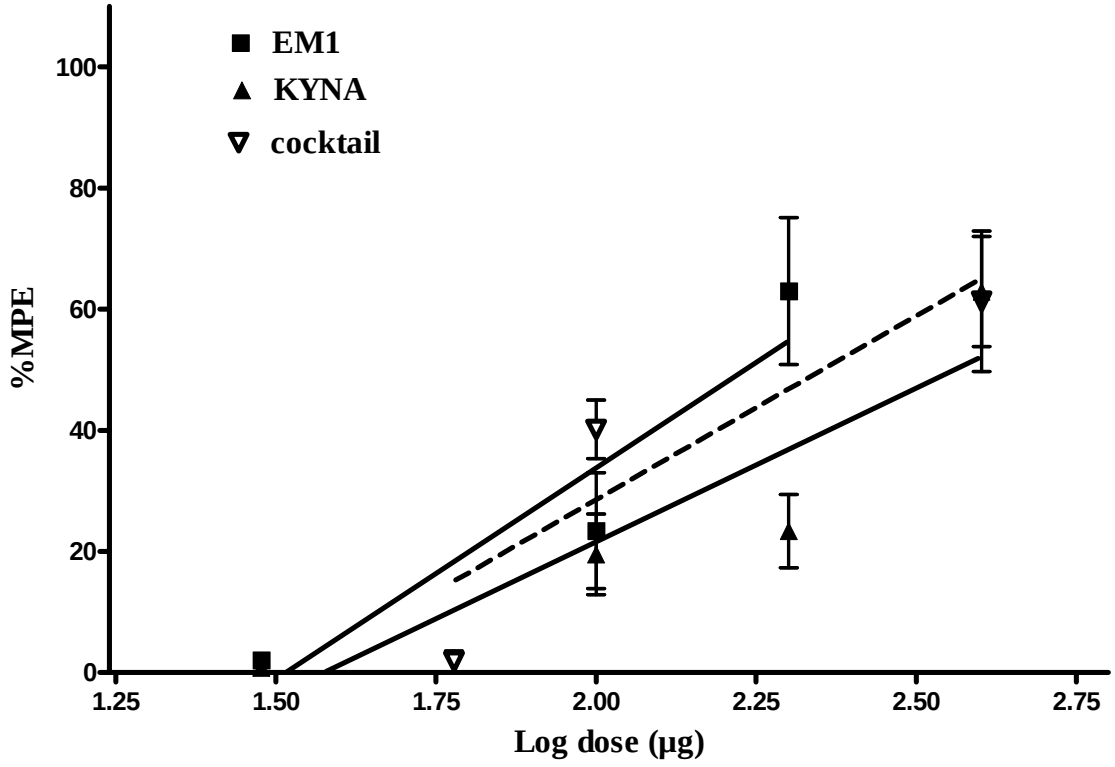

Figure 6.

The magnitude of the dose-dependent effects of EM1, KYNA by themselves and their combinations (mean of values at 30, 45 and 60 min).

2nd series:

EM1 produced again a dose-dependent antinociceptive effect, which developed gradually, and it reached its maximum between 45 and 60 min (Fig. 7). **Thus, 100 µg EM caused about 10 %MPE, while 300 µg caused a prolonged effect, which was about 50 %MPE at 45th and 60th min. ANOVA of AUC values showed significant differences between the groups revealing the dose-dependent effect of EM1** ($F_{3,56}$=41.86, p<0.0001; Fig. 8). The ED_{25} AUC value was 233 (CI: 198-268) µg.

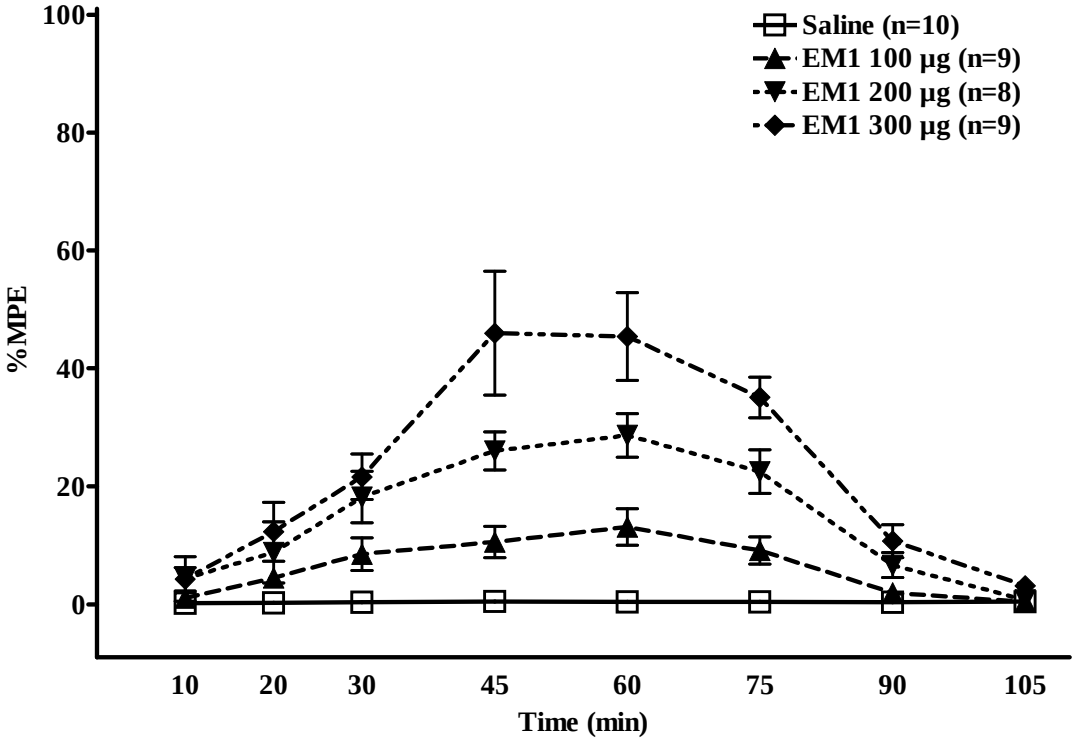

Figure 7.

Time course of the effects of EM1 (100, 200 and 300 µg) on the mechanical pain threshold on the inflamed side. Each point denotes the mean ± SEM of the results.

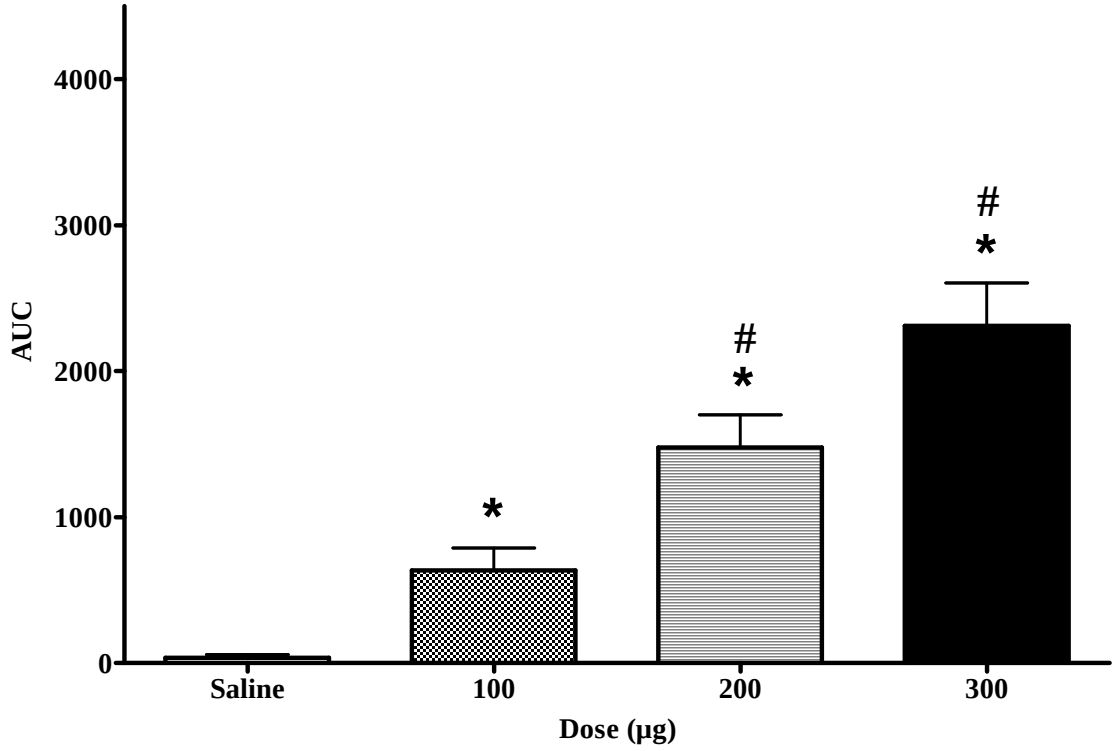

Figure 8.

The area under the curve (AUC) values of EM1 (100-300 µg) treatment. Symbol *indicates a significant (p<0.05) difference compared to the vehicle-treated group. # denotes a significant difference from EM1 (100 µg) treated groups.

2-AG by itself caused a dose-dependent antiallodynic effect, which also developed slowly. The highest dose produced a prolonged antinociception, and it achieved 55 %MPE at 75th min. (Fig. 9). **ANOVA of AUC values proved significant effects of treatment** (F6,53=10.65, ***p*<0.0001**), time (F7,371=41.37, p<0.0001), and interaction (F42,371=5.56, p<0.0001; Fig 10). **Its potency was higher compared with EM1**, i.e. the ED27 AUC value was 143 µg (CI: 100–163).

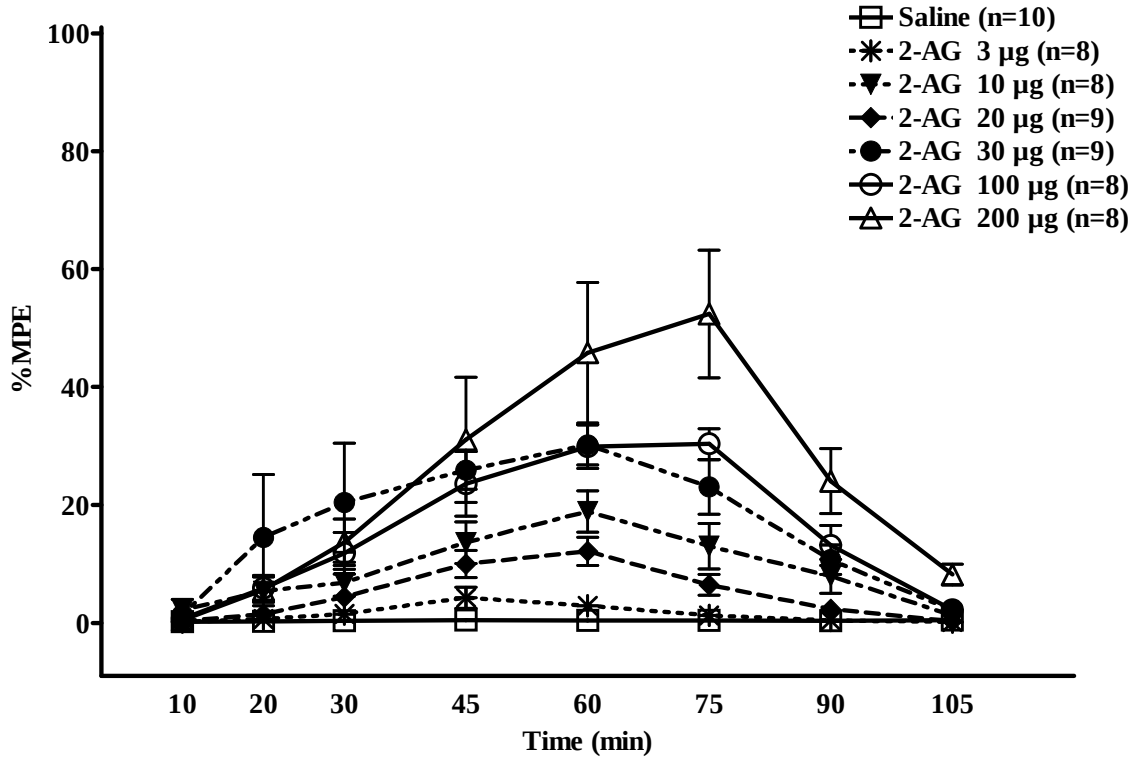

Figure 9.

Time course of the effects of 2-AG (3, 10, 20, 30, 100, 200 µg) on the mechanical pain threshold on the inflamed side. Each point denotes the mean ± SEM of the results.

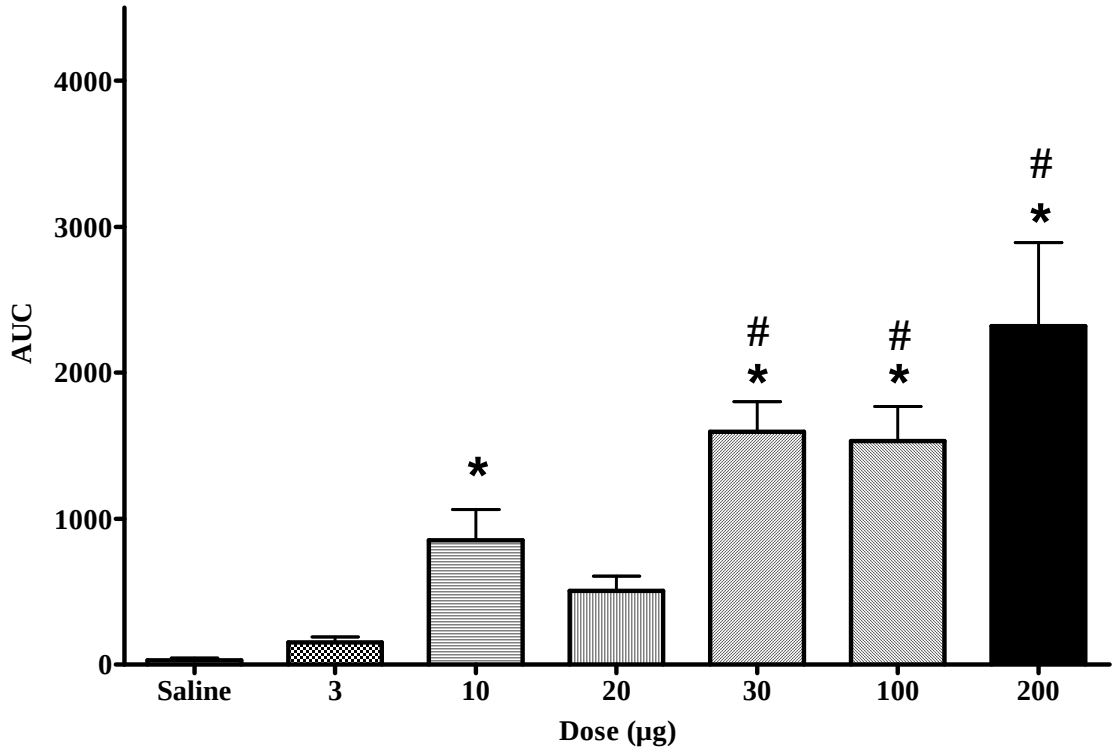

Figure 10.

AUC values os 2 AG treatments with different doses (3-200 µg). Symbol *indicates a significant (p<0.05) difference compared to the vehicle-treated group. # denotes a significant difference from 2-AG treated groups.

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 (Fig. 11, 12).

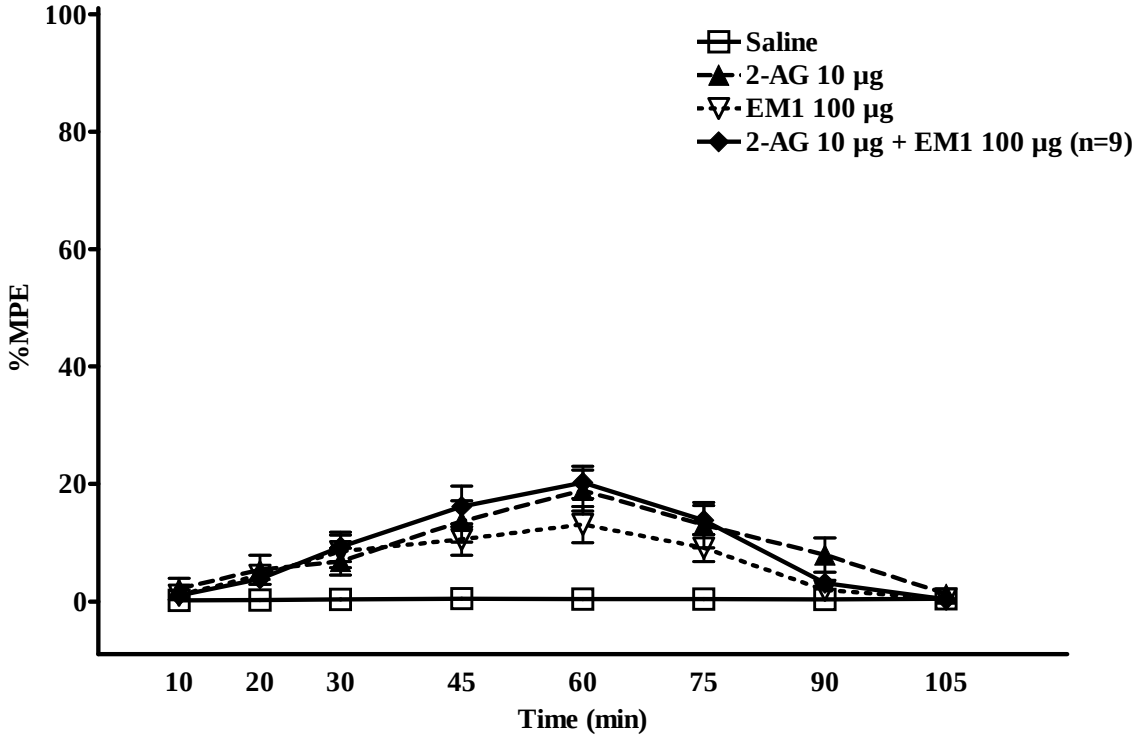

Figure 11.

Time course of the antinociceptive effects of EM1 (100 µg) and 2 AG (10 µg) and in combinations (10:1 fixed dose-ratio). Each point denotes the mean ± SEM of the results.

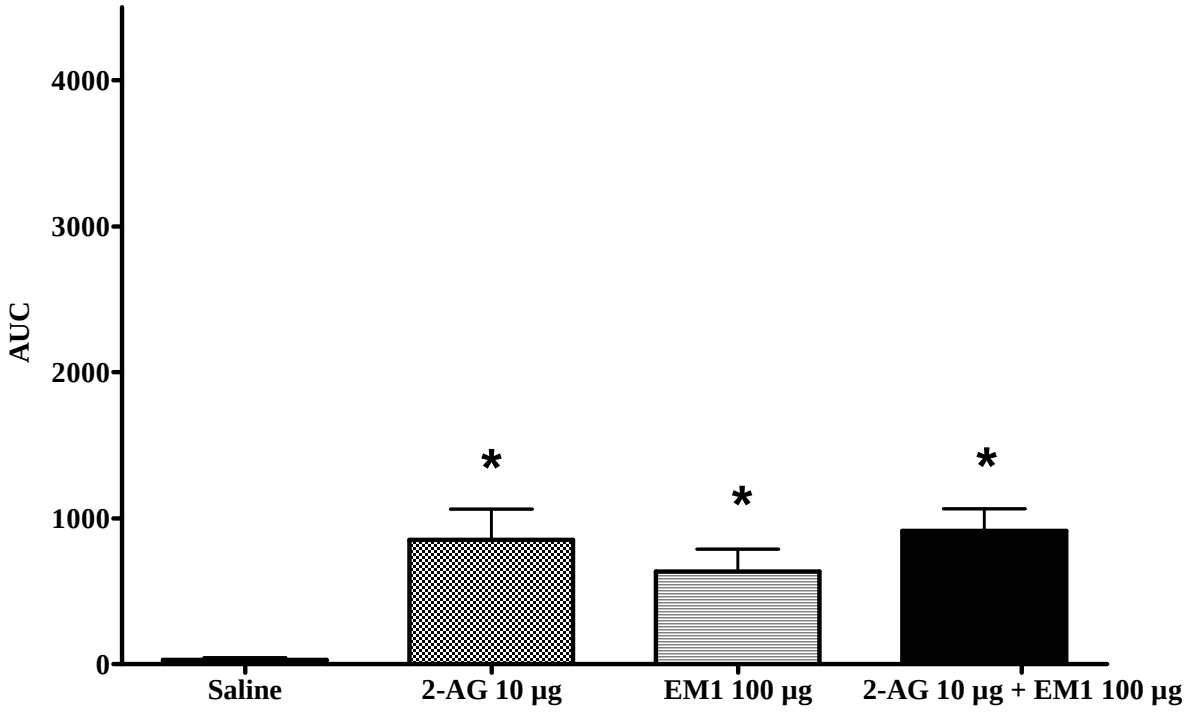

Figure 12

AUC values of EM1 (100 µg) and 2 AG (10 µg) and in combinations. Symbol *indicates a significant (p<0.05) difference compared to the vehicle-treated group.

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As regards the 20-200 µg 2-AG - EM1 combination, ANOVA revealed significant effects of treatment ($F3,32=25.64$, ***p*<0.0001**), time ($F7,224=51.99$, $p<0.0001$), and interaction ($F21,224=14.31$, $p<0.0001$). **This combination produced an increased antinociception compared to vehicle, 2-AG and EM1** (Fig. 13, 14).

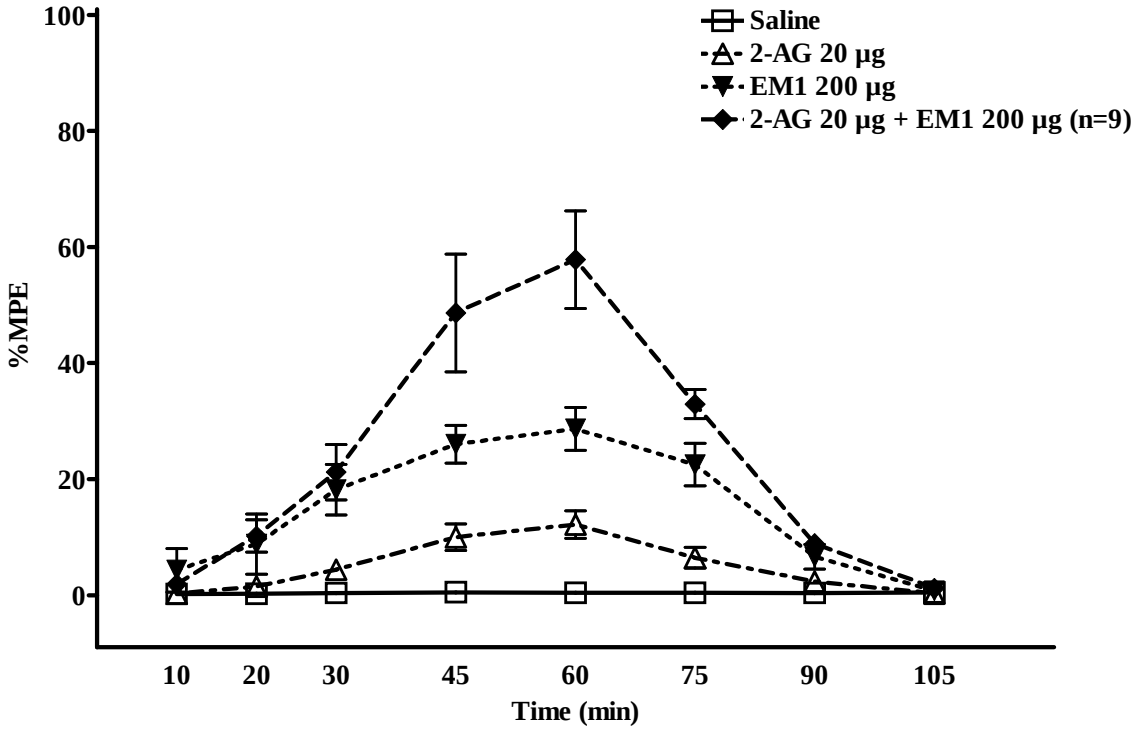

Figure 13.

Time course of the antinociceptive effects of EM1 (200 µg) and 2 AG (20 µg) and in combinations (10:1 fixed dose-ratio). Each point denotes the mean ± SEM of the results.

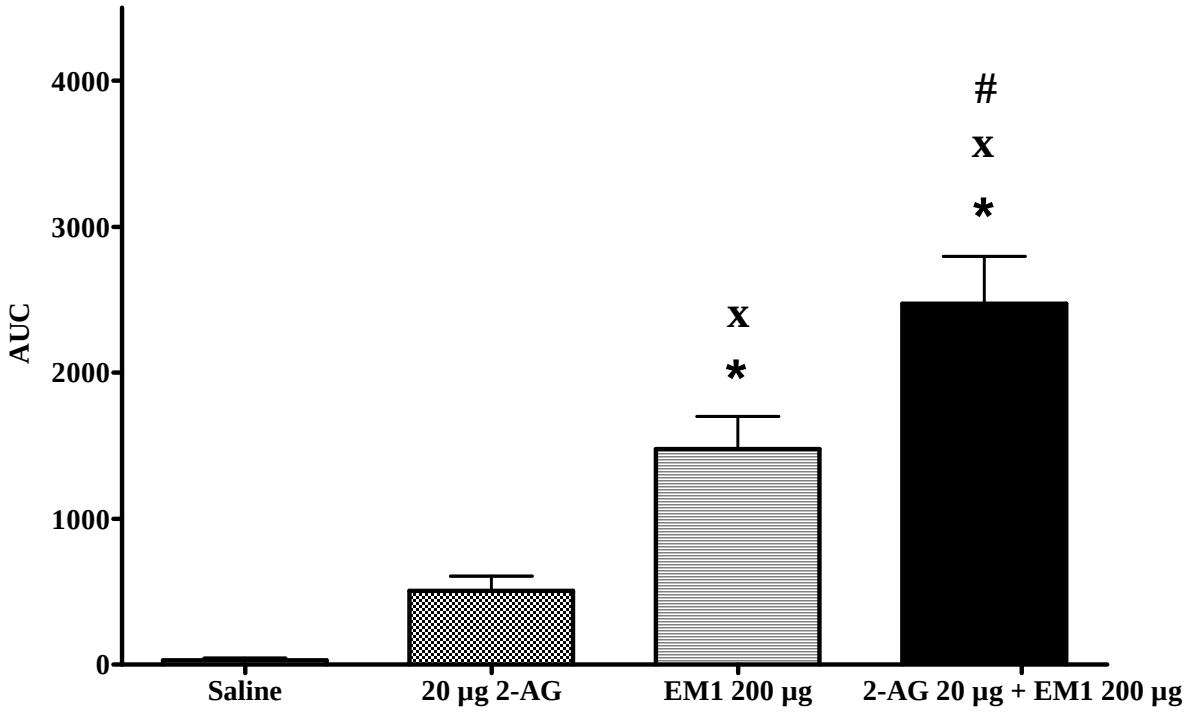

Figure 14.

AUC values of EM1 (200 µg) and 2 AG (20 µg) and in combinations. Symbol *indicates a significant (p<0.05) difference compared to the vehicle-treated group. # denotes a significant difference from EM1 treated groups. x denotes a significant difference from 2 AG treated groups.

Similarly, 30-300 µg 2-AG + EM1 also produced long-lasting and more effective antinociception compared to the single treatments (Fig. 15, 16). **ANOVA showed significant effects of treatment** (F3,34=29.03, ***p*<0.0001**), time (F7,238=29.84, p<0.0001), and interaction (F21,238=5.70, p<0.0001).

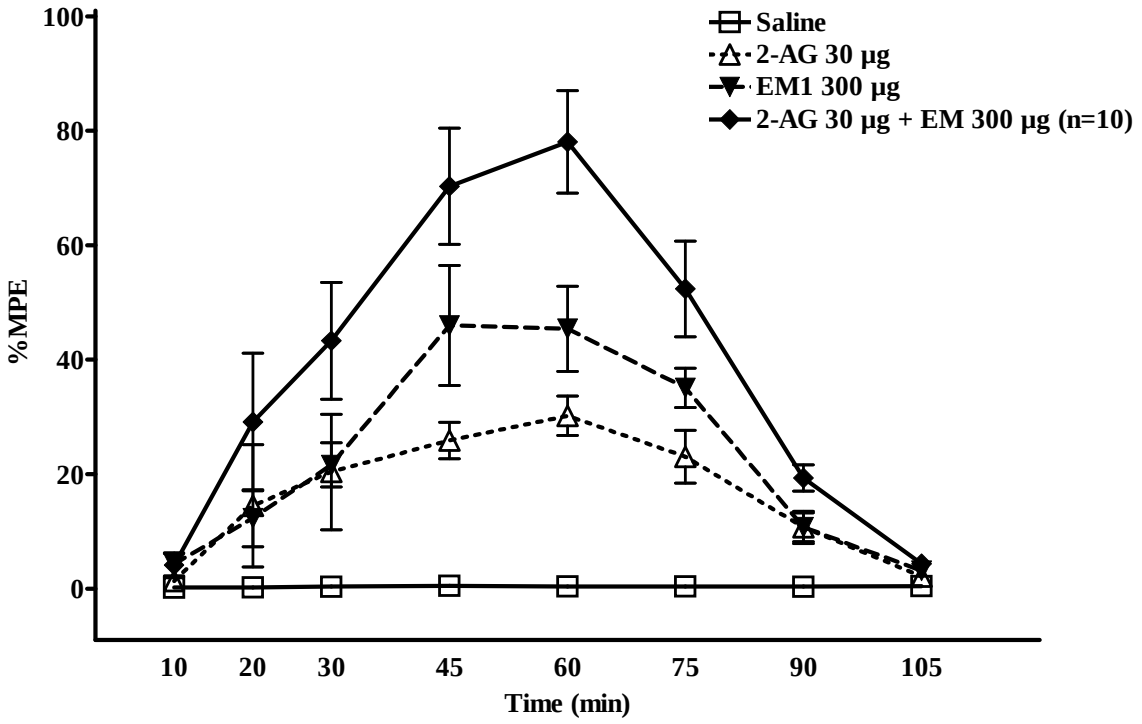

Figure 15.

Time course of the antinociceptive effects of EM1 (300 µg) and 2 AG (30 µg) and in combinations (10:1 fixed dose-ratio). Each point denotes the mean ± SEM of the results.

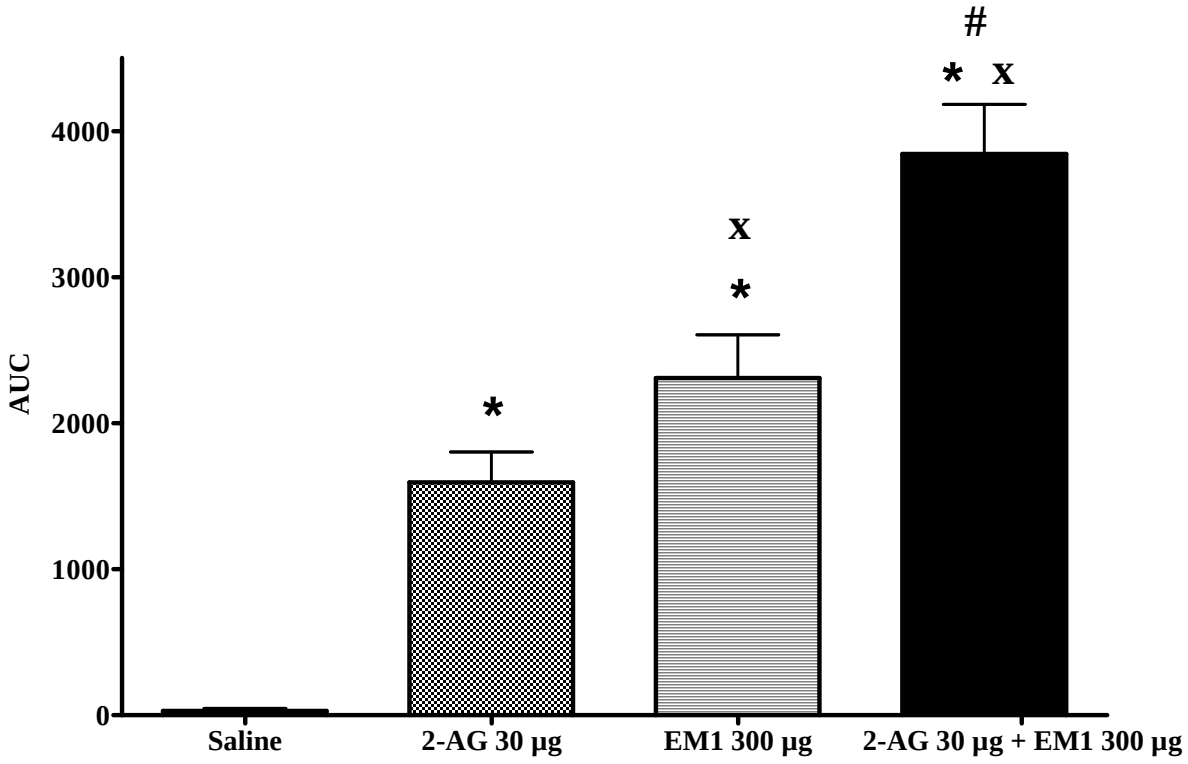

Figure 16.

AUC values of EM1 (300 µg) and 2 AG (30 µg) and in combinations. Symbol *indicates a significant (p<0.05) difference compared to the vehicle-treated group. # denotes a significant difference from EM1 treated groups. x denotes a significant difference from 2 AG treated groups.

As the ratio of the ED27 values of 2-AG/EM was 0,57, the doses of the combinations were calculated in this proportion (Tallarida et al., 1989). **The dose - response curve of the cocktail became more steeply compared to the EM and 2-AG lines** (Fig. 17). **The ED27 value was 99 µg (CI: 84–114), which did not differ significantly from the ED27 value for 2-AG, suggesting an additive interaction. However, since the curve for the combination was steeper compared to the single treatments, therefore, the largest dose combination produced significantly higher effect than the ligands by themselves.**

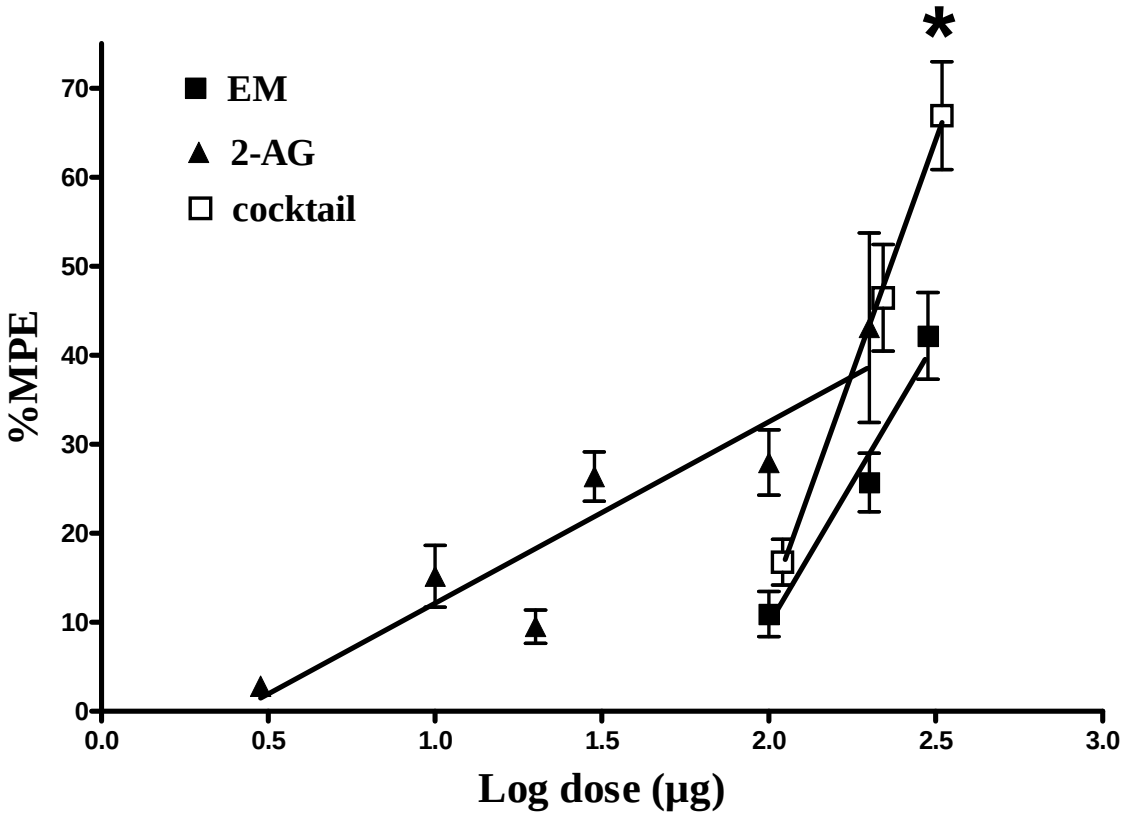

Figure 17.

The magnitude of the dose-dependent effects of EM1, 2-AG by themselves and their combinations. Symbol* indicates a significant (p<0.05) difference compared to the group treated with 200 µg 2-AG and EM1 by themselves.

We did not examine the motor behavior systematically, nor did we quantify it, but the animals' behaviors were observed, and there were no signs of altered behavior (immobility, flaccidity, excitation or motor weakness) after any treatments.

5 Discussion

Previous studies indicate that opioids can produce an additive or synergistic interactions with cannabinoids or NMDA antagonists primarily at central level (Cichewicz, 2004; Maldonado and Valverde, 2003; Welch and Eads, 1999).

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. The study 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.

Locally released opioid peptides at the site of injury are known to inhibit the inflammatory response and to reduce the pain associated with it (Stein et al., 1993). Only a few studies supported the beneficial effects of EM1 at peripheral level. IPL administration of both EM1 (40-160 µg) and EM2 (40-80 µg) decreased the mechanical allodynia after sciatic nerve injury in rats (Obara et al., 2004). The effects of the ligands were reversed by specific µ-opioid receptor antagonist drugs. Both ligands decreased the painful cold and warm hypersensitivity as well. Others have found that the IPL applied EM1 in low doses (0.3-1.25 µg) decreases Freund's adjuvant-induced mechanical allodynia (Labuz et al., 2006). The differences in the potency might be due to the alterations in the pain models, and/or the applied pain tests (von Frey vs. paw pressure test). Furthermore, the effect of EM1 might have been decreased by endopeptidases, since synovial fibroblasts are a rich source of these enzymes (Barsun et al., 2007; Bathon et al., 1992; Shane et al., 1999). EM1 can also decrease the joint inflammation and this effect may contribute to its antinociceptive potency (Straub et al., 2008). The effects of EM1 developed relatively slowly compared to other studies (Labuz et al., 2006; McDougall et al., 2004; Obara et al., 2004), which might be due to the differences in the routes of the application, the pain models and/or the investigated parameters (vascular reactivity vs. pain threshold). As regards the effects of EM1 on joint pain, a recent study has detected a decreased afferent nerve activity in response to noxious hyperrotation of the joint in anesthetized rats after

EM1 administration (Li et al., 2005). EM1 (60 µg) applied intra-arterially close to the knee joint, caused up to a 75% reduction in joint afferent nerve activity, but its effect was lost during chronic inflammation. The intra-articularly administered EM1 reduces knee joint blood flow through the action on unmyelinated primary afferent neurons, and this effect is not sustainable during advanced inflammation (McDougall et al., 2004). The loss of this response appears to be due to downregulation of µ-opioid receptors as a consequence of EM1 accumulation within the arthritic joint. Considering the action mechanism of EM1, it is suggested that the activation of µ-opioid receptors by EM1 can inhibit the release of pain producing substances (e.g. substance P) from primary sensory neurons (Stein et al., 1990a).

As regards the action mechanism of KYNA, the current study does not provide direct evidence for specific receptor involvement in mediating the efficacy of KYNA, but data from receptor binding studies performed in other laboratories do allow us to suggest potential receptor mechanisms which may be involved. Peripheral NMDA receptors play significant roles in sensory processing at peripheral level (Coggeshall and Carlton, 1998; Kinkelin et al., 2000). Recently, the possibility that glutamate may be released by neuronal endings in the inflamed knee joint has been demonstrated in rats, and its contribution to the hyperalgesic events initiated during the development of joint inflammation has strongly been supported through the activation of NMDA on primary sensory neurons (Coggeshall et al., 1998; Lawand et al., 2000). Since KYNA produces its effects mainly by the inhibition of NMDA receptors, therefore we suppose that primarily this effect might have led to its effectivity in this model, however, its antinociceptive potency and the side-effects could not have been predicted from the earlier results. We found that KYNA had lower potency but similar efficacy compared to EM1.

The inhibition of alpha7 nAChRs by KYNA also could have a role in its effects (Nemeth et al., 2005). It has been found that nAchRs play a role in modulating pain transmission both centrally and peripherally, however, the results are controverting (Damaj et al., 2000; Damaj, 2000). Stimulation of neuronal nAChR excites or sensitizes peripheral sensory nerve fibres but it has also been reported to mediate cholinergic antinociception (Bernardini et al., 2001; Gilbert et al., 2001). There is controversy about the localization of alpha7 nAChR at the periphery as well, since Haberberger et al. found these receptors on all nociceptive neurons (Haberberger et al., 2004), while Lang et al. could not have detected them (Lang et al., 2003). While the deficiency in this receptor did not influence pain sensitivity (Rashid et al., 2006), a recent study suggests that activation of alpha7

nAChR may elicit antinociceptive effects in an inflammatory pain model with peripheral mechanism (Wang et al., 2005b). Therefore, the results of inhibition of alpha7 nAChR are uncertain in the periphery, but it might be involved in the effects of KYNA. Furthermore, the cholinesterase inhibitor, neostigmine, has been injected directly into the knee joint, and such an approach also provides evidence for a cholinergic peripheral analgesia. Thus, intra-articular neostigmine partially suppresses mechanical hyperalgesia in the rat inflamed knee joint model (Buerkle et al., 1998) and produces some postoperative analgesia in patients undergoing knee surgery (Yang et al., 1998).

A recent study has shown that GPR35, a previously orphan GPR, functions as a receptor for the KYNA (Wang et al., 2006). KYNA elicits calcium mobilization and IP3 production in a GPR35-dependent manner in the presence of $G_{qi/o}$ chimeric G proteins, and also induces the internalization of GPR35. Expression analysis indicates that GPR35 is predominantly detected in immune cells and in the gastrointestinal tract, but it has also been found in the DRG on small- to medium-diameter neurons (Ohshiro et al., 2008). The results suggest that GPR35 may modulate nociception and continued study of this receptor will provide additional insight into the role of KYNA in pain perception. However, no in vivo data are available as regards the role of GPR35 in the effects of KYNA, and no specific antagonists have been developed until now. Regardless of the mechanism of action, the results clearly show that KYNA has antihyperalgesic potency at peripheral level. Therefore, KYNA can influence several systems which might be involved in the effects of KYNA on pain threshold.

The use of cannabinoids for the management of a wide range of painful disorders has been well documented at spinal, supraspinal, and peripheral levels (Guindon et al., 2007b; Hohmann, 2002; Pertwee, 2001). Peripheral nerve fibres 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 (Griffin et al., 1997; Pertwee, 2001). Thus, topically applied cannabinoids have provided effective analgesia in different pain models, and this effect is mediated by CB1 and CB2 activation (Agarwal et al., 2007; Nackley et al., 2003).

The antinociceptive properties of endogenous cannabinoids have been established in a number of experiments, but only some data suggest that these ligands can also be involved in the peripheral pain mechanisms (Calignano et al., 1998; Guindon et al., 2006b; Nackley

et al., 2003; Richardson et al., 1998b). The cannabinoid receptor system presents in the synovium; therefore, it may be an important therapeutic target for the treatment of pain and inflammation associated with osteoarthritis and rheumatoid arthritis, but only a few studies have investigated their effects at joint level (Richardson et al., 2008). Thus, a selective CB1 receptor agonist, arachidonyl-2-chloroethylamide, has been able to reduce the mechanosensitivity of afferent nerve fibers in control and osteoarthritic rat knee joints (Schuelert and McDougall, 2008). A close intra-arterial injection of anandamide to medial articular nerve has significantly increased the discharge of C-fibers by activation of transient receptor potential vanilloid-1 receptors (TRPV1) both in normal and arthritic rats (Gauldie et al., 2001). Another study has shown that anandamide produced dose-dependent increases in the rat knee joint blood flow, when it was applied on the surface of the joint (Lam et al., 2007). Since, anandamide can activate both the cannabinoid and TRPV1 receptors; both of these effects can influence the pain threshold (Starowicz et al., 2007).

Only few data have proved the antinociceptive potency of 2-AG. Systemic administration to mice, 2-AG (ED50=12.5 mg/kg) has caused antinociception in acute pain tests, immobility, reduction of spontaneous activity, and lowering of rectal temperature (Ben Shabat et al., 1998; Mechoulam et al., 1995). IPL injected 2-AG (0.01-100 μg) has decreased pain behavior in a dose-dependent manner in the late phase of formalin test in rats, and the antinociceptive effects of 2-AG have been prevented by AM630, a selective CB2 antagonist, but not by AM251, a selective CB1 receptor antagonist (Guindon et al., 2007a; Hohmann, 2007). It also decreased (0.001-100 μg) the mechanical and thermal hypersensitivity after nerve injury in the same race (Desroches et al., 2008). Moreover, the antinociceptive effects of 2-AG are prevented by a selective CB2 receptor antagonist, but not by a CB1 receptor antagonist in the formalin test, while both antagonists inhibit the antiallodynic and antihyperalgesic effects of 2-AG (Desroches et al., 2008; Guindon et al., 2007a). In our circumstances, higher doses of 2-AG were effective, which might be due to the differences between the pain models (neuropathy versus carrageenan-induced arthritis).

An important technique employed to decrease the side-effects is the use of combination of several agents in low doses that produce the same therapeutic effects as a single drug applied in a higher dose. In this respect, there is a growing body of experimental data, which indicates that NMDA antagonists potentiate the analgesic effect of opiates and may block or reduce the development of tolerance following long-term opiate administration (Dickenson, 1997; Joo et al., 2000; Nishizawa et al., 1998; Trujillo

and Akil, 1991; Wiesenfeld-Hallin, 1998). Controversially, there have been some reports that the NMDA antagonist MK-801 neither has an antinociceptive effect of its own, nor does it alter that of morphine (Trujillo et al., 1991), while other data indicate a simple additive interaction of MK-801 with morphine in the carrageenan-induced inflammatory pain model (Yamamoto et al., 1993). Some studies have investigated the interactions of EM1 with different drugs to improve its efficacy at central level (Csullog et al., 2001; Hao et al., 2000; Hao et al., 1999; Wang et al., 1999). The present study showed that the type of the interaction at peripheral level was additive. Since both opioid and glutamate receptors are present on the primary sensory neurons at the periphery, the co-activation and antagonism of these receptors could have a beneficial effect on the inhibition of pain sensation at doses which do not cause side-effects. Since the ratio of the applied drugs can also influence the type of the interactions (Tallarida et al., 1997), another ratio of these drugs may produce other type of interactions.

The acute administration of EM1 and/or KYNA did not influence the degree of edema in our circumstances. However, it cannot be excluded that their administration would produce not only antinociception but also antiinflammatory effect, and this may contribute to their antinociceptive effects (Khalil et al., 1999; Parada-Turska et al., 2006; Straub et al., 2008). Thus, opioid receptors have been demonstrated on primary sensory neurons and immune cells (Stein et al., 1990a; Stein et al., 1993), and locally released opioid peptides (including EM1) are known to inhibit the inflammatory response at the site of injury (McDougall et al., 2003; Stein et al., 1993; Straub et al., 2008). Similarly, KYNA can also control the inflammatory process by inhibition of the proliferation of synoviocytes (Parada-Turska et al., 2006).

The beneficial interaction between opioids and cannabinoids are well known after systemic and/or central administrations (Cichewicz and Mccarthy, 2003; Welch and Stevens, 1992), while only a few studies have investigated their interaction at peripheral level (Yesilyurt et al., 2003; Yesilyurt and Dogrul, 2004). These studies have applied synthetic drugs by a topical immersion method, and the acute heat pain threshold has been determined. It has been found that the topically applied cannabinoid potentiates the effect of morphine. We found that the coadministration of endogenous opioid and cannabinoid ligands produced additive interaction, and they effectively decreased the inflammatory allodynia in our model. Since both μ-opioid and CB receptors can be found in the synovial membrane, and/or on the nociceptive primary sensory neurons and these receptors are G-protein coupled ones, their coactivation can modify the level of cAMP and/or open

potassium channels and/or close calcium channels, and/or inhibit release of substance P and other pain-inducing ligands leading to a potentiated inhibition of the propagation of nociceptive stimuli (Ahluwalia et al., 2000; Jordan et al., 2000; Richardson et al., 2008; Stein et al., 1993).

6 General Conclusions

We proved the antinociceptive potency of EM1 at joint level in an inflammatory pain model.

We firstly demonstrated the antiallodynic potency of intra-articularly administered KYNA without any side-effects.

We showed the antinociceptive effect of 2-AG at peripheral level.

We have found that the combination of EM1 with KYNA produced additive antinociceptive interaction.

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.

7 Abbreviations used in the study

5HT	5-hydroxytryptamine
IL	interleukin
TNF	tumour necrosis factor
ATP	adenosine-triphoszphate
NGF	nerve growth factor
TRP	transient receptor potential
P2X	purinoreceptor
GPR	G-protein-coupled metabotropic receptors
cAMP	cyclic adenosine monophosphate
IP3	inositol triphosphate
PKA	protein kinase A
PKC	protein kinase C
SDH	spinal dorsal horn
ARC	arcuate nucleus
LAAH	lateral area of anterior hypothalamus
ACC	anterior cingulated cortex
PAG	periaqueductal grey matter
RVM	rostroventral medulla
NRM	nucleus raphe magnus
LC	locus ceruleus
NSAIDs	nonsteroidal anti-inflammatory drugs
COX	cyclooxygenase
ORL1	opioid receptor-like receptor
CNS	central nervous system
IPL	intraplantar
EMs	endomorphins
EM1	endomorphin-1
EM2	endomorphin-2
NMDA	N-methyl-d-aspartate
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
KA	kainic acid

KYNA	kynurenic acid
GlyRs	glycine receptors
GPR35	G protein-coupled receptor 35
CBs	Cannabinoids
CB1	Cannabinoid-1 receptor
CB2	Cannabinoid-2 receptor
AC	adenylate cyclase
MAPK	mitogen-activated protein kinase
AEA	arachidonoyl-ethanolamine (anandamide)
2-AG	2-arachidonoyl-glycerol
PEA	palmityl-ethanolamide
NAGly	N-arachidonoyl-glycine
OEA	N-oleoyl-ethanolamide
NAPE	N-acylphosphatidyl-ethanolamine
FAAH	fatty acid amide hydrolase
TRPV1	transient receptor potential vanilloid 1 receptor
PPARα	peroxisome proliferators-activated receptor α
MAGL	monoglyceride lipase
NTX	naltrexone
α7nAChRs	alpha7 nicotinic acetylcholine receptors

8. References

Agarwal, N., Pacher, P., Tegeder, I., Amaya, F., Constantin, C. E., Brenner, G. J., Rubino, T., Michalski, C. W., Marsicano, G., Monory, K., Mackie, K., Marian, C., Batkai, S., Parolaro, D., Fischer, M. J., Reeh, P., Kunos, G., Kress, M., Lutz, B., Woolf, C. J., Kuner, R., 2007. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. Nat. Neurosci. 10, 870-879.

Ahern, G. P., 2003. Activation of TRPV1 by the satiety factor oleoylethanolamide. J. Biol. Chem. 278, 30429-30434.

Ahluwalia, J., Urban, L., Capogna, M., Bevan, S., Nagy, I., 2000. Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. Neuroscience 100, 685-688.

Akil, H., Watson, S. J., Young, E., Lewis, M. E., Khachaturian, H., Walker, J. M., 1984. Endogenous opioids: biology and function. Annu. Rev. Neurosci. 7, 223-255.

Almasi, R., Szoke, E., Bolcskei, K., Varga, A., Riedl, Z., Sandor, Z., Szolcsanyi, J., Petho, G., 2008. Actions of 3-methyl-N-oleoyldopamine, 4-methyl-N-oleoyldopamine and N-oleoylethanolamide on the rat TRPV1 receptor in vitro and in vivo. Life Sci. 82, 644-651.

Bach, F. W., 1997. Beta-endorphin in the brain. A role in nociception. Acta Anaesthesiol. Scand. 41, 133-140.

Barin, A. K., McDougall, J. J., 2003. Endomorphin-1 causes synovial hypoaemia in rat knee joints via a capsaicin-sensitive neural pathway. Neurosci. Lett. 344, 21-24.

Barsun, M., Jajcanin, N., Vukelic, B., Spoljaric, J., Abramic, M., 2007. Human dipeptidyl peptidase III acts as a post-proline-cleaving enzyme on endomorphins. Biol. Chem. 388, 343-348.

Basbaum, A. I., Fields, H. L., 1984. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu. Rev. Neurosci. 7, 309-338.

Bathon, J. M., Proud, D., Mizutani, S., Ward, P. E., 1992. Cultured human synovial fibroblasts rapidly metabolize kinins and neuropeptides. J. Clin. Invest. 90, 981-991.

Behrens, F., Shepard, N., Mitchell, N., 1975. Alteration of Rabbit Articular-Cartilage by Intra-Articular Injections of Glucocorticoids. J. Bone Joint Surg. Am. 57, 70-76.

Ben Shabat, S., Fride, E., Sheskin, T., Tamiri, T., Rhee, M. H., Vogel, Z., Bisogno, T., De Petrocellis, L., Di Marzo, V., Mechoulam, R., 1998. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur. J. Pharmacol. 353, 23-31.

Bernardini, N., Sauer, S. K., Haberberger, R., Fischer, M. J. M., Reeh, P. W., 2001. Excitatory nicotinic and desensitizing muscarinic (M2) effects on C-nociceptors in isolated rat skin. J. Neurosci. 21, 3295-3302.

Besson, J. M., Chaouch, A., 1987. Peripheral and spinal mechanisms of nociception. Physiol. Rev. 67, 67-186.

Bisogno, T., 2008. Endogenous cannabinoids: structure and metabolism. J. Neuroendocrinol. 20, 1-9.

Bleakman, D., Alt, A., Nisenbaum, E. S., 2006. Glutamate receptors and pain. Semin. Cell Dev. Biol. 17, 592-604.

Bodnar, R. J., Klein, G. E., 2004. Endogenous opiates and behavior: 2003. Peptides 25, 2205-2256.

Bodnar, R. J., 2008. Endogenous opiates and behavior: 2007. Peptides 29, 2292-2375.

Boschi, G., Desiles, M., Reny, V., Rips, R., Wrigglesworth, S., 1983. Antinociceptive properties of thyrotropin releasing hormone in mice - comparison with morphine. Br. J. Pharmacol. 79, 85-92.

Buerkle, H., Boschin, M., Marcus, M. A. E., Brodner, G., Wusten, R., Van Aken, H., 1998. Central and peripheral analgesia mediated by the acetylcholinesterase-inhibitor neostigmine in the rat inflamed knee joint model. Anesth. Analg. 86, 1027-1032.

Burstein, S. H., 1999. The cannabinoid acids: nonpsychoactive derivatives with therapeutic potential. Pharmacol. Ther. 82, 87-96.

Burstein, S. H., Adams, J. K., Bradshaw, H. B., Fraioli, C., Rossetti, R. G., Salmonsen, R. A., Shaw, J. W., Walker, J. M., Zipkin, R. E., Zurier, R. B., 2007. Potential anti-inflammatory actions of the elmiric (lipoamino) acids. Bioorg. Med. Chem. 15, 3345-3355.

Calignano, A., La Rana, G., Giuffrida, A., Piomelli, D., 1998. Control of pain initiation by endogenous cannabinoids. Nature 394, 277-281.

Calignano, A., La Rana, G., Piomelli, D., 2001. Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. Eur. J. Pharmacol. 419, 191-198.

Carlton, S. M., Coggeshall, R. E., 1999. Inflammation-induced changes in peripheral glutamate receptor populations . Brain. Res. 820, 63-70.

Carlton, S. M., Hargett, G. L., Coggeshall, R. E., 1995. Localization and Activation of Glutamate Receptors in Unmyelinated Axons of Rat Glabrous Skin. Neurosci. Lett. 197, 25-28.

Carpenedo, R., Pittaluga, A., Cozzi, A., Attucci, S., Galli, A., Raiteri, M., Moroni, F., 2001. Presynaptic kynurenate-sensitive receptors inhibit glutamate release. Eur. J. Neurosci. 13, 2141-2147.

Caterina, M. J., Julius, D., 1999. Sense and specificity: a molecular identity for nociceptors . Curr. Opin. Neurobiol. 9, 525-530.

Catterall, W. A., 2002. Molecular mechanisms of gating and drug block of sodium channels. Novartis Found. Symp. 241, 206-225.

Chemin, J., Monteil, A., Perez-Reyes, E., Nargeot, J., Lory, P., 2001. Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. EMBO J. 20, 7033-7040.

Chen, P., Hu, S., Yao, J., Moore, S. A., Spector, A. A., Fang, X., 2005. Induction of cyclooxygenase-2 by anandamide in cerebral microvascular endothelium. Microvasc. Res. 69, 28-35.

Cichewicz, D. L., 2004. Synergistic interactions between cannabinoid and opioid analgesics. Life Sci. 74, 1317-1324.

Cichewicz, D. L., Mccarthy, E. A., 2003. Antinociceptive synergy between D^9-tetrahydrocannabinol and opioids after oral administration. J. Pharmacol. Exp. Ther. 304, 1010-1015.

Coderre, T. J., Katz, J., Vaccarino, A. L., Melzack, R., 1993. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. Pain 52, 259-285.

Coggeshall, R. E., Carlton, S. M., 1997. Receptor localization in the mammalian dorsal horn and primary afferent neurons. Brain Res. Rev. 24, 28-66.

Coggeshall, R. E., Carlton, S. M., 1998. Ultrastructural analysis of NMDA, AMPA, and kainate receptors on unmyelinated and myelinated axons in the periphery. J. Comp. Neurol. 391, 78-86.

Coggeshall, R. E., Zhou, S. T., Carlton, S. M., 1997. Opioid receptors on peripheral sensory axons. Brain Res. 764, 126-132.

Correll, G. E., Maleki, J., Gracely, E. J., Muir, J. J., Harbut, R. E., 2004. Subanesthetic ketamine infusion therapy: A retrospective analysis of a novel therapeutic approach to complex regional pain syndrome. Pain Med. 5, 263-275.

Costa, B., Comelli, F., Bettoni, I., Colleoni, M., Giagnoni, G., 2008. The endogenous fatty acid amide, palmitoylethanolamide, has anti-allodynic and anti-hyperalgesic effects in a murine model of neuropathic pain: involvement of CB1, TRPV1 and PPAR gamma receptors and neurotrophic factors. Pain 139, 541-550.

Coutaux, A., Adam, F., Willer, J. C., Le Bars, D., 2005. Hyperalgesia and allodynia: peripheral mechanisms. Joint Bone Spine 72, 359-371.

Cravatt, B. F., Giang, D. K., Mayfield, S. P., Boger, D. L., Lerner, R. A., Gilula, N. B., 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384, 83-87.

Csullog, E., Joo, G., Toth, G., Dobos, I., Benedek, G., Horvath, G., 2001. Antinociceptive effect of continuous intrathecal administration of endomorphin-1. Pain 94, 31-38.

Dafny, N., Dong, W. Q., Prieto-Gomez, C., Reyes-Vazquez, C., Stanford, J., Qiao, J. T., 1996. Lateral hypothalamus: site involved in pain modulation. Neuroscience 70, 449-460.

Damaj, M. I., 2000. The involvement of spinal Ca^{2+}/calmodulin-potein kinase II in nicotine-induced antinociception in mice. Eur. J. Pharmacol. 404, 103-110.

Damaj, M. I., Meyer, E. M., Martin, B. R., 2000. The antinociceptive effects of alpha7 nicotinic agonists in an acute pain model. Neuropharmacology 39, 2785-2791.

De Petrocellis, L., Chu, C. J., Moriello, A. S., Kellner, J. C., Walker, J. M., Di Marzo, V., 2004. Actions of two naturally occurring saturated N-acyldopamines on transient receptor potential vanilloid 1 (TRPV1) channels. Br. J. Pharmacol. 143, 251-256.

De Petrocellis, L., Starowicz, K., Moriello, A. S., Vivese, M., Orlando, P., Di Marzo, V., 2007. Regulation of transient receptor potential channels of melastatin type 8 (TRPM8): effect of cAMP, cannabinoid CB1 receptors and endovanilloids. Exp. Cell Res. 313, 1911-1920.

Desroches, J., Guindon, J., Lambert, C., Beaulieu, P., 2008. Modulation of the anti-nociceptive effects of 2-arachidonoyl glycerol by peripherally administered FAAH and MGL inhibitors in a neuropathic pain model. Br. J. Pharmacol. 155, 913-924.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946-1949.

Dhawan, B. N., Cesselin, F., Raghubir, R., Reisine, T., Bradley, P. B., Portoghese, P. S., Hamon, M., 1996. International union of pharmacology. XII. classification of opioid receptors. Pharmacol Rev 48, 567-592.

Di Marzo, V., Breivogel, C. S., Tao, Q., Bridgen, D. T., Razdan, R. K., Zimmer, A. M., Zimmer, A., Martin, B. R., 2000. Levels, metabolism, and pharmacological activity of anandamide in CB_1 cannabinoid receptor knockout mice. Evidence for non-CB_1, non-CB_2 receptor-mediated actions of anandamide in mouse brain. J. Neurochem. 75, 2434-2444.

Di Marzo, V., Melck, D., Bisogno, T., De Petrocellis, L., 1998. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends Neurosci. 21, 521-528.

Dickenson, A. H., 1997. NMDA receptor antagonists: interactions with opioids. Acta Anaesthesiol. Scand. 41, 112-115.

Dickenson, A. H., Chapman, V., Green, G. M., 1997. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. Gen. Pharmacol. 28, 633-638.

Dinh, T. P., Carpenter, D., Leslie, F. M., Freund, T. F., Katona, I., Sensi, S. L., Kathuria, S., Piomelli, D., 2002. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc. Natl. Acad. Sci. U S A 99, 10819-10824.

Doan, T., Massarotti, E., 2005. Rheumatoid arthritis: An overview of new and emerging therapies. J. Clin. Pharmacol. 45, 751-762.

Dogrul, A., Gul, H., Akar, A., Yildiz, O., Bilgin, F., Guzeldemir, E. (2003). Topical cannabinoid antinociception: synergy with spinal sites. Pain 105: 11-16.

Dray, A., 1995. Inflammatory mediators of pain. Br. J. Anaesth. 75, 125-131.

Fields, H., 2004. State-dependent opioid control of pain. Nat. Rev. Neurosci. 5, 565-575.

Fields,H.L., Basbaum,A.I., 1999. Central nervous system mechanisms of pain modulation. In: Wall,P.D., Melzack,R. (Eds.), Textbook of pain. Churchill Livingstone, Edinburgh, pp. 309-329.

Fields,H.L., Liebskind,J.C., Dickenson,A.H., 1994. Pain research and management. IASP Press, Seattle.

Fisher, K., Coderre, T. J., Hagen, N. A., 2000. Targeting the N-methyl-D-aspartate receptor for chronic pain management: Preclinical animal studies, recent clinical experience and future research directions. J. Pain Symptom Manage. 20, 358-373.

Fitch, R. W., Kuhn, J. E., 2008. Intraarticular lidocaine versus intravenous procedural sedation with narcotics and benzodiazepines for reduction of the dislocated shoulder: a systematic review. Acad. Emerg. Med. 15, 703-708.

Fu, J., Gaetani, S., Oveisi, F., Lo Verme, J., Serrano, A., de Fonseca, F. R., Rosengarth, A., Luecke, H., Di Giacomo, B., Tarzia, G., Piomelli, D., 2003. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. Nature 425, 90-93.

Furst, S., 1999. Transmitters involved in antinociception in the spinal cord. Brain Res. Bull. 48, 129-141.

Ganong, A. H., Lanthorn, T. H., Cotman, C. W., 1983. Kynurenic acid inhibits synaptic and acidic amino acid-induced responses in the rat hippocampus and spinal cord. Brain Res. 273, 170-174.

Gauldie, S. D., McQueen, D. S., Pertwee, R., Chessell, I. P., 2001. Anandamide activates peripheral nociceptors in normal and arthritic rat knee joints. Br. J. Pharmacol. 132, 617-621.

Germolec, D. R., Frawley, R. P., Evans, E., 2010. Markers of inflamation. Methods Mol. Biol. 598, 53-73.

Gilbert, S. D., Clark, T. M., Flores, C. M., 2001. Antihyperalgesic activity of epibatidine in the formalin model of facial pain. Pain 89, 159-165.

Grazia Cascio, M., Minassi, A., Ligresti, A., Appendino, G., Burstein, S., Di Marzo, V., 2004. A structure-activity relationship study on N-arachidonoyl-amino acids as possible endogenous inhibitors of fatty acid amide hydrolase. Biochem. Biophys. Res. Commun. 314, 192-196.

Griffin, G., Fernando, S. R., Ross, R. A., Mckay, N. G., Ashford, M. L. J., Shire, D., Huffman, J. W., Yu, S., Lainton, J. A. H., Pertwee, R. G., 1997. Evidence for the presence of CB2-like cannabinoid receptors on peripheral nerve terminals. Eur. J. Pharmacol. 339, 53-61.

Grubb, B. D., 2004. Activation of sensory neurons in arthritic joint. Novartis Found. Symp. 260, 28-36.

Guindon, J., Desroches, J., Beaulieu, P., 2007a. The antinociceptive effects of intraplantar injections of 2-arachidonoyl glycerol are mediated by cannabinoid CB2 receptors. Br. J. Pharmacol. 150, 693-701.

Guindon, J., Hohmann, A. G., 2007b. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. Br. J. Pharmacol. 153, 319-334.

Guindon, J., LoVerme, J., De Lean, A., Piomelli, D., Beaulieu, P., 2006a. Synergistic antinociceptive effects of anandamide, an endocannabinoid, and nonsteroidal anti-inflammatory drugs in peripheral tissue: a role for endogenous fatty-acid ethanolamides? Eur. J. Pharmacol. 550, 68-77.

Guindon, J., De Lean, A., Beaulieu, P., 2006b. Local interactions between anandamide, an endocannabinoid, and ibuprofen, a nonsteroidal anti-inflammatory drug, in acute and inflammatory pain. Pain 121, 85-93.

Gupta, A., Bodin, L., Holmstrom, B., Berggren, L., 2001. A systematic review of the peripheral analgesic effects of intraarticular morphine. Anesth. Analg. 93, 761-770.

Haberberger, R. V., Bernardini, N., Kress, M., Hartmann, P., Lips, K. S., Kummer, W., 2004. Nicotinic acetylcholine receptor subtypes in nociceptive dorsal root ganglion neurons of the adult rat. Auton. Neurosci. 113, 32-42.

Hansen, H. S., Artmann, A., 2008. Endocannabinoids and nutrition. J. Neuroendocrinol. 20, 94-99.

Hao, S., Takahata, O., Iwasaki, H., 2000. Intrathecal endomorphin-1 produces antinociceptive activities modulated by alpha 2-adrenoceptors in the rat tail flick, tail pressure and formalin tests. Life Sci. 66, 195-200.

Hao, S. L., Takahata, O., Iwasaki, H., 1999. Isobolographic analysis of interaction between spinal endomorphin-1, a newly isolated endogenous opioid peptide, and lidocaine in the rat formalin test. Neurosci. Lett. 276, 177-180.

Harrington, L., Schneider, J. I., 2006. Atraumatic joint and limb pain in the elderly. Emerg. Med. Clin. North Am. 24, 389-412.

Hassan, A. H. S., Ableitner, A., Stein, C., Herz, A., 1993. Inflammation of the rat paw enhances axonal-transport of opioid receptors in the sciatic-nerve and increases their density in the inflamed tissue. Neuroscience 55, 185-195.

Hayes, C. S., Heinrich, S. D., Craver, R., Macewen, G. D., 1990. Subacute Osteomyelitis. Orthopedics 13, 363-366.

Hejazi, N., Zhou, C., Oz, M., Sun, H., Ye, J. H., Zhang, L., 2006. Delta9-tetrahydrocannabinol and endogenous cannabinoid anandamide directly potentiate the function of glycine receptors. Mol. Pharmacol. 69, 991-997.

Hepper, C. T., Halvorson, J. J., Duncan, S. T., Gregory, A. J. M., Dunn, W. R., Spindler, K. P., 2009. The efficacy and duration of intra-articular corticosteroid injection for knee osteoarthritis: a systematic review of level I studies. J. Am. Acad. Orthop. Surg. 17, 638-646.

Heyneman, C. A., Lawless-Liday, C., Wall, G. C., 2000. Oral versus topical NSAIDs in rheumatic diseases: a comparison. Drugs 60, 555-574.

Hilmas, C., Pereira, E. F., Alkondon, M., Rassoulpour, A., Schwarcz, R., Albuquerque, E. X., 2001. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. J. Neurosci. 12, 7463-7473.

Hirota, K., Lambert, D. G., 1996. Ketamine: its mechanism(s) of action and unusual clinical uses. Br. J. Anaesth. 77, 441-444.

Hocking, G., Cousins, M. J., 2003. Ketamine in chronic pain management: an evidence-based review. Anesth. Analg. 97, 1730-1739.

Hohmann, A. G., 2002. Spinal and peripheral mechanisms of cannabinoid antinociception: behavioral, neurophysiological and neuroanatomical perspectives. Chem. Phys. Lipids 121, 173-190.

Hohmann, A. G., 2007. Inhibitors of monoacylglycerol lipase as novel analgesics. Br. J. Pharmacol. 150, 673-675.

Hohmann, A. G., Briley, E. M., Herken, Herkenham, M., 1999. Preo- and postsynaptic distribution of cannabinoid and mu opioid receptors in rat spinal cord. Brain Res. 822, 17-25.

Hohmann, A. G., Suplita, R. L., 2006. Endocannabinoid mechanisms of pain modulation. AAPS J. 8, E693-E708.

Horvath, G., 2000. Endomorphin-1 and endomorphin-2. Pharmacology of the selective endogenous mu-opioid receptor agonists. Pharmacol. Ther. 88, 437-463.

Howlett, A. C., Breivogel, C. S., Childers, S. R., Deadwyler, S. A., Hampson, R. E., Porrino, L. J., 2004. Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology 47, 345-358.

Huang, S. M., Bisogno, T., Petros, T. J., Chang, S. Y., Zavitsanos, P. A., Zipkin, R. E., Sivakumar, R., Coop, A., Maeda, D. Y., De Petrocellis, L., Burstein, S., Di Marzo, V., Walker, J. M., 2001. Identification of a new class of molecules, the arachidonyl amino acids, and characterization of one member that inhibits pain. J. Biol. Chem. 276, 42639-42644.

Huang, S. M., Bisogno, T., Trevisani, M., Al-Hayani, A., De Petrocellis, L., Fezza, F., Tognetto, M., Petros, T. J., Krey, J. F., Chu, C. J., Miller, J. D., Davies, S. N., Geppetti, P., Walker, J. M., Di Marzo, V., 2002. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc. Natl. Acad. Sci. U S A 99, 8400-8405.

Ibrahim, M. M., Porreca, F., Lai, J., Albrecht, P. J., Rice, F. L., Khodorova, A., Davar, G., Makriyannis, A., Vanderah, T. W., Mata, H. P., Malan, T. P. Jr., 2005. CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. Proc. Natl. Acad. Sci. U S A 102, 3093-3098.

Jackson, D. L., Graff, C. B., Richardson, J. D., Hargreaves, K. M., 1995. Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats. Eur. J. Pharmacol. 284, 321-325.

Jensen, T. S., Yaksh, T. L., 1989. Comparison of the antinociceptive effect of morphine and glutamate at coincidental sites in the periaqueductal gray and medial medulla in rats. Brain Res. 476, 1-9.

Ji, G., Neugebauer, V., 2008. Pro- and anti-nociceptive effects of corticotropin-releasing factor (CRF) in central amygdala neurons are mediated through different receptors. J. Neurophysiol. 99, 1201-1212.

Joo, G., Horvath, G., Klimscha, W., Kekesi, G., Dobos, I., Szikszay, M., Benedek, G., 2000. The effects of ketamine and its enantiomers on the morphine- or dexmedetomidine-induced antinociception after intrathecal administration in rats. Anesthesiology 93, 231-241.

Jordan, B. A., Cvejic, S., Devi, L. A., 2000. Opioids and their complicated receptor complex. Neuropsychopharmacology 23, S5-18.

Kalso, E., Tramer, M. R., Carroll, D., McQuay, H. J., Moore, R. A., 1997. Pain relief from intra-articular morphine after knee surgery: A qualitative systematic review. Pain 71, 127-134.

Katz, N., Sun, S., Johnson, F., Stauffer, J., 2010. ALO-01 (morphine sulfate and naltrexone hydrochloride) extended-release capsules in the treatment of chronic pain of osteoarthritis of the hip or knee: pharmacokinetics, efficacy, and safety. J. Pain 11, 303-311.

Kekesi, G., Joo, G., Csullog, E., Dobos, I., Klimscha, W., Toth, K., Benedek, G., Horvath, G., 2002. The antinociceptive effect of intrathecal kynurenic acid and its interaction with endomorphin-1 in rats. Eur. J. Pharmacol. 445, 93-96.

Khalil, Z., Sanderson, K., Modig, M., Nyberg, F., 1999. Modulation of peripheral inflammation by locally administered endomorphin-1. Inflamm. Res. 48, 550-556.

Kidd, B. L., Photiou, A., Inglis, J. J., 2004. The role of inflammatory mediators on nociception and pain in arthritis. Novartis Found. Symp. 260, 122-133.

Kinkelin, I., Brocker, E. B., Koltzenburg, M., Carlton, S. M., 2000. Localization of ionotropic glutamate receptors in peripheral axons of human skin. Neurosci Lett 283, 149-152.

Kohli, P., Levy, B. D., 2009. Resolvins and protectins: mediating solutions to inflammation. Br. J. Pharmacol. 158, 960-971.

Kohno, M., Hasegawa, H., Inoue, A., Muraoka, M., Miyazaki, T., Oka, K., Yasukawa, M., 2006. Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. Biochem. Biophys. Res. Commun. 347, 827-832.

Kondo, S., Kondo, H., Nakane, S., Kodaka, T., Tokumura, A., Waku, K., Sugiura, T., 1998. 2-arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through Ca2+-dependent and -independent mechanisms. FEBS Lett. 429, 152-156.

Kozak, K. R., Rowlinson, S. W., Marnett, L. J., 2000. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. J. Biol. Chem. 275, 33744-33749.

Labuz, D., Berger, S., Mousa, S. A., Zollner, C., Rittner, H. L., Shaqura, M. A., Segovia-Silvestre, T., Przewlocka, B., Stein, C., Machelska, H., 2006. Peripheral antinociceptive effects of exogenous and immune cell-derived endomorphins in prolonged inflammatory pain. J. Neurosci. 26, 4350-4358.

Labuz, D., Mousa, S. A., Schafer, M., Stein, C., Machelska, H., 2007. Relative contribution of peripheral versus central opioid receptors to antinociception. Brain Res. 1160, 30-38.

Lam, F. F. Y., Luk, P. W. S., Ng, E. S. K., 2007. Pharmacological characterization of receptor types mediating the dilator action of anandamide on blood vessels of the rat knee joint. Life Sci. 80, 1495-1502.

Lambert, D. G., 2008. The nociceptin/orphanin FQ receptor: a target with broad therapeutic potential. Nat. Rev. Drug. Discov. 7, 694-710.

Lang, P. M., Burgstahler, R., Sippel, W., Irnich, D., Schlotter-Weigel, B., Grafe, P., 2003. Characterization of neuronal nicotinic acetylcholine receptors in the membrane of unmyelinated human C-fiber axons by in vitro studies. J. Neurophysiol. 90, 3295-3303.

Lawand, N. B., McNeaney, T., Westlund, K. N., 2000. Amino acid release into the knee joint: key role in nociception and inflammaton. Pain 86, 69-74.

Lawand, N. B., Willis, W. D., Westlund, K. N., 1997. Excitatory amino acid receptor involvement in peripheral nociceptive transmission in rats. Eur. J. Pharmacol. 324, 169-177.

Lawson, S. N., 1996. Peptides and cutaneous polymodal nociceptor neurones. Prog. Brain. Res. 113, 369-385.

Lever, I. J., Rice, A. S. C., 2007. Cannabinoids and pain. Handb. Exp. Pharmacol. 177, 265-306.

Li, Z. M., Proud, D., Zhang, C. F., Wiehler, S., McDougall, J. J., 2005. Chronic arthritis down-regulates peripheral mu-opioid receptor expression with concomitant loss of endomorphin 1 antinociception. Arthritis Rheum. 52, 3210-3219.

Likar, R., Schafer, M., Paulak, F., Sittl, R., Pipam, W., Schalk, H., Geissler, D., Bernatzky, G., 1997. Intraarticular morphine analgesia in chronic pain patients with osteoarthritis. Anesth. Anal. 84, 1313-1317.

Lo Verme, J., Fu, J., Astarita, G., La Rana, G., Russo, R., Calignano, A., Piomelli, D., 2005. The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. Mol. Pharmacol. 67, 15-19.

Lo Verme, J., Russo, R., La Rana, G., Fu, J., Farthing, J., Mattace-Raso, G., Meli, R., Hohmann, A., Calignano, A., Piomelli, D., 2006. Rapid broad-spectrum analgesia through activation of peroxisome proliferator-activated receptor-alpha. J. Pharmacol. Exp. Ther. 319, 1051-1061.

Loh, H. H., Tseng, L. F., Wei, E., Li, C. H., 1976. Beta-endorphin is a potent analgesic agent. Proc. Natl. Acad. Sci. U S A 73, 2895-2898.

Lozovaya, N., Yatsenko, N., Beketov, A., Tsintsadze, T., Burnashev, N., 2005. Glycine receptors in CNS neurons as a target for nonretrograde action of cannabinoids. J. Neurosci. 25, 7499-7506.

Mackie, K., 2008. Cannabinoid receptors: where they are and what they do. J. Neuroendocrinol. 20, 10-14.

Maekawa, K., Minami, M., Yabuuchi, K., Toya, T., Katao, Y., Hosoi, Y., Onogi, T., Satoh, M., 1994. In-situ hybridization study of mu-opioid and kappa-opioid receptor messenger-RNAs in the rat spinal-cord and dorsal-root ganglia. Neurosci. Lett. 168, 97-100.

Maingret, F., Patel, A. J., Lazdunski, M., Honore, E., 2001. The endocannabinoid anandamide is a direct and selective blocker of the background K(+) channel TASK-1. EMBO J. 20, 47-54.

Malan, T. P. Jr., Ibrahim, M. M., Vanderah, T. W., Makriyannis, A., Porreca, F., 2002. Inhibition of pain responses by activation of CB_2 cannabinoid receptors. Chem. Phys. Lipids 121, 191-200.

Maldini, B., Miskulin, M., Antolic, S., Goranovic, T., Sakic-Zdravcevic, K., Gudelj, G., 2010. Local or spinal anesthesia in acute knee surgery. Coll. Antropol. 34, 247-254.

Maldonado, R., Valverde, O., 2003. Participation of the opioid system in cannabinoid-induced antinociception and emotional-like responses. Eur. Neuropsychopharmacol. 13, 401-410.

Marnett, J. L., Kalgutkar, A. S., 1999. Cyclooxigenase 2 inhibitors: discovery, selectivity and the future. Trends Pharmacol. Sci. 20, 465-469.

Martin, B. R., Mechoulam, R., Razdan, R. K., 1999. Discovery and characterization of endogenous cannabinoids. Life Sci. 65, 573-595.

McDougall, J. J., Baker, C. L., Hermann, P. M., 2003. Attenuation of knee joint inflammation by peripherally administered endomorphin-1. J. Mol. Neurosci. 22, 125-137.

McDougall, J. J., Larson, S. E. M., 2006. Nociceptin/orphanin FQ evokes knee joint pain in rats via a mast cell independent mechanism. Neurosci. Lett. 398, 135-138.

McDougall, J. J., Barin, A. K., McDougall, C. M., 2004. Loss of vasomotor responsiveness to the mu-opioid receptor ligand endomorphin-1 in adjuvant monoarthritic rat knee joints. Am. J. Physiol. 286, R634-R641.

Mechoulam,R., Ben Shabat,S., Hanus,L., Fride,E., Bayewitch,M., Vogel,Z., 1996. Endogenous cannabinoid ligands. In: AIDS, drugs of abuse, and the neuroimmune axis. Plenum Press, New York, pp. 95-101.

Mechoulam, R., Ben Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., Gopher, A., Almog, S., Martin, B. R., Compton, D. R., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. 50, 83-90.

Meller, S. T., 1996. Ketamine: Relief from chronic pain through actions at the NMDA receptor? Pain 68, 435-436.

Menetrey, D., Basbaum, A. I., 1987. The distribution of substance P-, enkephalin- and dynorphin- immunoreactive neurons in the medulla of the rat and their contribution to bulbospinal pathways. Neuroscience 23, 173-187.

Millan, M. J., 1999. The induction of pain: an integrative review. Prog. Neurobiol. 57, 1-164.

Millan, M. J., 2002. Descending control of pain. Prog. Neurobiol. 66, 355-474.

Minami, M., Maekawa, K., Yabuuchi, K., Satoh, M., 1995. Double in situ hybridization study on coexistence of mu-opioid, delta-opioid and kappa-opioid receptor messenger-RNAs with preprotachykinin-A messenger-RNA in the rat dorsal-root ganglia. Mol. Brain. Res. 30, 203-210.

Moreland, L. W., Curtis, J. R., 2009. Systemic Nonarticular Manifestations of Rheumatoid Arthritis: Focus on Inflammatory Mechanisms. Semin. Arthritis Rheum. 39, 132-143.

Moroni, F., Russi, P., Lombardi, G., Beni, M., Carla, V., 1988. Presence of kynurenic acid in the mammalian brain. J. Neurochem. 51, 177-180.

Mousa, S. A., Machelska, H., Schäfer, M., Stein, C., 2002. Immunhistochemical localization of endomorphin-1 and endomorphin-2 in immune cells and spinal cord in a model of inflammatory pain. J. Neuroimmunol. 126, 5-15.

Nackley, A. G., Suplita II, R. L., Hohmann, A. G., 2003. A peripheral cannabinoid mechanism suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. Neuroscience 117, 659-670.

Nemeth, H., Toldi, J., Vecsei, L., 2005. Role of kynurenines in the central and peripherial nervous systems. Curr. Neurovasc. Res. 2, 249-260.

Neugebauer, V., Li, W., Bird, G. C., Han, J. S., 2004. The amygdala and persistent pain. Neuroscientist 10, 221-234.

Nishizawa, M., Yaksh, T. L., Weber, E., 1998. Effects of intrathecal NMDA and non-NMDA antagonists on acute thermal nociception and their interaction with morphine. Anesthesiology 89, 715-722.

Obara, I., Przewlocki, R., Przewlocka, B., 2004. Local peripheral effects of mu-opioid receptor agonists in neuropathic pain in rats. Neurosci. Lett. 360, 85-89.

Obara, I., Przewlocki, R., Przewlocka, B., 2005. Spinal and local peripheral antiallodynic activity of Ro64-6198 in neuropathic pain in the rat. Pain 116, 17-25.

Oh, D. Y., Yoon, J. M., Moon, M. J., Hwang, J.-I., Choe, H., Lee, J. Y., Kim, J. I., Kim, S., Rhim, H., O'Dell, D. K., Walker, J. M., Na, H. S., Lee, M. G., Kwon, H. B., Kim, K., Seong, J. Y., 2008. Indentification of farnesyl pyrophosphate and N-arachidonylglycine as endogenous ligands for GPR92. J. Biol. Chem. 283, 21054-21064.

Ohshiro, H., Tonai-Kachi, H., Ichikawa, K., 2008. GPR35 is a functional receptor in rat dorsal root ganglion neurons. Biochem. Biophys. Res. Commun. 365, 344-348.

Okamoto, Y., Wang, J., Morishita, J., Ueda, N., 2007. Biosynthetic pathways of the endocannabinoid anandamide. Chem. Biodivers. 4, 1842-1857.

Oztuna, V., Eskandari, M., Bugdayci, R., Kuyurtar, F., 2007. Intra-articular injection of tenoxicam in osteoarthritic knee joints with effusion. Orthopedics 30, 1039-1042.

Palkovits, M., 2000. Stress-induced expression of co-localized neuropeptides in hypothalamic and amygdaloid neurons. Eur. J. Pharmacol. 405, 161-166.

Pan, H. L., Wu, Z. Z., Zhou, H. Y., Chen, S. R., Zhang, H. M., Li, D. P., 2008. Modulation of pain transmission by G-protein-coupled receptors. Pharmacol. Ther. 117, 141-161.

Papathanassiou, N. P., 1994. Intraarticular Use of Tenoxicam in Degenerative Osteoarthritis of the Knee-Joint. J. Int. Med. Res. 22, 332-337.

Parada-Turska, J., Rzeski, W., Zgrajka, W., Majdan, M., Kandefer-Szerszen, M., Turski, W., 2006. Kynurenic acid, an endogenous constituent of rheumatoid arthritis synovial fluid, inhibits proliferation of synoviocytes in vitro. Rheumatol. Int. 26, 422-426.

Pawlak, D., Takada, Y., Urano, T., Takada, A., 2000. Serotonergic and kynurenic pathways in rats exposed to foot shock. Brain Res. Bull. 52, 197-205.

Pertovaara, A., Wei, H., Hämäläinen, M. M., 1996. Lidocaine in the rostroventromedial medulla and the periaqueductal gray attenuates allodynia in neuropathic rats. Neurosci. Lett. 218, 127-130.

Pertwee, R. G., 2001. Cannabinoid receptors and pain. Prog. Neurobiol. 63, 569-611.

Pertwee, R. G., 2007. GPR55: a new member of the cannabinoid receptor clan? Br. J. Pharmacol. 152, 984-986.

Peter-Szabo, M., Kekesi, G., Nagy, E., Sziver, E., Benedek, G., Horvath, G., 2007. Quantitative characterization of a repeated acute joint inflammation model in rats. Clin. Exp. Pharmacol. Physiol. 34, 520-526.

Pilcher, W. H., Joseph, S. A., Mcdonald, J. V., 1988. Immunocytochemical localization of pro-opiomelanocortin neurons in human-brain areas subserving stimulation analgesia. J. Neurosurg. 68, 621-629.

Placzek, E. A., Okamoto, Y., Ueda, N., Barker, E. L., 2008. Mechanisms for recycling and biosynthesis of endogenous cannabinoids anandamide and 2-arachidonylglycerol. J. Neurochem. 107, 987-1000.

Porter, A. C., Sauer, J. M., Knierman, M. D., Becker, G. W., Berna, M. J., Bao, J. Q., Nomikos, G. G., Carter, P., Bymaster, F. P., Leese, A. B., Felder, C. C., 2002. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. J. Pharmacol. Exp. Ther. 301, 1020-1024.

Prusakiewicz, J. J., Kingsley, P. J., Kozak, K. R., Marnett, L. J., 2002. Selective oxygenation of N-arachidonylglycine by cyclooxygenase-2. Biochem. Biophys. Res. Commun. 296, 612-617.

Rainville, P., Duncan, G. H., Price, D. D., Carrier, B., Bushnell, M. C., 1997. Pain affect encoded in human anterior cingulate but not somatosensory cortex. Science 277, 968-971.

Rashid, H., Furue, H., Yosihimura, M., Ueda, H., 2006. Tonic inhibitory role of alpha4beta2 subtype of nicotinic acetylcholine receptors on nociceptive transmission in the spinal cord in mice. Pain 125, 125-135.

Reinscheid, R. K., Nothacker, H.-P., Bourson, A., Ardati, A., Henningsen, R. A., Bunzow, J. R., Grandy, D. K., Langen, H., Monsma, F. J. Jr., Civelli, O., 1995. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. Science 270, 792-794.

Reinscheid, R. K., Nothacker, H.-P., Civelli, O., 2000. The orphanin FQ/nociceptin gene: structure, tissue distribution of expression and functional implications obtained from knockout mice. Peptides 21, 901-906.

Rice, A. S., Fraquhar-Smith, W. P., Nagy, I., 2002. Endocannabinoids and pain: spinal and peripheral analgesia in inflammation and neuropathy. Prostaglandins Leukot. Essent. Fatty Acids 66, 243-256.

Richardson, D., Pearson, R. G., Kurian, N., Latif, M. L., Garle, M. J., Barrett, D. A., Kendall, D. A., Scammell, B. E., Reeve, A. J., Chapman, V., 2008. Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. Arthritis Res. Ther. 10, R43.

Richardson, J. D., Aanonsen, L., Hargreaves, K. M., 1998a. Antihyperalgesic effects of spinal cannabinoids. Eur. J. Pharmacol. 345, 145-153.

Richardson, J. D., Kilo, S., Hargreaves, K. M., 1998b. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors. Pain 75, 111-119.

Rimmerman, N., Bradshaw, H. B., Hughes, H. V., Chen, J. S., Hu, S. S., McHugh, D., Vefring, E., Jahnsen, J. A., Thompson, E. L., Masuda, K., Cravatt, B. F., Burstein, S.,

Vasko, M. R., Prieto, A. L., O'Dell, D. K., Walker, J. M., 2008. N-Palmitoyl glycine, a novel endogenous lipid that acts as a modulator of calcium influx and nitric oxide production in sensory neurons. Mol. Pharmacol. 74, 213-224.

Rittner, H. L., Brack, A., Stein, C., 2008. Pain and the immune system. Br. J. Anaesth. 101, 40-44.

Romero-Sandoval, A., Nutile-McMenemy, N., DeLeo, J. A., 2008. Spinal microglial and perivascular cell cannabinoid receptor type 2 activation reduces behavioral hypersensitivity without tolerance after peripheral nerve injury. Anesthesiology 108, 722-734.

Ronai, A. Z., Szemenyei, E., Kató, E., Kocsis, L., Orosz, Gy., Al-Khrasani, M., Tóth, G., 2006. Endomorphin synthesis in rat brain from intracerebroventricularly injected $^{[3H]}$Tyr-Pro: a possible biosynthetic route for endomorphins. Regul. Pept. 134, 54-60.

Rosseland, L. A., Stubhaug, A., Skoglund, A., Breivik, H., 1999. Intra-articular morphine for pain relief after knee arthroscopy. Acta Anaesthesiol. Scand. 43, 252-257.

Rossier, J., French, E. D., Rivier, C., Ling, N., Guillemin, R., Bloom, F., 1977. Foot-shock induced stress increases beta-endorphin levels in blood but not brain. Nature 270, 618-620.

Ryberg, E., Larsson, N., Sjogren, S., Hjorth, S., Hermansson, N. O., Leonova, J., Elebring, T., Nilsson, K., Drmota, T., Geasley, P. J., 2007. The orphan receptor GPR55 is a novel cannabinoid receptor. Br. J. Pharmacol. 152, 1092-1101.

Saario, S. M., Laitinen, J. T., 2007. Therapeutic potential of endocannabinoid-hydrolysing enzyme inhibitors. Basic Clin. Pharmacol. Toxicol. 101, 287-293.

Sakurada, S., Hayashi, T., Yuhki, M., Fujimura, T., Murayama, K., Yonezawa, A., Sakurada, C., Takeshita, M., Zadina, J. E., Kastin, A. J., Sakurada, T., 2000. Differential antagonism of endomorphin-1 and endomorphin-2 spinal nociception by naloxonazine and 3-methoxynaltraxone. Brain Res. 881, 1-8.

Sandkuhler, J., 1996. The organization and function of endogenous antinociceptive system. Prog. Neurobiol. 50, 49-81.

Sawynok, J., Reid, A., 2002. Modulation of formalin-induced behaviors and edema by local and systemic administration of dextromethorphan, memantine and ketamine. Eur. J. Pharmacol. 450, 153-162.

Schaible, H. G., Richter, F., Ebersberger, A., Boettger, M. K., Vanegas, H., Natura, G., Vazquez, E., von Banchet, G. S., 2009. Joint pain. Exp. Brain Res. 196, 153-162.

Schuelert, N., McDougall, J. J., 2008. Cannabinoid-mediated antinociception is enhanced in rat osteoarthritic knees. Arthritis Rheum. 58, 145-153.

Schwarcz, R., Pellicciari, R., 2002. Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. J. Pharmacol. Exp. Ther. 303, 1-10.

Serhan, C. N., 2006. Novel chemical mediators in the resolution of inflammation:resolvins and protectins. Anesthesiol. Clin. 24, 341-364.

Shane, R., Wilk, S., Bodnar, R. J., 1999. Modulation of endomorphin-2-induced analgesia by dipeptidyl peptidase IV. Brain Res. 815, 278-286.

Sheskin, T., Hanus, L., Slager, J., Vogel, Z., Mechoulam, R., 1997. Structural requirements for binding of anandamide-type compounds to the brain cannabinoid receptor. J. Med. Chem. 40, 659-667.

Sim, L. J., Liu, Q., Childers, S. R., Selley, D. E., 1998. Endomorphin-stimulated [35S]GTPgammaS binding in rat brain: evidence for partial agonist activity at mu-opioid receptors. J. Neurochem. 70, 1567-1576.

Simpson, D. A., Headley, P. M., Lumb, B. M., 2008. Selective inhibition from the anterior hypothalamus of C- versus A-fibre mediated spinal nociception. Pain 136, 305-312.

Sinis, N., Birbaumer, N., Gustin, S., Schwarz, A., Bredanger, S., Becker, S. T., Unertl, K., Schaller, H. E., Haerle, M., 2007. Memantine treatment of complex regional pain syndrome - A preliminary report of six cases. Clin. J. Pain 23, 237-243.

Sipe, J. C., Arbour, N., Gerber, A., Beutler, E., 2005. Reduced endocannabinoid immune modulation by a common cannabinoid 2 (CB2) receptor gene polymorphism: possible risk for autoimmune disorders. J. Leukoc. Biol. 78, 231-238.

Sloan, P., Babul, N., 2006. Extended-release opioids for the management of chronic non-malignant pain. Expert Opin. Drug Deliv. 3, 489-497.

Smith, D. J., Perrotti, J. M., Crisp, T., Cabral, M. E. Y., Long, J. T., Scalzitti, J. M., 1988. The mu opiate receptor is responsible for descending pain inhibition originating in the periaqueductal gray region of the rat brain. Eur. J. Pharmacol. 156, 47-54.

Snider, W. D., McMahon, S. B., 1998. Tackling pain at the source: new ideas about nociceptors. Neuron 20, 629-632.

Starowicz, K., Nigam, S., Di Marzo, V., 2007. Biochemistry and pharmacology of endovanilloids. Pharmacol. Ther. 114, 13-33.

Stein, A., Yassouridis, A., Szopko, C., Helmke, K., Stein, C., 1999. Intraarticular morphine versus dexamethasone in chronic arthritis. Pain 83, 525-532.

Stein, C., 1993. Peripheral mechanisms of opioid analgesia. Anesth. Analg. 76, 182-191.

Stein, C., Gramsch, C., Hassan, A. H. S., Przewlocki, R., Parsons, C. G., Peter, K., Herz, A., 1990a. Local opioid receptors mediating antinociception in inflammation: endogenous ligands. Prog. Clin. Biol. Res. 328, 425-427.

Stein, C., Gramsch, C., Herz, A., 1990b. Intrinsic mechanisms of antinociception in inflammation: local opioid receptors and beta-endorphin. J. Neurosci. 10, 1292-1298.

Stein, C., Hassan, A. H. S., Lehrberger, K., Giefing, J., Yassouridis, A., 1993. Local analgesic effects of endogenous opioid peptides. Lancet 342, 321-324.

Stein, C., Hassan, A. H. S., Przewlocki, R., Gramsch, C., Peter, K., Herz, A., 1990c. Opioids from immunocytes interact with receptors on sensory nerves to inhibit nociception in inflammation. Proc. Natl. Acad. Sci. U S A 87, 5935-5939.

Stein, C., Millan, M. J., Shippenberg, T. S., Peter, K., Herz, A., 1989. Peripheral opioid receptors mediating antinociception in inflammation. Evidence for involvement of mu, delta and kappa receptors. J. Pharmacol. Exp. Ther. 248, 1269-1275.

Stone, T. W., 1993. Neuropharmacology of quinolinic and kynurenic acids. Pharmacol. Rev. 45, 309-379.

Stone, T. W., 2000. Development and therapeutic potential of kynurenic acid and kynurenine derivatives for neuroprotection. Trends Pharmacol. Sci. 21, 149-154.

Straub, R. H., Wolff, C., Fassold, A., Hofbauer, R., Chover-Gonzalez, A., Richards, L. J., Jessop, D. S., 2008. Antiinflammatory role of endomorphins in osteoarthritis, rheumatoid arthritis, and adjuvant-induced polyarthritis. Arthritis Rheum. 58, 456-466.

Sugiura, T., Kishimoto, S., Oka, S., Gokoh, M., 2006. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. Prog. Lipid Res. 45, 405-446.

Sun, Y. G., Lundeberg, T., Yu, L. C., 2003. Involvement of endogenous beta-endorphin in antinociception in the arcuate nucleus of hypothalamus in rats with inflammation. Pain 104, 55-63.

Szabo, B., 2008. Pharmacology of cannabinoid receptors. Biotrend Rev. 2, 1-13.

Tallarida, R. J., Porreca, F., Cowan, A., 1989. Statistical analysis of drug-drug and site-site interactions with isobolograms. Life Sci. 45, 947-961.

Tallarida, R. J., Stone, Jr. D. J., Raffa, R. B., 1997. Efficient designs for studying synergistic drug combinations. Life Sci. 61, 417-425.

Tasto, J. P., Elias, D. W., 2007. Adhesive capsulitis. Sports Med. Arthrosc. 15, 216-221.

Theis, K. A., Murphy, L., Hootman, J. M., Helmick, C. G., Yelin, E., 2007. Prevalence and correlates of arthritis-attributable work limitation in the US population among persons ages 18-64: 2002 National Health Interview Survey Data. Arthritis Rheum. 57, 355-363.

Treede, R.-D., Meyer, R. A., Raja, S. N., Campbell, J. N., 1992. Peripheral and central mechanisms of cutaneous hyperalgesia. Prog. Neurobiol. 38, 397-421.

Trujillo, K. A., Akil, H., 1991. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251, 85-87.

Turski, W. A., Schwarcz, R., 1988. On the disposition of intrahippocampally injected kynurenic acid in the rat. Exp. Brain Res. 71, 563-567.

Tverskoy, M., Oren, M., Vaskovich, M., Dashkovsky, I., Kissin, I., 1996. Ketamine enhances local anesthetic and analgesic effects of bupivacain by peripheral mechanism: a study in postoperative patients. Neurosci. Lett. 215, 5-8.

Unlu, Z., Ay, K., Tuzun, C., 2006. Comparison of intra-articular tenoxicam and oral tenoxicam for pain and physical functioning in osteoarthritis of the knee. Clin. Rheumatol. 25, 54-61.

Urbanska, E. M., Chmielewski, M., Kocki, T., Turski, W. A., 2000. Formation of endogenous glutamatergic receptors antagonist kynurenic acid - differences between cortical and spinal cord slices. Brain Res. 878, 210-212.

Vaccarino, A. L., Olson, G. A., Olson, R. D., Kastin, A. J., 2000. Endogenous opiates: 1998. Peptides 20, 1527-1574.

van Jonbergen, H. P. W., Poolman, R. W., van Kampen, A., 2010. Isolated patellofemoral osteoarthritis. Acta Orthop. 81, 199-205.

van Zonneveld, A. J., de Boer, H. C., van der Veer, E. P., Rabelink, T. J., 2010. Inflammation, vascular injury and repair in rheumatoid arthritis. Ann. Rheum. Dis. 69, 57-60.

Vane, J. R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drog. Nat. New Biol. 231, 232-235.

Vecsei, L., Beal, M. F., 1991. Comparative behavioral and pharmacological studies with centrally administered kynurenine and kynurenic acid in rats. Eur. J. Pharmacol. 196, 239-246.

Walker, J. M., Hohmann, A. G., 2005. Cannabinoid mechanisms of pain suppression. Handb. Exp. Pharmacol. 168, 509-554.

Walker, J. M., Hohmann, A. G., Martin, W. J., Strangman, N. M., Huang, S. M., Tsou, K., 1999. The neurobiology of cannabinoid analgesia. Life Sci. 65, 665-673.

Walker, J. M., Krey, J. F., Chen, J. S., Vefring, E., Jahnsen, J. A., Bradshaw, H., Huang, S. M., 2005. Targeted lipidomics: fatty acid amides and pain modulation. Prostaglandins Other Lipid Mediat. 77, 35-45.

Walker, J. M., Krey, J. F., Chu, C. J., Huang, S. M., 2002. Endocannabinoids and related fatty acid derivates in pain modulation. Chem. Phys. Lipids 121, 159-172.

Wall, P.D., Melzack,R., 1999. Textbook of pain, fourth ed. Churchill Livingstone, Edinburgh.

Walter, L., Stella, N., 2004. Cannabinoids and neuroinflammation. Br. J. Pharmacol. 141, 775-785.

Wang, J. H., Simonavicius, N., Wu, X. S., Swaminath, G., Reagan, J., Tian, H., Ling, L., 2006. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. J. Biol. Chem. 281, 22021-22028.

Wang, X., Miyares, R. L., Ahern, G. P., 2005a. Oleoylethanolamide excites vagal sensory neurones, induces visceral pain and reduces short-term food intake in mice via capsaicin receptor TRPV1. J. Physiol. 564, 541-547.

Wang, Y., Su, D. M., Wang, R. H., Liu, Y., Wang, H., 2005b. Antinociceptive effects of choline against acute and inflammatory pain. Neuroscience 132, 49-56.

Wang, Y.-Q., Zhu, C.-B., Wu, G.-C., Cao, X.-D., Wang, Y., Cui, D. F., 1999. Effects of orphanin FQ on endomorphin-1 induced analgesia. Brain Res. 835, 241-246.

Warncke, T., Jorum, E., Stubhaug, A., 1997. Local treatment with the N-methyl-D-aspartate receptor antagonist ketamine, inhibit development of secondary hyperalgesia in man by a peripheral action. Neurosci. Lett. 227, 1-4.

Watterson, G., Howard, R., Goldman, A., 2004. Peripheral opioids in inflammatory pain. Arch. Dis. Child. 89, 679-681.

Wei, H., Panula, P., Pertovaara, A., 1998. A differential modulation of allodynia, hyperalgesia and nociception by neuropeptide FF in the periaqueductal gray of neuropathic rats: interactions with morphine and naloxone. Neuroscience 86, 311-319.

Welch, S. P., Eads, M., 1999. Synergistic interactions of endogenous opioids and cannabinoid systems. Brain Res. 848, 183-190.

Welch, S. P., Stevens, D. L., 1992. Antinociceptive activity of intrathecally administered cannabinoids alone, and in combination with morphine, in mice. J. Pharmacol. Exp. Ther. 262, 10-18.

Wiesenfeld-Hallin, Z., 1998. Combined opioid-NMDA antagonist therapies. Drugs 55, 1-4.

Wiles, A. L., Pearlman, R. J., Rosvall, M., Aubrey, K. R., Vandenberg, R. J., 2006. N-Arachidonyl-glycine inhibits the glycine transporter, GLYT2a. J. Neurochem. 99, 781-786.

Williams, N. H., Amoakwa, E., Burton, K., Hendry, M., Lewis, R., Jones, J., Bennett, P., Neal, R. D., Andrew, G., Wilkinson, C., 2010. The Hip and Knee Book: developing an active management booklet for hip and knee osteoarthritis. Br. J. of Gen. Pract. 60, 113-120.

Wood, P. B., 2009. Enhanced pain perception in rheumatoid arthritis: Novel considerations. Curr. Pain and Headache Rep.13, 434-439.

Yaksh, T. L., Dirig, D. M., Malmberg, A. B., 1998. Mechanism of action of nonsteroidal anti-inflammatory drugs. Cancer Invest. 16, 509-527.

Yamamoto, T., Shimoyama, N., Mizuguchi, T., 1993. The effects of morphine, MK-801, an NMDA antagonist, and CP-96,345, an NK-1 antagonist, on the hyperesthesia evoked by carageenan injection in the rat paw. Anesthesiology 78, 124-133.

Yang, L. C., Chen, L. M., Wang, C. J., Buerkle, H., 1998. Postoperative analgesia by intra-articular neostigmine in patients undergoing knee arthroscopy. Anesthesiology 88, 334-339.

Yang, Z., Aubrey, K. R., Alroy, I., Harvey, R. J., Vandenberg, R. J., Lynch, J. W., 2009. Subunit-specific modulation of glycine receptors by cannabinoids and N-arachidonyl-glycine. Biochem. Pharmacol. 76, 1014-1023.

Yesilyurt, O., Dogrul, A., 2004. Lack of cross-tolerance to the antinociceptive effects of systemic and topical cannabinoids in morphine-tolerant mice. Neurosci. Lett. 371, 122-127.

Yesilyurt, O., Dogrul, A., Gul, H., Seyrek, M., Kusmez, O., Ozkan, Y., Yildiz, O., 2003. Topical cannabinoid enhances topical morphine antinociception. Pain 105, 303-308.

Zadina, J. E., Hackler, L., Ge, L. J., Kastin, A. J., 1997. A potent and selective endogenous agonist for the mu-opiate receptor. Nature 386, 499-502.

Zhang, J., Hoffert, C., Vu, H. K., Groblewski, T., Ahmad, S., O'Donnell, D., 2003. Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. Eur. J. Neurosci. 17, 2750-2754.

Zhang, J., Chen, C., 2008. Endocannabinoid 2-arachidonoylglycerol protects neurons by limiting COX-2 elevation. J. Biol. Chem. 283, 22601-22611.

Zhao, M., Wang, J. Y., Jia, H., Tang, J. S., 2007. Roles of different subtypes of opioid receptors in mediating the ventrolateral orbital cortex opioid-induced inhibition of mirror-neuropathic pain in the rat. Neuroscience 144, 1486-1494.

Zhou, S., Komak, S., Du, J., Carlton, S. M., 2001. Metabotropic glutamate 1alpha receptors on peripheral primary afferent fibers: their role in nociception. Brain Res. 913, 18-26.

Zhou, S. T., Bonasera, L., Carlton, S. M., 1996. Peripheral administration of NMDA, AMPA or KA results in pain behaviors in rats. NeuroReport 7, 895-900.

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10. Publications

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

Mecs L., Tuboly G., Toth K., Nagy E., Nyari T., Benedek G., Horvath G. Peripheral antinociceptive effect of 2-arachidonoyl-glycerol and its interaction with endomorphin-1 in arthritic rat ankle joints. *Clin. Exp. Pharmacol. Physiol*. 37: 544-550 2010. Impact factor: 1.936

Abstracts related to the Thesis

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

Horvath G, Mecs L, Tuboly G., Benedek G. Interaction of topically applied endomorhin-1 with kinurenic acid. *European Opioid Conference*. Ferrara, Italy, April 9-11, 2008. (Abstract)

Mecs L, Benedek G, Horvath G. Endogén lingandok alkalmazása izületi fájdalom csökkentésére. *Magyar Ortopéd Társaság 51. kongresszusa*. Székesfehérvár június 19-21. 2008. *Magyar Traumatológia Ortopédia Kézsebészet Plasztkai Sebészet*. 51:40 . (Abstract)

Horvath G, Mecs L, Tuboly G, Benedek G. Antinociceptive interactions of topically applied endogenous ligands. *6th FENS Forum of European Neuroscience Geneva,* Switzerland, July 12-16, 2008. (Abstract)

Mecs L. Benedek G., Horvath G. Antinociceptive interactions of topically applied endogenous ligands. *MÉT LXXII Vándorgyűlése*. Debrecen, június 4-6, 2008. *Acta Physiol. Hung*. 96:103-104. 2009 (Abstract) Impact factor: 0.750

Mecs L., Tuboly G., Benedek G., Horvath G. Interaction of topically applied endogenous ligands (endomorphin-1 and 2-arachidonoyl glycerol) in joint pain model. *MITT XII. Congress*, Budapest, Hungary, January 22-24, 2009, Clin. Neurosci/Ideggyógy. Szle. (Abstract)

Mecs L, Tuboly G., Toth K., Horvath G. The peripheral antinociceptive effect of the endocannabinoid 2-AG and endomorphin-1. *Six SICOT/SIROT Annual International Conference*, Pattaya, Thailand, October 29 - November 1, 2009. (Abstract)

Mecs L., Toth K., Wellinger K., Tuboly G., Kekesi G., Horvath G. Peripheral effects of endogenous ligands in the rat inflamed joint model. *2009 World Congress on Osteoarthritis*, Montreal, Canada, September 10-13. 2009. Osteoarthritis and Cartilage 17.S1:S29.2009 (Abstract) Impact factor: 3.888

Mecs L., Tuboly G., Toth K., Benedek G., Horvath G. Az izületi fájdalom csökkentése lokálisan alkalmazott endomorphinnal és endocannabinoiddal. *Magyar Ortopéd Társaság 52. Kongresszusa.* Szolnok 2009, június 25-27.

11. ANNEXES