

The Role of the Endogenous Cannabinoid System in Peripheral Analgesia

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Abstract: The therapeutic potential of cannabinoids has been studied and investigated through centuries, although many interesting discoveries have emerged from this field in the past decades. Indeed, peripheral analgesic effects of cannabinoids are a new avenue of treatment since they are avoiding the deleterious central side effects of systemic administration. Recently, it has been demonstrated that cannabinoid receptors (more specifically CB₁ and CB₂ receptors) and their endogenous ligands are present at the peripheral level, especially in different layers of skin, and mostly, in the epidermis and dermis. Those findings are reinforcing and confirming the efficacy of peripheral administration of cannabinoids used to alleviate pain in many different animal models. However, many studies have shown that the endocannabinoid system interacts with other receptors and pathways to modulate pain at the peripheral level. Thereof, the main goal of this review is to explain, in a better way, the different interactions regarding the cannabinoid system with other cellular components of its environment, its involvement in the modulation of pain at the peripheral level and, more precisely, in different layers of the skin.

Keywords: Cannabinoids, endocannabinoids, anandamide, primary afferent neurone, analgesia, pain, opioids.

INTRODUCTION

In order to interact with our environment, there is a need to recognize and to react to harmful stimuli in order to avoid them. In fact, the skin is considered to be more than a protection against external stimuli. Indeed, it is defined as an organ associated with four essential functions: protection, sensitivity, thermoregulation and metabolism [1]. The skin is constituted of three major layers: the epidermis, the dermis and the hypodermis. The epidermis is further subdivided into the five following strata: stratum corneum, lucidum, granulosum, spinosum and germinativum (or basal). Cutaneous sensory nerves are the specialized detectors (also known as nociceptors) which respond to noxious stimuli (thermal, chemical and mechanical) and innervate the epidermis and the dermis [1-4]. Thus, nociceptors correspond to free nerve endings that are divided into two groups: the unmyelinated polymodal C fibers and the thinly myelinated A δ fibers [5, 6]. These afferent nerve fibers are transmitting sensory stimuli *via* dorsal root ganglia, further activating the spinal cord and communicating to specific areas of the central nervous system that will result in the perception of different types of pain [5, 6]. Thereof, C fibers are associated with the burning sensation and are slowly conducting nociceptive information (0.5-2 m/s), whereas the larger A δ fibers are responsible for the briefer sharp pain and are more rapidly conducting (5-20 m/s) [5, 6]. Both C and A δ fibers respond to a variable range of stimuli such as: physical (trauma, heat, cold, osmotic changes, mechanical stimulation, ultraviolet light), as well as, chemical (toxic agents, allergens, proteases, microbes) agents [6, 7]. Therefore, the present work will show the peripheral aspect (free nerve endings) of the transmission, in relation, to the modulation of pain through the endocannabinoid system, and other related interacting components or signaling pathways.

Indeed, the cannabis, and its medical use, have been extensively studied since the middle of the 19th century [8]. The discovery of endogenous cannabinoids (anandamide and 2-arachidonoylglycerol) which are the most studied ones, cannabinoid receptors (mainly CB₁ in the nervous system and CB₂ in the immune system) and their antagonists, and also the generation of knock out mice for those receptors have helped the research development of this new field [9-12]. Anandamide or arachidonylethanolamide (AEA) biosynthesis occurs from a phospholipid precursor, N-arachidonoylphosphatidylethanolamine (NAPE), synthesized from phosphati-

dylethanolamine and phosphatidylcholine by an N-acyl-transferase (NAT). NAPE is then hydrolyzed to anandamide by specific phospholipase D [8, 13, 14]. 2-arachidonoylglycerol (2-AG) is formed from phospholipids to a diacylglycerol (DAG) precursor that is catalyzed by a phospholipase C (PLC) which is followed by the hydrolysis of DAG by a diacylglycerol lipase (DAGL) [15, 16]. Furthermore, FAAH (fatty acid amide hydrolase) [17] and MGL (monoacylglycerol lipase) [18] are the enzymes that degrade anandamide and 2-AG, respectively. Although the biosynthesis/metabolism of both endocannabinoids has been simplified to maintain the focus of this review, it is important to mention that alternative pathways exist and have been studied [19-22]. Indeed, new avenues involve the metabolism of anandamide and 2-AG by cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 enzymes, further adding to the complexity of endocannabinoid metabolism [19, 21, 23, 24]. The endogenous cannabinoids as well as their cannabinoid receptors are localized in the pain pathways from the periphery to the central nervous system (CNS) [25-27]. The analgesic effects of cannabinoids are supported by many studies involving different animal models. Thus, the endocannabinoid system and its interaction with other receptors/pathways can modulate nociception at peripheral level, and this will be mainly discussed in inflammatory pain models.

LOCALIZATIONS OF CANNABINOID RECEPTORS IN PRIMARY AFFERENT NEURONS

Previous studies have demonstrated that cannabinoid receptors are found in the periphery in dorsal root ganglia (DRG) (CB₁ ARNm using double-label *in situ* hybridization [26]; DRG neurons cultures [28]; CB₁ and CB₂ using immunohistochemistry (IHC) [29]; CB₁ and CB₂ using Western blot [30]). Indeed, it has been shown that cells from DRG synthesized cannabinoid receptors, and transported them towards the peripheral terminals of primary afferent neurons (detection of CB₁ ARNm in DRG using *in situ* hybridization histochemistry [31, 32], densities of cannabinoid receptors using *in vitro* receptor binding and emulsion autoradiography [32]). Additional evidence for the presence of cannabinoid receptors on primary afferent neurons has been obtained (CB₁ using IHC [33]; CB₁ and CB₂ using IHC [34]). Thereof, CB₁ and CB₂ receptors were found in large myelinated and small unmyelinated human cutaneous nerve fibers [34]. Cannabinoid CB₁ receptors were also identified in the keratinocytes of the stratum spinosum and granulosum and also on mast cells and macrophages [34]. The CB₂ cannabinoid receptors were identified in keratinocytes of the stratum granulosum (CB₂ using IHC [35]), the stratum basal [34] and suprabasal layers of the epidermis and hair follicles (CB₁ and CB₂

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using Western blot and IHC [36]), as well as on mast cells and macrophages [34, 37 for a review]. CB₁ and CB₂ receptors are also found on other types of cells and adnexal structures like sweat glands, sebaceous cells and others [34, 37]. Even though cannabinoid receptors have been found at different levels in the periphery, their localization is linked to the validity of highly specific antibodies. Indeed, the specificity of some antibodies has been questioned particularly the commercially available ones [38]. Therefore, the reader needs to be aware of this information in order to better judge the studies presented above, and the specificity of antibodies should be addressed in future studies. Cannabinoid receptors produce their analgesic effect by inhibiting the release of neurotransmitters by inhibition of adenylate cyclase and activation of mitogen-activated protein kinases; the CB₁ receptors also inhibit the Ca²⁺ channel and activate the K⁺ channel [39 for a review].

PERIPHERAL ANTINOCICEPTIVE EFFECTS OF ENDOGENOUS COMPOUNDS RELATED TO AEA

In fact, further evidence for the presence of CB₁ and CB₂ cannabinoid receptors in the skin comes from the data indicating the antinociception produced by peripheral administration of cannabinoids. Indeed, local injection of anandamide or palmitoylethanolamide (PEA, a fatty-acid amide), at doses devoid of systemic effects, reduced painful behavior in inflammatory rat models and these effects were mediated by CB₁ and CB₂ cannabinoid receptors [40-44]. Moreover, anandamide and PEA are found in the paw skin at the amounts that could activate the cannabinoid receptors [40-44]. Furthermore, peripheral administration of 2-AG produced a dose-dependent antinociceptive effect mediated by the CB₂ receptors when administered before formalin in the formalin test [45] and WIN 55,212-2, a synthetic cannabinoid agonist, reduced pain behaviour in the capsaicin or carrageenan model implicating CB₁ and CB₂ receptors [46, 47, respectively]. Local administration of AM1241, a selective agonist for the CB₂ receptors, reversed the hyperalgesic effect of carrageenan [48, 49] or of capsaicin [49, 50], which demonstrated that peripheral CB₂ cannabinoid receptors are able to modulate nociceptive processes. Furthermore, PEA administered topically increased the level of PEA in the paw and reduced the inflammation in the carrageenan model [51]. PEA has anti-inflammatory and antinociceptive properties [40]. The anti-inflammatory effects have been shown to depend on PPAR- α receptor activation, since PEA does not activate CB₂ cannabinoid receptors, even though, its antinociceptive effect is blocked by SR144528 [52]. This effect produced by SR144528 seemed to be related to an interaction with PPAR- α receptors pathway [52]. In another study, it was also shown that macrophages participate in the biosynthesis and inactivation of 2-AG when they are activated by ionomycin or lipopolysaccharides (LPS) [16]. Indeed, it was demonstrated that LPS stimulated the production of anandamide in rat macrophages [53-55] and of 2-AG in platelets although the mechanism responsible for this effect has not been elucidated [53]. The peripheral antinociceptive effects of cannabinoids are quite interesting and should be studied extensively in future years since they are devoid of the psychotropic effects that are attributed to the systemic administrations of these substances [27, 56].

INHIBITION OF ENDOCANNABINOIDS DEGRADATION ENZYMES

Although the inhibition of the formation of prostaglandins, by blocking the activity of cyclooxygenases (COX), is the main mechanism of the analgesic action of nonsteroidal anti-inflammatory drugs (NSAIDs), some of these drugs might have other independent effects. Indeed, it has been demonstrated that FAAH, the enzyme that degrades anandamide, activity is inhibited by NSAIDs such as ibuprofen, ketorolac, and flurbiprofen using *in vitro* studies in rat brain [57, 58] (Fig. 1). Therefore, it has been

demonstrated that the local administration of a non-selective COX inhibitor (diclofenac) [59], a selective COX-1 inhibitor (resveratrol) [59] or some selective COX-2 inhibitors (lumiracoxib, nimesulide, meloxicam) [60-62] produced an antinociceptive effect in the formalin test. Furthermore, it has also been demonstrated that the peripheral administration of a selective COX-2 inhibitors (celecoxib) [63] reversed hyperalgesia caused by carrageenan. However, it has been shown that other inhibitors of FAAH like methyl arachidonyl fluorophosphonate (MAFP) and URB597 produced an antinociceptive effect in inflammatory pain models when administered systematically (MAFP in formalin test [64]; URB597 in complete Freund's adjuvant [65]). Therefore, it has been demonstrated in the formalin test that the combination of non-selective COX inhibitor (ibuprofen) or selective COX-2 inhibitor (rofecoxib) with anandamide given locally, produced a synergistic analgesic effect in the formalin test which is mediated by CB₁ and partially by CB₂ cannabinoid receptors [43, 44]. In the same perspective, the local administration of URB602, a MGL inhibitor [66], produced a dose-dependent antinociceptive effect in the formalin test that is mediated by CB₁ and CB₂ receptors [45]. Finally, the combination of 2-AG with URB602, given locally, produced an additive antinociceptive effect in the formalin test [45]. Indeed, this modulation of the endogenous cannabinoids by blocking their degradation enzymes conferred a better antinociceptive effect than endocannabinoids given alone.

ANANDAMIDE AS AN ENDOVANILLOID

Anandamide is an endocannabinoid activating CB₁ and CB₂ receptors but it can also act as an endovanilloid on TRPV1 (transient receptor potential vanilloid 1) receptors which are sensitive to capsaicin (the pungent chemical in chilli peppers), protons and heat [67-70]. It seems that capsaicin and anandamide share the same binding site [71], but anandamide needs to be at high concentrations to activate those TRPV1 receptors [72]. The activation of TRPV1 receptors creates an increase in the cellular entry of Ca²⁺ and depolarizes the cell which will liberate CGRP (calcitonin gene-related peptide) and substance P causing a vasodilatation [68]. In fact, at high concentrations anandamide can have opposite effect, when activating TRPV1 receptors, than those produced by activation of cannabinoid receptors. Therefore, it seems that a functional relation exists between TRPV1 and CB₁ receptors at DRG [33], spinal and brain [73] levels when those two receptors are expressed in the same cell. It will be interesting to study more intensively this relationship, at the peripheral level, like in the paw. Indeed, TRPV1 activation displayed a pronociceptive role as demonstrated by the absence of acute nocifensive behavior following intraplantar injection of a PKC activator in TRPV1 knock out mice [74]. Thus, PKC activation elicited nociception exclusively through TRPV1 receptor activation [74]. More importantly, the activity of TRPV1 receptors was enhanced/induced by AEA and bradykinin in a manner that is dependent on PKC activation [75]. An interesting fact is that the local (paw) administration of capsazepine, an antagonist of TRPV1 receptors, produced a significant nociceptive effect in the formalin test [44]. It has also been shown that following PKC activation OEA (oleoylethanolamide), a fatty-acid amide, can activate TRPV1 receptors in neurons [76]. However, the local administration of anandamide combined with the NSAIDs (ibuprofen or rofecoxib) increased the level of anandamide, PEA and OEA (oleoylethanolamide) in a greater way than each compound given alone and this increase was not mediated by TRPV1 receptors [44].

ENDOCANNABINOID SYSTEM IMPLICATION WITH OTHER PAIN MEDIATORS

An important pain mediator, nerve growth factor (NGF) has long been recognized as a critical factor supporting the development of neurons in the peripheral nervous system, which includes

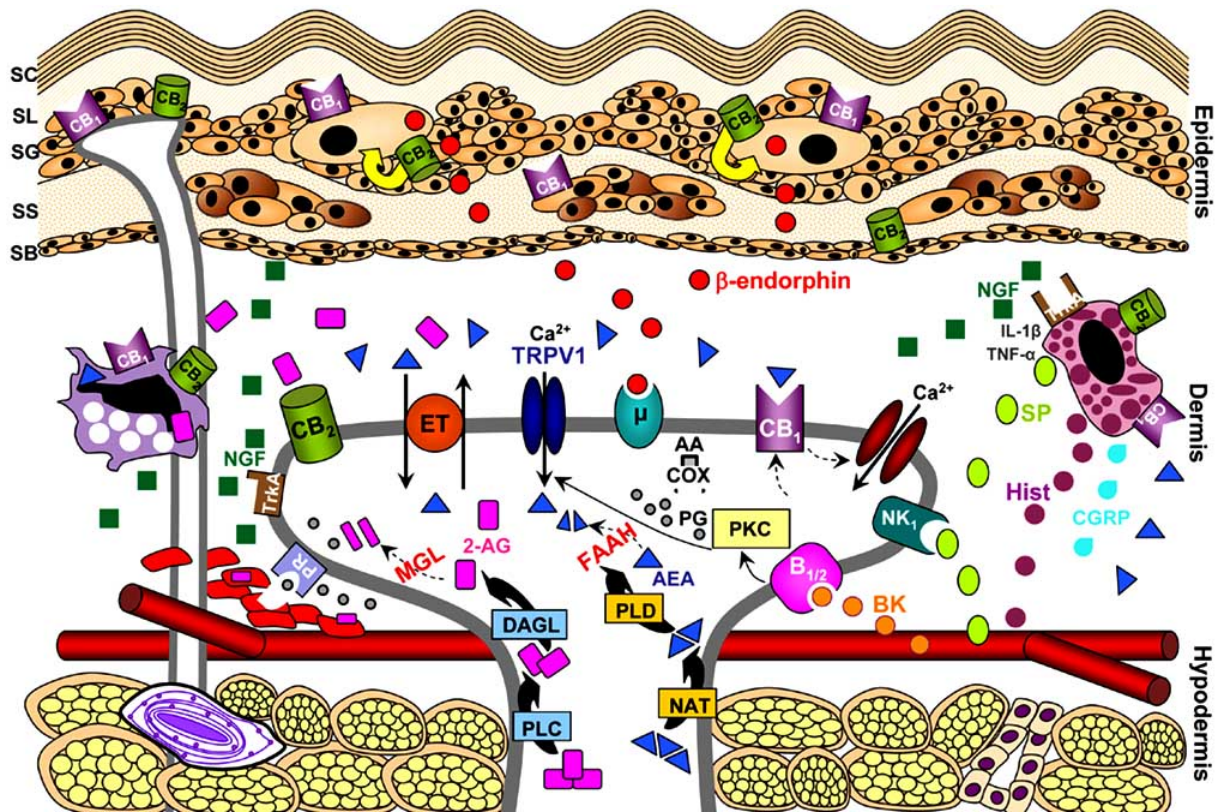


Fig. (1). Neurobiological interactions between the endogenous cannabinoids and other receptor systems in the skin for the modulation of inflammatory pain.

In the epidermis, the cells represented in the SC, SL, SG, SS and SB are mainly keratinocytes, but, in the SG, SS and SB, there is an afferent nerve fiber going into these layers of the epidermis. In the dermis, there is another afferent nerve fiber beside the smaller one. In the right side, there is a mast cell (pink) and, in the left part of the figure, a macrophage (purple). In the hypodermis, blood vessels and platelets (red rectangle cells) are shown. There is also a sudoriparous gland (sweat gland) shown on the right side that is surrounded by adipose cells (fat tissue) and, on the left side, Pacini cells (receptors of pressure sensation) are depicted that are represented surrounded by adipose cells. The other components of the figure are given in the abbreviations list.

nearly all nociceptors [4, 5, 77]. Its painful effect is mediated through the activation of high affinity TrkA receptors located on primary afferent nociceptors [78] (Fig. 1). NGF plays a pivotal role in a model of visceral hyperalgesia, the injection of intra-vesical NGF produced bladder hyperalgesia that was attenuated by the systemic administration of anandamide [79]. This antihyperalgesic effect was mediated by CB₁ and CB₂ receptors [79]. This experiment suggests that the endogenous cannabinoid system can modulate the NGF components involved in inflammatory processes. Therefore, a possible explanation for the CB₂ anti-inflammatory effect could be attributed to attenuation in the induction of mast cell degranulation and neutrophil accumulation by NGF which is known to contribute to inflammatory hyperalgesia [80]. Mast cells, macrophages and various other cell types express the NGF [81] and these cells are particularly important in inflammation. Mast cells expressing TrkA respond to NGF by proliferating and releasing inflammatory mediators including IL-1 β and TNF- α [82]. Other than that, most of the NGF, that is constitutively expressed, is produced by epithelial cells that are mostly keratinocytes in the skin [81]. NGF strongly regulates the expression of receptors expressed by nociceptors such as TRPV1 receptors *in vitro* and *in vivo* and also in several ion channels [77]. Other pain mediators implicated with the endocannabinoid system are nitric oxide (NO) and cytokines. Indeed, the endocannabinoid system interacts with the NO pathway and influences NO production in a neuropathic pain model [83]. Moreover, the involvement of cytokines in pain and hyperalgesia has been studied extensively and they are also influencing the cannabinoid system [84 for a review]. Thereof, the information above confirms a link between the endocannabinoid system and other pain

mediators like NGF, nitric oxide and cytokines. They are linked to interact together to modulate inflammatory processes at the peripheral level.

INTERACTION BETWEEN THE ENDOCANNABINOID AND OPIOID SYSTEMS

Cannabinoid agonists may cause the release of endogenous opioids since a functional interplay between the endocannabinoid and opioid systems in the modulation of analgesic responses has been proposed by numerous studies (*in vivo* studies involving mice after intrathecal injections [85]; *in vivo* studies in rats with spinal perfusion [86]; *in vivo* studies in mice after subcutaneous injections [87]; *in vivo* studies in mice after oral administration [88]). In fact, the activation of CB₂ cannabinoid receptors by AM1241 stimulates the release from keratinocytes of β -endorphins acting at neuronal μ -opioid receptors to inhibit nociception (CB₂ using IHC [35]) (Fig. 1). The CB₂ immunolabeling was detected on β -endorphin-containing keratinocytes in the stratum granulosum throughout the epidermis of the hindpaw. This mechanism implicated the local release of β -endorphin, where CB₂ receptors are present, leading to anatomical interaction between cannabinoid and opioid systems [35]. The presence of cannabinoid CB₂ receptor in keratinocytes has been reported previously (CB₁ and CB₂ using Western blot and IHC [36]), and more recently (CB₁ and CB₂ using IHC [34]). Furthermore, it has been demonstrated that, following intraplantar injection of carrageenan, the hypoalgesic effects of selective COX-2 inhibitors (celecoxib, rofecoxib and SC236 were administered systemically (subcutaneous or oral administration) or locally (into the paw)

are abolished by pretreatment with naltrexone (an opioid receptor antagonist given systematically (subcutaneous or oral administration) or locally (into the paw) (*in vivo* studies in rats [89])). In this inflammatory model, it was concluded that the antinociceptive effects of selective COX-2 inhibitors involved the participation of endogenous opioids. Thus, the interaction between the cannabinoid and the opioid system needs to be investigated further at the peripheral level.

CONCLUSION

It was demonstrated, from different studies presented herein, that the endocannabinoid system can interact with other pathways, and receptor systems, at the peripheral level to modulate pain. However, further studies are necessary to obtain a better understanding of all the mechanisms involved in the peripheral modulation of pain. We discussed some mechanisms of action involved, but the elucidation of many others is awaited in order to improve our understanding of the analgesic effect of local administration of cannabinoids. Indeed, the antinociceptive effects of cannabinoids given at the peripheral level are explained by the action on cannabinoid receptors, but also by the interaction with other receptors and signaling pathways as shown in the Fig. (1). In the next years, pain modulation, at the peripheral level, may be exploited in order to avoid the deleterious central side-effects of systemic administration of cannabinoids.

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ABBREVIATIONS

2-AG	=	2-arachidonoylglycerol
AA	=	Arachidonic acid
AEA	=	Anandamide
B _{1/2}	=	Bradykinin _{1/2} receptors
BK	=	Bradykinin
Ca ²⁺	=	Calcium
CB ₁	=	Cannabinoid receptor 1
CB ₂	=	Cannabinoid receptor 2
CGRP	=	Calcitonin gene-related peptide
COX	=	Cyclooxygenase
DAGL	=	Diacylglycerol lipase
ET	=	Endocannabinoid membrane transporter
FAAH	=	Fatty acid amide hydrolase
Hist	=	Histamine
IL-1 β	=	Interleukin-1 β
MGL	=	Monoacylglycerol lipase
NAT	=	N-acyl transferase
NGF	=	Nerve growth factor
NK ₁	=	Neurokinin ₁ receptor
PG	=	Prostaglandins
PKC	=	Protein kinase C
PLC	=	Phospholipase C
PLD	=	Phospholipase D

PR	=	Prostanoid receptors
SB	=	Stratum basal (or germinativum)
SC	=	Stratum corneum
SG	=	Stratum granulosum
SL	=	Stratum luteum
SP	=	Substance P
SS	=	Stratum spinosum
TNF- α	=	Tumor necrosis factor alpha
TrkA	=	Tyrosine kinase A receptor
TRPV1	=	Transient receptor potential vanilloid 1
μ	=	μ opioid receptor.

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