

The Endocannabinoid System as a Potential Therapeutic Target for Pain Modulation

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Although cannabis has been used for pain management for millennia, very few approved cannabinoids are indicated for the treatment of pain and other medical symptoms. Cannabinoid therapy re-gained attention only after the discovery of endocannabinoids and fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), the enzymes playing a role in endocannabinoid metabolism. Nowadays, research has focused on the inhibition of these degradative enzymes and the elevation of endocannabinoid tonus locally; special emphasis is given

on multi-target analgesia compounds, where one of the targets is the endocannabinoid degrading enzyme. In this review, I provide an overview of the current understanding about the processes accounting for the biosynthesis, transport and metabolism of endocannabinoids, and pharmacological approaches and potential therapeutic applications in this area, regarding the use of drugs elevating endocannabinoid levels in pain conditions. (*Balkan Med J* 2014;31:115-20).

Key Words: 2-AG, anandamide, endocannabinoids, FAAH, MAGL, pain

Cannabinoids are a group of chemical compounds that produce their effects via activating cannabinoid receptors; they include the phytocannabinoids (herbal cannabinoids/natural cannabinoids found in the cannabis plant), synthetic cannabinoids, and endogenous cannabinoids (endocannabinoids) (1, 2). Cannabis, also known as marijuana, has been used as both a recreational and medicinal drug for centuries. Pain management is the most important among the medicinal purposes, and has been drawing intense attention following the discovery of cannabinoid receptors and their endogenous ligands, endocannabinoids (3-5). Δ^9 -tetrahydrocannabinol (THC) and cannabidiol, the critical components of the cannabis plant, and the synthetic cannabinoids and their analogues have been shown to exert strong analgesic action both in preclinical and clinical studies (6-9). In this review, after a brief introduction to cannabinoid receptors, phytocannabinoids and synthetic cannabinoids, I provide an overview of what is currently known about the synthesis, release, degradation and biological actions of endocannabinoids, regarding their role in pain modulation, and describe the recent evidence of the promising results of augmentation of endogenous cannabinoid tonus for the treatment of pain.

CANNABINOID RECEPTORS AND THE SITE OF ACTION OF CANNABINOID

To date, two subtypes of cannabinoid receptors, termed cannabinoid-1 (CB_1) and cannabinoid-2 (CB_2) receptors, have been cloned (10, 11). CB_1 receptors are most abundantly expressed in the central nervous system (CNS), most densely in motor and limbic regions, and in areas that are involved in pain transmission and modulation, such as periaqueductal grey (PAG), rostral ventromedial medulla (RVM), spinal cord dorsal horn, and in the periphery. CB_1 receptors are generally located pre-synaptically on axons and terminals of neurons and mediate the inhibition of neurotransmitter release by the inhibition of adenylate cyclase, blockade of voltage-dependent calcium channels, and/or by the activation of potassium channels and mitogen-activated protein kinase. CB_2 receptors, on the other hand, are found mainly, but not exclusively, outside the CNS, predominantly in peripheral tissues with immune functions, and most densely in the spleen. Similar to CB_1 receptors, CB_2 receptors are also G-protein coupled, inhibits adenylate cyclase and produce cellular inhibition, but neither blockade of calcium channels



nor activation of potassium channels mediates this inhibitory effect (Figure 1) (1, 2, 12-14).

Phytocannabinoids, synthetic cannabinoids and endocannabinoids are thought to produce their anti-nociceptive action primarily through CB₁ receptors, located at the supraspinal, spinal and peripheral levels (15, 16). Activation of descending inhibition by presynaptic inhibition of GABAergic and glutamatergic transmission in the PAG and modulation of on- and off-cells in the RVM seems to play pivotal roles in supraspinal analgesia (15, 17, 18). CB₁ receptors found in presynaptic afferent terminals and on the terminals of intrinsic neurons and efferent supraspinal neurons are likely to mediate anti-nociception at the spinal level (15, 17). Topical cannabinoid anti-nociception and its synergy with spinal sites have also been suggested (19). CB₂ receptors, although not as extensively studied as the CB₁ receptors, are also proposed to play a role in the anti-nociceptive effects of cannabinoids (20).

PHYTOCANNABINOID AND SYNTHETIC CANNABINOID

Almost 80 of the chemical compounds in the cannabis plant, named phytocannabinoids, have the structure of a cannabinoid. Of these, THC is the best characterised and the primary psychoactive component of the plant. THC has an impact on many pathophysiological processes, including anti-nociception, through the activation of CB₁ and CB₂ receptors. However, its clinical utility is limited due to its unwanted CNS effects, which are mediated via brain CB₁ receptors (5, 14). Cannabidiol, another important phytocannabinoid gaining attention recently, has a very low affinity at CB₁ and CB₂ receptors. Unlike THC, it does not cause any psychoactivity, but exerts many positive pharmacological effects, including anti-anxiety, anti-epileptic, anti-bacterial, anti-inflammatory, anti-cancer and anti-diabetic properties (14, 21, 22). In addition to its wide therapeutic spectrum, cannabidiol is proposed to reverse some of the central side effects of THC (21-23). Nabiximols (Sativex®), a herbal cannabis extract containing THC and cannabidiol at a 1:1 ratio in an oromucosal spray, has been approved for the treatment of neuropathic pain and spasticity associated with multiple sclerosis and intractable cancer pain (24).

Dronabinol (Marinol®), a synthetic THC, and its analogue nabilone (Cesamet®) are the currently available synthetic cannabinoids. Dronabinol and nabilone have been approved for chemotherapy-associated emesis in Canada and USA for many years. In addition, nabilone is indicated for anorexia associated with AIDS-related weight loss (14, 25). Recently, a clinical trial regarding the efficacy of nabilone in diabetic neuropathy has also ended in success (26). Rimonabant, a CB₁ receptor antagonist/inverse agonist, was approved for

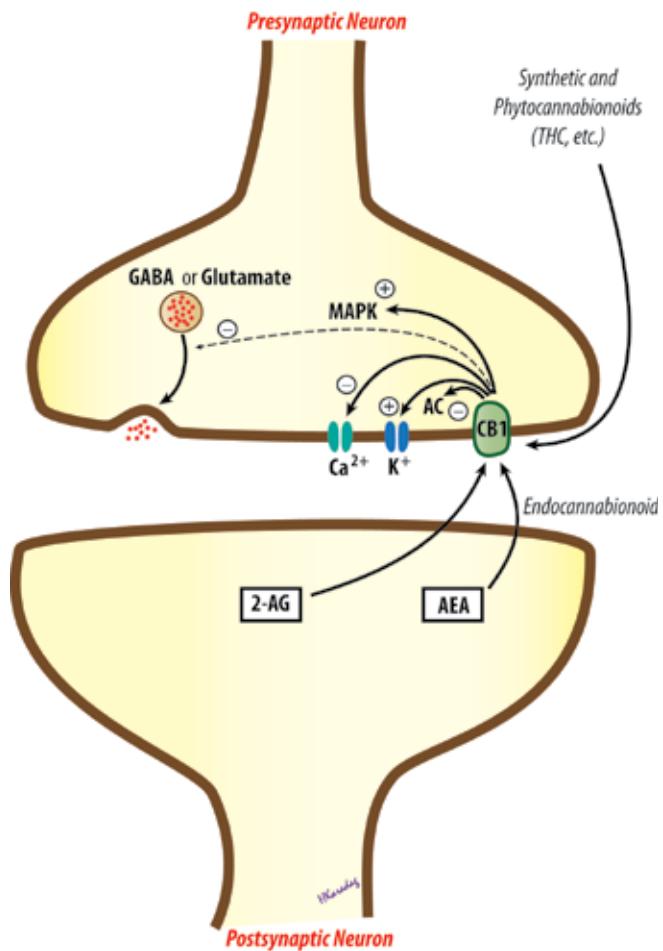


FIG. 1. Transduction mechanisms for cannabinoid CB₁ receptors. AC, adenylyl cyclase; AEA, arachidonyl ethanolamide, anandamide; 2-AG, 2-arachidonoylglycerol; CB₁, cannabinoid receptor 1; GABA, gamma-aminobutyric acid; MAPK, mitogen-activated protein kinase; THC, tetrahydrocannabinol.

obesity and smoking cessation, but withdrawn because of the increased incidence of depression.

ENDOCANNABINOID: SYNTHESIS, RELEASE, TRANSPORT, AND METABOLISM

The endocannabinoid system is comprised of cannabinoid CB₁ and CB₂ receptors, endogenous agonists of these receptors, "endocannabinoids", and the processes playing a role in biosynthesis, release, transport and metabolism of these endogenous lipid-signalling molecules. Arachidonyl ethanolamide (AEA, anandamide), 2-arachidonoylglycerol (2-AG), O-arachidonyl ethanolamine (virodhamine), N-arachidonyl dopamine (NADA), and 2-arachidonyl glyceryl ether (noladin ether) are the putative endocannabinoids. Of these endogenous metabolites of eicosanoid fatty acids, AEA and 2-AG

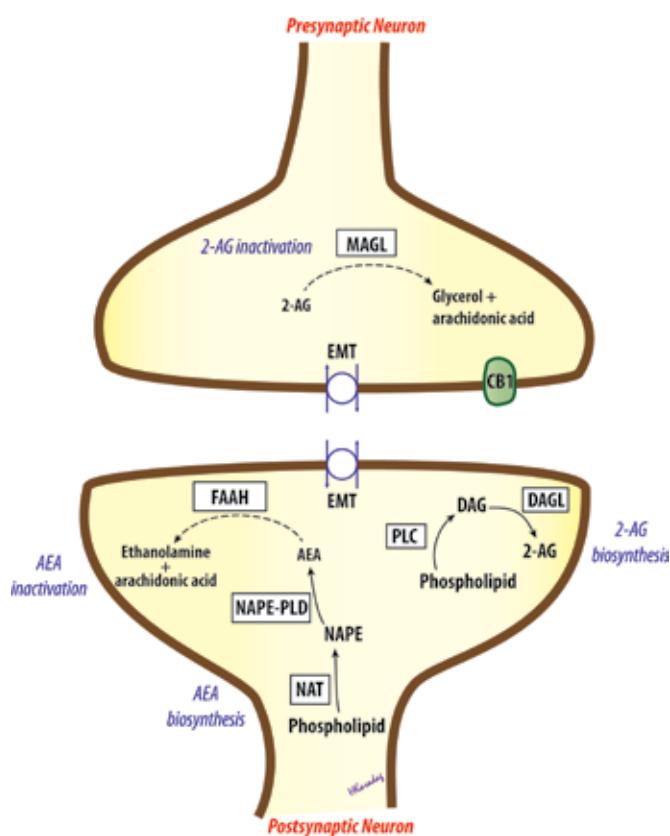


FIG. 2. Biosynthesis and inactivation of endocannabinoids. AEA, arachidonyl ethanolamide, anandamide; 2-AG, 2-arachidonoylglycerol; CB₁, cannabinoid receptor 1; DAG, diacylglycerol; DAGL, diacylglycerol lipase; EMT, endocannabinoid membrane transporter; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE, N-acyl-phosphatidylethanolamine; NAPE-PLD, NAPE-selective phospholipase D; NAT, N-acyltransferase; PLC, phospholipase C.

are the best characterised (4, 5, 14). Endocannabinoids activate CB₁ and/or CB₂ receptors to modulate physiological and pathological conditions, including memory, appetite, immune function, sleep, stress response, thermoregulation, addiction, and no wonder analgesia. AEA and NADA are also shown to have an affinity to transient receptor potential vanilloid 1 (TRPV1) receptors (4, 14, 27).

Endocannabinoids are highly lipophilic compounds that are not stored in vesicles after production. Unlike classical cannabinoids, such as THC, they are substantially synthesised “on demand” from membrane phospholipids, and released immediately. Both AEA and 2-AG are produced at post-synaptic neurons. A two-step process is proposed as the main biosynthetic pathway for AEA. First, phosphatidylethanolamine, a membrane phospholipid, is converted to N-acyl- phosphatidylethanolamine (NAPE) by a calcium-dependent N-acyltransferase (NAT). Then, an NAPE-selective phospholipase D (NAPE-PLD) catalyses NAPE to form N-acylethanolamines, such as AEA. For 2-AG, the major pathway also consists of a two-step

process: diacylglycerol (DAG) is initially produced from inositol phospholipids by the phospholipase C enzyme, and subsequently hydrolysed to 2-AG by a diacylglycerol lipase (DAGL). Alternative enzymatic pathways also appear to be involved in the biosynthesis of both AEA and 2-AG. Following their release from depolarised post-synaptic neurons, endocannabinoids are regarded to act as retrograde messengers, and activate CB₁ receptors on pre-synaptic terminals. Their overall effect may be excitatory or inhibitory, depending on a reduction in neurotransmitter release, mostly as the result of pre-synaptic inhibition of GABA or glutamatergic transmission (Figure 2) (4, 14, 27-29).

After activating cannabinoid receptors, endocannabinoids are removed from the extracellular space by a process of cellular uptake. Not much is known about the uptake of 2-AG, but a specific “endocannabinoid membrane transporter” appears to mediate the removal of AEA, although this has not yet been cloned (14, 28, 30, 31). Then, AEA is hydrolysed to arachidonic acid and ethanolamine in post-synaptic neurons, whereas 2-AG is hydrolysed to arachidonic acid and glycerol in pre-synaptic neurons, predominantly by the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. Accordingly, FAAH appears to terminate endocannabinoid action during synthesis, while MAGL seems to play role after receptor activation. The endocannabinoids AEA and 2-AG are also metabolised via oxidation by the enzymes cyclooxygenase (COX), lipoxygenase, and cytochrome P-450 (4, 14, 28, 29).

MODULATION OF THE ENDOCANNABINOID SYSTEM

The central side effects of exogenous cannabinoids, most of which are related to CB₁ receptors in the central nervous system (CNS), directed researchers to look for alternative strategies. Peripherally restricted CB₁ agonists and/or selective CB₂ receptor agonists deserve attention, since they are expected to minimise unwanted CNS effects mediated via CB₁ receptors. There are reports indicating that selective peripheral CB₁ agonists are effective in controlling conditions, such as chronic neuropathic pain and spasticity in multiple sclerosis (32, 33); however, no evidence of analgesic efficacy was observed for a peripherally acting CB₁/CB₂ receptor agonist in the human capsaicin pain model (34). Selective CB₂ receptor agonists have also undergone clinical trials and found to be beneficial, but unwanted effects, such as immune depression, have prevented their use in the clinic (35, 36). Combining low doses of cannabinoids with other analgesics, such as opioids or NSAIDs, is also promising. Reducing the cannabinoid and opioid/NSAID dose needed may provide an advantageous treatment strategy by enhancing pain relief whilst minimising the incidence of adverse effects (6, 7, 37).

Elevating endocannabinoid levels appears to be the most striking strategy for developing analgesic drugs with cannabinoid properties but devoid of central psychotropic effects (38–40). During pain states, endocannabinoids are only synthesised and metabolised in the CNS structures involved in pain transmission and modulation. Thus, augmenting endocannabinoid levels in these structures will only effect CB receptors in these areas and possibly reduce central side effects. Inhibition of the degradative enzymes FAAH and MAGL, and inhibition of endocannabinoid cellular uptake are the pharmacological strategies used to modulate the endocannabinoid system and elevate endocannabinoid levels locally (3, 5, 22, 38, 40).

Fatty acid amide hydrolase inhibitors, such as URB597 and OL135, are shown to be effective at reducing sensitivity to pain in acute and chronic experimental pain models (41, 42). However, in a recent clinical trial, PF-04457845, an irreversible FAAH inhibitor, failed to induce effective analgesia in patients with knee osteoarthritis (43). It is noteworthy that AEA, at higher concentrations, activates TRPV1 channels, which are known to be involved in nociception, and that the COX pathway represents an alternative metabolic route for AEA. Thus, COX-1 and COX-2 inhibitors, and TRPV1 antagonists seem to be potential second targets to combine with FAAH inhibitors (22, 39, 44). Dual FAAH/TRPV1 blockers, such as N-arachidonoyl-serotonin (AA-5-HT) and OMDM198, are also efficacious in animal studies, but this multi-target strategy has not yet reached the clinic (45, 46).

Inhibition of MAGL using pharmacological agents, such as URB602, JZL184 and OMDM169, also elicits anti-nociceptive activity in experimental models, but tolerance may develop with fully effective doses of MAGL inhibitors (47). Dual FAAH/MAGL inhibition and using peripherally restricted inhibitors of FAAH and MAGL are also among the promising strategies (38, 39, 48). Moving to the inhibition of endocannabinoid cellular uptake, there are some potential therapeutic effects observed with the use of membrane transporter inhibitors in animal models (30, 38, 40). This approach has once again gained attention after the recent discovery of FAAH-like anandamide transporter (49), but clinical research remains to be determined.

It is worth mentioning that endocannabinoid system seems to mediate anti-nociceptive effects of 2 analgesics used worldwide, paracetamol and dipyrone. Paracetamol is metabolised in the brain by FAAH into AM404, which reinforces the activity of the descending serotonergic system through the endocannabinoid system via CB₁ receptors (50, 51). FAAH also appears to be responsible for the formation of two novel arachidonyl-conjugated metabolites of dipyrone (52). Metabolites of these classical analgesics then possibly activate CB receptors or inhibit endocannabinoid metabolism (53–55), but clinical research is needed to determine how many of these findings also apply to humans.

CONCLUSION

The discovery of CB₁ and CB₂ receptors, their endogenous ligands (endocannabinoids), and the processes responsible for the biosynthesis, release, transport and metabolism of these compounds were a huge help in understanding the role of endocannabinoids in various physiological and pathological conditions, including pain modulation. Elevating endocannabinoid levels locally by the inhibition of endocannabinoid degrading enzymes, FAAH and MAGL, using pharmacological agents, and thereby reducing the unwanted central effects of exogenous cannabinoids, also made an additional contribution to this knowledge. Some of the most important future directions in this field are: (i) developing peripherally restricted CB₁ agonists, (ii) inventing new CB₂ agonists, (iii) combining cannabinoids with other analgesics, and (iv) elevating endocannabinoid levels using multi-target drugs such as FAAH/MAGL, FAAH (MAGL)/COX and FAAH (MAGL)/TRPV1 dual blockers. Clinical trials will demonstrate the value of these approaches.

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