Cannabinoids and Neuropathic Pain

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Abstract: After a brief overview of the endocannabinoid system (CB receptors, and endocannabinoids) and of the cannabinergic ligands, some general issues related to cannabinoids and pain are commented. Finally, the most important findings regarding cannabinoids and neuropathic pain are discussed in detail.

Keywords: Cannabinoid, neuropathic pain, analgesia.

INTRODUCTION

The therapeutic and psychotropic properties of the hemp plant *Cannabis sativa*, have been known for centuries. The compounds responsible for these actions are the cannabinoids, tricyclic structures derived from the benzopyran ring of which the most representative is (-)-Δ^9-tetrahydrocannabinol, Δ^9-THC, main component of the plant isolated and characterized in 1964 [1]. These compounds interact with the cannabinoid receptors of which up to now two have been characterized. The endogenous cannabinoids and other compounds, mainly heterocycles, also bind to these receptors, so that the term cannabinoid has now been extended to include all these substances. Many recent publications have dealt with general aspects of the cannabinoid system, cannabinergic ligands and potential therapeutical applications [2-4]. Nevertheless, in this review concerning cannabinoids and neuropathic pain a small introduction to the subject follows.

THE ENDOCANNABINOID SYSTEM

The Cannabinoid Receptors

To date, two types of cannabinoid receptors have been identified [5]: the CB1, cloned in 1990; and the CB2, cloned in 1993. Recent studies and the use of cannabinoid CB1 knockout mice have indicated the possible existence of additional cannabinoid receptor subtypes [6, 7]. Evidence for a "CB3" receptor has been provided in a study analyzing milk intake and survival in newborn cannabinoid CB1 knockout mice [8]. Cannabinoid receptors are widely distributed in various mammalian tissues. The CB1 receptors are found mainly in the central nervous system (CNS) but they are also present in some peripheral neurones and in certain non-neuronal tissues. CB2 receptors are found in immune cells where they are supposed to mediate an immunosuppressant effect.

The CB1 receptor has been cloned from rat [9], mouse and human tissues [10] and exhibits 97 to 99% aminoacid sequence identity along species. The CB2 receptor [11] exhibits overall 44% homology with the CB1 but about 68% within the transmembrane region. Both CB receptor types belong to the large family of G-protein coupled receptors (GPCRs) [12] controlling a wide variety of signal transduction. GPCRs are integral membrane proteins characterized by seven hydrophobic transmembrane helixes. CB1 receptors are also coupled through G proteins to several types of calcium and potassium channels.

Endocannabinoids

The identification of the cannabinoid receptors led to the discovery of the endogenous cannabinoid ligands, the most important being anandamide (AEA) [13] and 2-arachydonoylglycerol (2-AG) [14]. Three other endocannabinoids have also been reported: noladin ether [15], virodhamine [16] and *N*-arachidonoyl-dopamine (NADA) [17], but the first two have been the most studied up to date. Fig. (**1**).

The regulation mechanisms of the endocannabinoids involve two processes: transport of the endocannabinoids across the cell membrane and their degradation. The uptake is proposed to be mediated by a transporter (ANT or AMT) [18] or to be a passive diffusion process. Once in the cell, endocannabinoids are hydrolyzed by the enzyme anandamide amidohydrolase (FAAH) [19]. It should be mentioned that while FAAH has been fully characterized, the X ray data having been recently published [20], the existence of AMT is still the subject of controversy, with different authors providing evidence for [18] and against its existence [21].

The endocannabinoid system, integrated by the cannabinoid receptors and the endocannabinoids, together with two cannabinergic proteins, AMT and FAAH, are important therapeutic targets and the subject of intense research in medicinal chemistry [22]. Endocannabinoids have been implicated in a variety of physiological functions including pain reduction, motor regulation, learning, memory, reward and appetite stimulation [23], and so, inhibitors of the two proteins can be used for therapeutic purposes.

Two cannabinoid pharmaceuticals are in clinical use as anti-emetics and to stimulate appetite, Marinol® and Cesamet® [24]. Other potential therapeutic applications for

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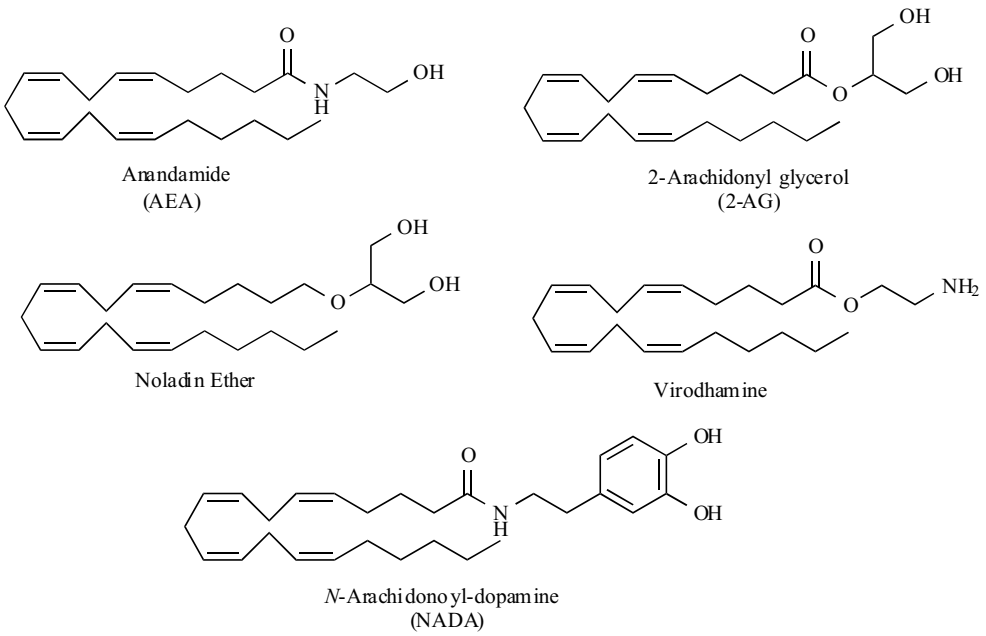


Fig. (1). Structures of endogeneous cannabinoid ligands.

cannabinoid agonists include multiple sclerosis and spinal cord injury [25, 26], brain injury [27], glaucoma [28], bronchial asthma, vasodilation [29], treatment of malignant gliomas [30, 31] and pain [32]. CB1 receptor antagonists/inverse agonists also have potential use for improving cognitive and memory dysfunction associated with Alzheimer's disease [33] and as appetite suppressants, rimonabant (SR141716) being in phase III clinical trials.

CANNABINOID LIGANDS

There are many chemically different structures capable of binding to cannabinoid receptors. Although different authors use different classifications of these ligands [34, 35], for simplification purposes, in this review, we will group them in three major classes.

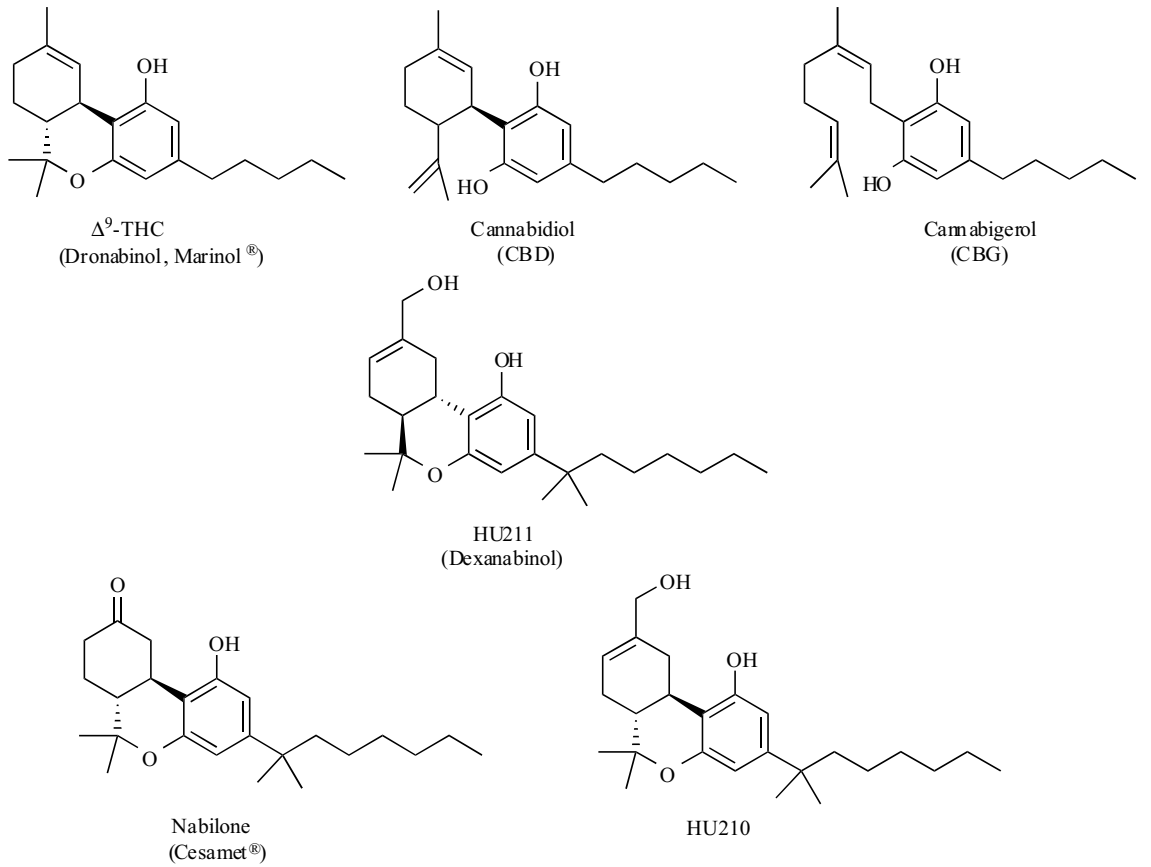
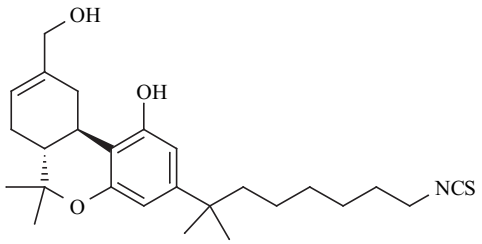
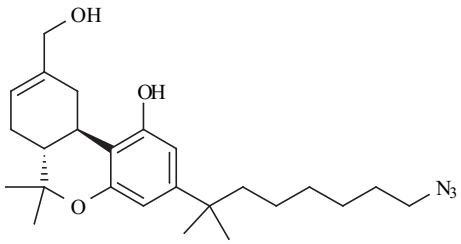


Fig. (2). Structures of some natural and classical cannabinoids.



AM708



AM836

Fig. (3). Structures of some Δ^8-THC analogues.

Naturally Occurring Cannabinoids and Related Structures

Apart from Δ^9-THC already mentioned, two other cannabinoids are also present in the plant, cannabigerol (CBG) and cannabidiol (CBD), in which there is a renewed interest because of its high therapeutic potential and non-psychotropic nature [36]. Other classical or tricyclic cannabinoids include nabilone which together with dronabinol are the two commercially available substances. Dexanabinol, HU211, is in phase III clinical trials for traumatic brain injury [37] Fig. (**2**).

Recently, Makriyannis *et al.* have reported novel cannabinoids having selectivity for the CB2 receptor such as AM1714 [38], and affinity ligands which have reactive groups which make them useful probes for studying the active sites of the receptor such as AM836, and AM708 [39]. Huffman has recently described novel Δ^8-THC analogues with greater affinity for the CB2 receptor [40]. Novel 1',1'-chain substituted Δ^8-tetrahydrocannabinols have also appeared in the literature [41] Fig. (**3**).

Along these years many analogues have been synthesized such as nantradol, a considerably modified cannabinoid with potent analgesic activity, which will be referred to later. The so-called non-classical cannabinoids which are usually bicyclic structures, such as CP55940 that is an important pharmacological tool, and HU308 [42], have also been the subject of research Fig. (**4**).

Endocannabinoids and Related Structures

Many analogues of the endogenous cannabinoids, anandamide and 2-arachidonoylglycerol have been synthesized [43], and the structure-activity relationships have been thoroughly studied [44, 45]. Improved metabolically stable derivatives such as methanandamide [46] and O689 [47] have also been prepared. Inhibitors of the carrier protein AMT have also been described [48] such as AM404 [49], UCM707 [50], and VDM11 [51]. Boger has studied FAAH substrate specifity [52] and synthesized new inhibitors [53] Fig. (**5**).

Endogenous cannabinoids such as anandamide bind also to the vanilloid VR1 receptor. The similarity between capsaicin, agonist of the VR1 receptor and some inhibitors of AMT led Di Marzo *et al.* to the development of some hybrid molecules such as arvanil [54] with high affinity for the CB1 receptor and capable of inhibiting anandamide uptake Fig. (**6**).

Heterocycles

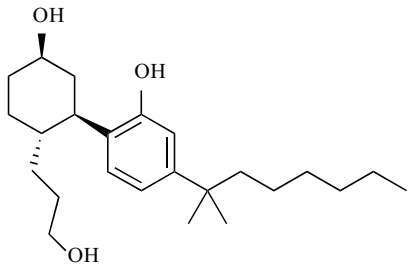
The third group of cannabinoid ligands includes different families of diverse structural classes of which the most important will be highlighted:

Diarylpyrazoles

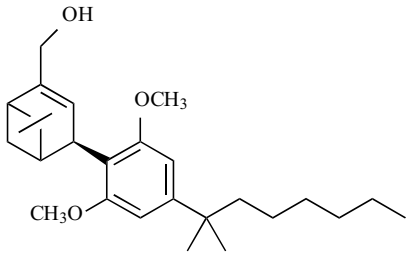
Originally developed by Sanofi, among them can be found the CB1 antagonist/inverse agonist SR141716, and the CB2 antagonist/inverse agonist SR144582. The structure activity relationships have been published for 1,5-diaryl pyrazoles, in general [55] and for SR141716 [56]. Analogues with lower lipophilicity have recently been described [57]. Fig. (**7**).

Aminoalkylindoles

The first compounds were developed at Sterling Winthrop as potential anti-inflammatory agents. WIN55212 is a potent CB1 and CB2 agonist with a slight selectivity for the CB2. Recently, C-3 amidoindole derivatives that selectively bind to the CB2 receptor have been published



CP55940



HU308

Fig. (4). Structures of some non-classical cannabinoids.

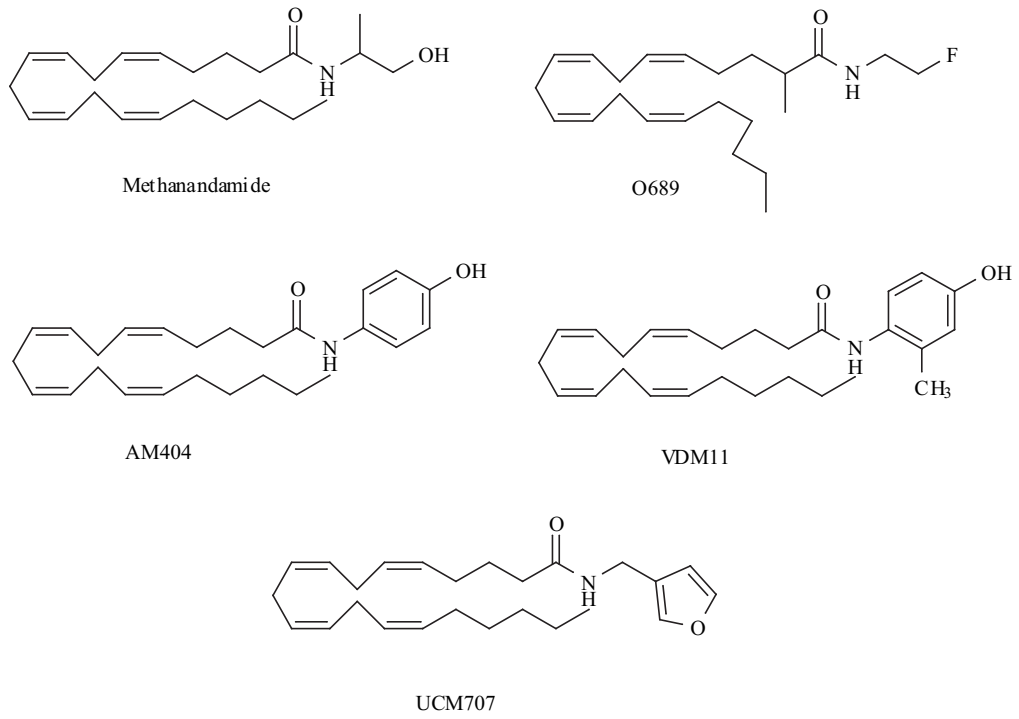


Fig. (5). Structures of some endocannabinoid derivatives.

[58]. Huffman has reported hybrid structures combining structural elements of traditional cannabinoids and cannabimimetic indoles [59], and has used 3-indolyl-1-naphthylmethanes for studying the aromatic stacking interactions with the CB1 receptors [60] Fig. (**7**).

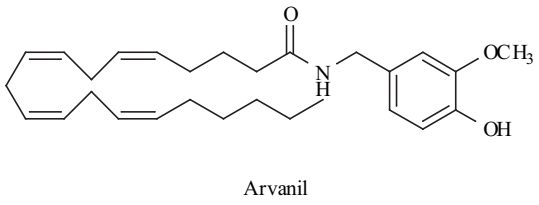


Fig. (6). Structure of an VR1 ligand / AMT inhibitor.

Miscellaneous Structures

These include diarylether sulfonyl ester derivatives such as BAY387271 [61], 3-alkyl-5-arylimidazolidine diones [62], diaryl 1,2,4-triazoles from our group [63], and 1,4-dihydroindeno[1,2-*c*]pyrazoles [64]. All of these have recently been reported and shown cannabinergic properties.

CANNABINOIDS AND PAIN

Cannabis extracts have been used for centuries for therapeutic applications including pain relief. It was widely used as analgesic in the 19th century and at the beginning of the 20th century was claimed to be the best remedy for migraine headache. Basic research concerning the involvement of the cannabinoid receptors and the endogenous ligands in pain has advanced considerably in the last decade, but this has not been paralleled by the development of novel analgesics.

One of the cannabinoid structures which was extensively studied as analgesic is nantradol, a considerably modified

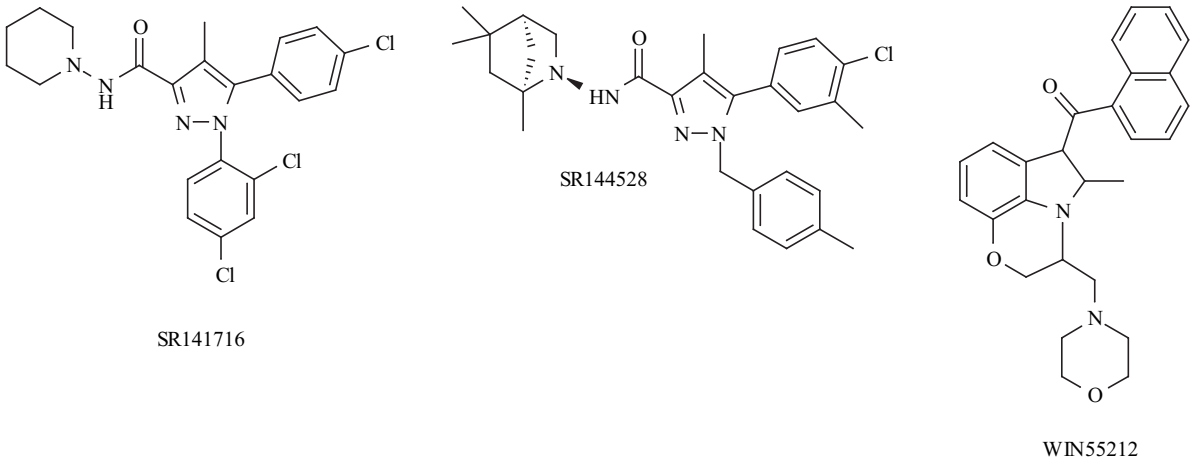


Fig. (7). Structures of some pyrazoles and aminoalkylindole.

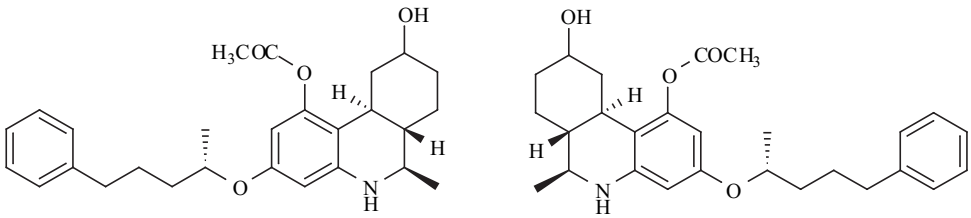


Fig. (8). Structure of the two isomers of nantradol.

structure, from Pfizer, with a nitrogen replacing the oxygen group of the dihydropyran ring. Originally synthesized as a 50:50 mixture of two pairs of racemic diastereoisomers (dextro and levo) and the pair with the opposite chirality at 2', it showed greater potency than morphine in some analgesic tests in animals. After the enantiomers were separated, levonantradol turned out to be 100 times more active than the other enantiomer, dextronantradol, and four times as active as the original mixture. Nevertheless, and in spite of its promising pharmacological profile, it had to be withdrawn because of its psychotropic effects [65] Fig. (**8**).

The biochemical basis of the involvement of the cannabinoid system in pain is now well established. Evidence has been provided for the fact that cannabinoid receptor agonists are active in animal models of acute, inflammatory and nerve injury induced pain, and that the antinociceptive effects of the cannabinoids are displayed after systemic, spinal, supraspinal and peripheral administration. Several recent publications have dealt with the subject of cannabinoids and analgesia [66-69]. Special mention should be made to the review by Pertwee which extensively deals with the knowledge of cannabinoid receptors and pain up to 2001 [32] and so, this paper will summarize the literature having appeared after that date.

Recently, Hohmann [69] has reviewed the spinal and peripheral mechanisms of cannabinoid antinociception and has come to the following conclusions:

i) cannabinoids suppress nociceptive processing through supraspinal, spinal and peripheral mechanisms;

ii) cannabinoids suppress neuronal hyperexcitability and central sensitization;

iii) endogenous cannabinoids suppress pain. The role of endocannabinoids in pain modulation has extensively been dealt with by several authors (for recent publications see Walker [70, 71], and Rice [67]).

iv) cannabinoids suppress hyperalgesia and allodynia through actions on the CB1 and CB2 receptors.

v) cannabinoid receptors are anatomically localized to modulate nociceptive transmission through actions in the spinal cord and periphery.

This same author has recently provided direct evidence that a peripheral cannabinoid mechanism suppresses spinal fos protein behaviour and pain behaviour in a rat model of inflammation [72].

Special interest is now being paid to the inhibition of pain responses by activation of CB2 receptors [73, 74] since the use of CB2 agonists could be a possibility for the treatment of acute and chronic pain without psychoactive effects [75]. This is especially relevant for the treatment of neuropathic pain and will therefore be dealt with in the next section.

Finally, two issues relevant to cannabinoid analgesia should be mentioned: One is the relationship between opioids and cannabinoids. Several functional studies have shown the relationship between these two systems [76], and this continues to be the subject of research. For example, there have been recent reports dealing with cannabinoids and dynorphin, and it seems that antinociception produced by spinal cannabinoids is mediated directly through the activation of cannabinoid receptors without the requirement of dynorphin release or activation of κ opioid receptors [77].

The other important issue when dealing with cannabinoid analgesia is the fact that chronic administration of cannabinoids results in the development of tolerance and dependence. According to Maldonado [78], although there is a large amount of information on cannabinoid dependence in animals, there remain several important issues to be clarified. Tolerance, dependence and motivational responses induced by anandamide seem to be different from the responses induced by exogenous cannabinoid agonists, and so the neurobiological mechanisms and the specific receptors involved must be further studied.

CANNABINOIDS AND NEUROPATHIC PAIN

Neuropathic pain is one of the main challenges of pain therapy. Actually, there are no effective treatments, due to its complex or unknown aetiology and to the reduced effectiveness of the most potent clinically used analgesics, the opioids. On the other hand, there are no good experimental models to study this kind of pain, and so, the search for new useful approaches, for the treatment of neuropathic pain, is an important target, both for clinical as well as basic researchers. In this context, cannabinoids are one of the new promising proposals.

The cannabinoid system is one of the endogenous systems that may modulate pain perception. It has been previously described that natural and synthetic cannabinoids may be used in the treatment of pain and their efficacy in the treatment of neuropathic pain is under evaluation at the moment [67] and most of the reviews focused on cannabinoids and pain include some interesting chapters about neuropathic pain [32, 69, 73, 79].

It is generally accepted that the slight opioid effectiveness in the treatment of neuropathic pain may be due, at least in part, to the reduction of the number of opioid

receptors in the spinal dorsal horn after chronic nerve injuries [80]. Regarding the cannabinoid system, it has been established that peripheral nerve injuries are not associated with such a depletion of CB1 receptors [81, 82]. Even more, there is an up regulation of CB1 receptors in the thalamus following peripheral nerve injuries [83]. This data supports the interest in the evaluation of cannabinoids as an alternative for the treatment of neuropathic pain and the involvement of the endocannabinoid system in the modulation of neuropathic pain perception is being widely studied.

Most of the experimental work has been done using several models of chronic constriction to injury of the rat sciatic nerve, or spinal nerve ligation to evaluate the effect of cannabinoids on thermal hyperalgesia or mechanical and cold allodynia. The targets of these works are: to confirm the effectivity, to determine the subtype of receptors, and to analyze mechanisms and anatomic structures involved in the cannabinoid antinociceptive activity.

Concerning the anatomical localization of cannabinoid mechanisms related with the control of neuropathic pain, there are a few but interesting data. It has been demonstrated that partial sciatic nerve ligation activates descending antinociceptive pathways related with the activation of *nucleus reticularis gigantocellularis pars alpha* [84] and that there is a CB1 receptor mediated inhibitory system which is activated in response to chronic noxious input. This descending modulation may be blocked by microinjection of the cannabinoid antagonist SR141716. These data confirm the participation of the *nucleus reticularis gigantocellularis pars alpha* in the descending nociceptive modulation, and also demonstrate that CB1 receptor activation mediates this antinociceptive effect [85]. Other central structures are probably involved for, as described above [83]; there is an up regulation of CB1 receptors in the thalamus following peripheral nerve injury that can contribute to increase the analgesic efficacy of cannabinoids in chronic neuropathic pain.

Interestingly, peripheral localizations for the antinociceptive cannabinoid activity have also been suggested [86] because of the antinociceptive effect of low doses of WIN55212 peripherally administered, and since peripheral, but not central, administration of the CB1 antagonist SR141716 was able to block the antihyperalgesia induced by WIN55212.

These results are in agreement with those reported for CB1 [87] and CB2 [88] agonists demonstrating that peripheral, but not systemic or central administration, of low doses of anandamide or palmioylethanolamide inhibited carrageenin induced thermal hyperalgesia by activation of CB receptors. In addition, the administration of SR141716 enhances thermal hyperalgesia and mechanical allodynia in hind paws of rats that have been rendered hyperalgesic by unilateral sciatic nerve ligation, at doses that does not alter sensitivity in naive animals without major side effects [89]. These findings support the selective role of CB1 receptor activation in the modulation of neuropathic pain perception.

Effects of cannabinoid agonists at the spinal level have also been investigated using spinal nerve (L4-L5) ligation model of neuropathic pain [90]. *In vivo* extracellular recording of A- and C- fiber evoked responses of dorsal horn neurons in nerve injured and sham operated rats were performed and a number of potential actions were evaluated after spinal or systemic administration of HU210. This CB1 agonist significantly reduced C-fiber mediated responses of spinal neurons in sham operated but not in nerve injured rats. The inhibitory effects of HU210 were blocked by systemic administration of SR141716 confirming the CB1 participation [91]. Regarding effects on Aδ-fiber, HU210 significantly reduced evoked neuronal responses, in both sham operated and nerve injured rats. The loss of effect of spinal HU210 on C-fiber, but not Aδ-fiber, mediated responses following injury indicate that CB receptors on C-fiber are functionally down-regulated. On the other hand, Aβ-fiber evoked responses of dorsal horn neurons were not influenced by HU210. Taking together these data and considering that there is evidence supporting the effectiveness of CB1 cannabinoid agonists on behavioral (hyperalgesia and allodynia) induced by nerve injury [87-89], Chapman [91] suggests that Aδ-fiber could mediate actions of cannabinoids on neuropathic induced pain.

In addition, the participation of peripheral CB receptors has been studied investigating the ability of cannabinoid agonists to inhibit neuropeptide release from primary afferent fibers in the skin. Isolated paw skin provides an *in vitro* model to study peripheral terminals of primary afferent neurons. Tissues obtained from diabetic rats were used as a model of peripheral neuropathy, which is a frequent complication of diabetes mellitus and the effects of endogenous and synthetic cannabinoids in the capsaicin-evoked CGRP release from isolated hindpaw skin of diabetic and control rats were studied. Capsazepine or neonatal capsaicin treatments abolished the response to capsaicin in control animals, suggesting that this effect is mediated by vanilloid receptors [92]. Anandamide inhibits capsaicin evoked CGRP release in non-diabetic rats [87], however neither CB1 nor CB2 antagonists were able to inhibit this effect; from this, it could be suggested that anandamide could act through non-CB1 or CB2 receptors [93]. The administration of the CB1/CB2 agonist, CP55940, inhibited capsaicin evoked CGRP release, in both diabetic and non-diabetic rats, and CB1 antagonist SR141716, but not the CB2 receptor antagonist SR144528, attenuated the effect of the agonist [93]. These results are in agreement with the hypothesis that CB1 receptors play a role in the modulation of the perception of peripheral neuropathies.

Most of the reported data support the role of CB1 receptors in the effect of cannabinoid on neuropathic models. Nevertheless there are also evidences suggesting the involvement of CB2 receptors [75]. Although these receptors have not been detected on neuronal tissues, there is evidence for the antinociceptive effectiveness of CB2 agonists on acute nociceptive [94, 95] and inflammatory [74, 96, 97] pain and, what is more important, the analgesic effect was not complicated with behavioral impairments (inhibition of ambulation or induction of catalepsy or hypothermia). Concerning neuropathic pain, there are data supporting the role of CB2 receptors: systemic or topic administration of AM1241, a CB2 selective agonist, is able to reverse the tactile and thermal hypersensitivity induced by nerve constriction [98].

Side effects are one of the main problems to propose the use of cannabinoids. Although doses needed to reverse mechanical allodynia in rat are smaller than those needed to induce behavioral impairment and at these doses WIN55212 even lacks antinociceptive effect, the effect on thermal hyperalgesia is reached at doses that affect motor performance [86]. The reason for these differences is unclear and could indicate different roles for CB receptors. Even more there are data supporting that peripheral mechanisms underlie the antinociceptive effect of CB1 cannabinoid agonists and evidence of CB2 mediated analgesic effects. Taken together all these findings permit to suggest that it could be possible to develop cannabinoid analgesics lacking psychotropic effects.

CONCLUSION AND PERSPECTIVES

For centuries, cannabis extracts have been used for therapeutic applications including pain relief. Nowadays, there is considerable evidence for cannabinoid analgesia in various animal models of pain, including acute antinociceptive, inflammatory and neuropathic pain. Cannabinoid agonists are therefore potential compounds to be developed as analgesics provided some of their drawbacks are overcome. These include sufficient bioavailability, the possible development of tolerance and dependence, and especially, the separation of the psychotropic from the analgesic effects. In this respect, CB2 receptor agonists, which do not produce CNS effects, may represent a promising therapeutic approach for pain treatment.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

AEA	=	Anandamide
ANT or AMT	=	Anandamide transporter
2-AG	=	2-Arachydonoylglycerol
CBD	=	Cannabidiol
CBG	=	Cannabigerol
CB1	=	Cannabinoid receptor 1
CB2	=	Cannabinoid receptor 2
CB3	=	Cannabinoid receptor 3
CNS	=	Central nervous system
FAAH	=	Anandamide amidohydrolase
GPCRs	=	G-Protein coupled receptors
NADA	=	*N*-Arachidonoyl-dopamine
Δ^8-THC	=	Δ^8-Tetrahydrocannabinol
Δ^9-THC	=	(-)-Δ^9-Tetrahydrocannabinol
VR1	=	Vanilloid receptor 1

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