Targeting the Cannabinoid System to Produce Analgesia

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Contents

Abstract Cannabinoid receptors are present at key sites involved in the relay and modulation of nociceptive responses. The analgesic effects of the cannabinoid CB_1 receptor are well described. The widespread distribution of these receptors in the brain does, however, also explain the side-effects associated with CB_1 receptor agonists. The cannabinoid CB_2 receptor also produces analgesic effects in models of acute, inflammatory and neuropathic pain. The sites and mechanisms of CB_2 receptor-mediated analgesia are described herein. In addition to targeting cannabinoid receptors directly, protection of endocannabinoids (eCBs) from metabolism also produces analgesic effects. Indeed, reports that noxious stimulation elevates levels of eCBs in the spinal cord and brain provide further rationale for this approach. The effects of inhibition of fatty acid amide hydrolase (FAAH) on nociceptive responses in models of inflammatory and neuropathic pain are discussed.

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Abbreviations

2AG	2-Arachidonoylglycerol
AEA	*N*-Arachidonoylethanolamine; Anandamide
CB_1	Cannabinoid-1 receptor
CB_2	Cannabinoid-2 receptor
CCI	Chronic constriction injury
CFA	Complete Freund's adjuvant
COX-2	Cyclooxygenase type 2
DGL	Diacylglycerol lipase
DRG	Dorsal root ganglion
FAAH	Fatty acid amide hydrolase
i.p.	Intraperitoneal administration
i.pl.	Intraplantar administration
MAPK	Mitogen activated protein kinase
MGL	Monoacylglycerol lipase
NAAA	*N*-Acylethanolamine hydrolysing acid amidase
NADA	*N*-Arachidonoyl dopamine
NAE	*N*-Acylethanolamines
NAPE	*N*-Acylphosphatidylethanolamine
OA	Osteoarthritis
OEA	*N*-Oleoylethanolamine
PAG	Periaqueductal grey
PEA	*N*-Palmitoyl ethanolamine
PLC	Phospholipase C
PLD	Phospholipase D
p.o.	Oral administration
RA	Rheumatoid arthritis
SNL	Spinal nerve ligation
Δ^9-THC	Δ^9-Tetrahydrocannabinol
TRPV1	Transient receptor potential vanilloid type 1

1 Introduction

The anti-nociceptive effects of cannabinoids are well documented (Hohmann 2002; Iversen and Chapman 2002; Jhaveri et al. 2007a, b; Pertwee 2001; Rice et al. 2002; Walker and Huang 2002). The analgesic effects of cannabinoids are, however, often limited by psychoactive side-effects. In the last decade, rapid scientific progress has

revealed an endogenous cannabinoid system, which consists of cannabinoid receptors, endogenous cannabinoid ligands and their synthesizing and metabolising enzymes. This progress has led to the investigation of the individual components of the cannabinoid system as targets for producing analgesia and other medicinal effects, with minimal side-effects. This chapter will discuss the application of these approaches to the development of novel analgesics.

2 The Endogenous Cannabinoid System

Two cannabinoid receptors, the cannabinoid-1 (CB_1) and cannabinoid-2 (CB_2) have been identified, cloned and pharmacologically characterised (see Mackie 2006). Both receptors are $G_{i/o}$-protein coupled receptors negatively coupled to adenylyl cyclase and positively coupled to mitogen activated protein kinase (MAPK). A third receptor, GPR55, binds a number of cannabinoid ligands and therefore has been proposed as a member of the cannabinoid receptor family (Brown 2007; Johns et al. 2007; Lauckner et al. 2008; Ryberg et al. 2007).

CB_1 receptors are associated with neuronal tissue, with high density in the central, peripheral and autonomic nervous system (Egertova and Elphick 2000; Herkenham et al. 1991; Tsou et al. 1998). CB_1 receptors are also present at lower densities in the heart, lung, testis, ovary, bone marrow, thymus, uterus and immune cells (Galiegue et al. 1995). CB_1 receptor density is moderate to high in regions involved in pain transmission and modulation, such as the dorsal root ganglion (DRG), spinal cord, thalamus, periaqueductal grey (PAG), amygdala and rostroventromedial medulla (Tsou et al. 1998). The effects of cannabinoid agonists on brain function have been investigated with functional magnetic resonance imaging studies. Systemic administration of a non-selective CB_1/CB_2 agonist increased regional cerebral blood flow, an indirect index of brain activity, in cortical regions, hippocampus, PAG, nucleus accumbens and striatum (Chin et al. 2008). Thus, the brain regions activated by the cannabinoid ligand correspond well to those regions identified by autoradiographic approaches.

Given that the bulk of the unwanted effects of cannabinoids arises due to activation of CB_1 receptors, recent research has focused on the potential for CB_2 receptor agonists as analgesics. At high densities, CB_2 receptors are primarily on immune tissues. Nevertheless, a putative role of the CB_2 receptor in the nervous system is becoming apparent. Although early studies indicated an absence of CB_2 receptors in the central nervous system, recent work has reported the presence of CB_2 mRNA in the spinal cord of control rats (Beltramo et al. 2006) and brain tissue (Gong et al. 2006; Van Sickle et al. 2005). The functional role of CB_2 receptors in the CNS is unclear. A functional imaging study demonstrated that CB_2 receptor antagonism did not alter brain activation evoked by systemic administration of a non-selective cannabinoid agonist (Chin et al. 2008). These data suggest there is little CB_2-mediated cannabinoid-induced brain activity under control conditions.

2.1 Endocannabinoids

Currently, five endogenous cannabinoid receptor ligands or eCBs have been discovered, of which anandamide (AEA) was the first to be identified (Devane et al. 1992). Since then, 2-arachidonoylglycerol (2AG; Mechoulam et al. 1995), noladin ether (Hanus et al. 2001), virodhamine (Porter et al. 2002) and *N*-arachidonoyldopamine (NADA; Huang et al. 2002) have been identified. ECBs are synthesized de novo and their actions are rapidly terminated by being taken up into cells where they are metabolised by enzymatic hydrolysis.

2.2 Endocannabinoid Synthesis

Several different pathways have been suggested to contribute to the synthesis of the *N*-acylethanolamines (NAEs) AEA, *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA) from *N*-acylphosphatidylethanolamine (NAPE). In addition to the established NAPE-phospholipase D (PLD) pathway, two alternative pathways via phospholipase C (PLC)-PTPN22 (Liu et al. 2008) and αβ hydrolase (αβH4)-GDE1 (Simon and Cravatt 2008) are able to generate NAEs. These multiple pathways may subserve differential synthesis of NAEs, as it has been suggested that NAPE-PLD mainly generates saturated *N*-acylethanolamines (NAEs) such as PEA (Leung et al. 2006). Tissue distribution of these synthetic enzyme pathways may also vary. AEA and PEA biosynthesis in the CNS is suggested to be predominantly via the αβH4-GDE1 pathway (Simon and Cravatt 2008). In macrophages, PLC-mediated cleavage of NAPE to phosphoanandamide prior to PTPN22-mediated dephosphorylation to NAE has been described (Liu et al. 2006).

2.3 Endocannabinoid Metabolism

AEA and other NAEs are mainly hydrolysed by FAAH (Cravatt et al. 1996; Deutsch and Chin 1993) whilst 2AG is mainly metabolised by monoacylglycerol lipase (MGL; Dinh et al. 2002). Although FAAH and MGL are the main enzymes for metabolism of AEA and 2AG, enzymes such as cyclooxygenase type 2 (COX-2; for review see Fowler 2007) also metabolise AEA and 2AG. In addition, *N*-acylethanolamine hydrolysing acid amidase (NAAA) can also metabolise AEA and PEA (Tsuboi et al. 2007).

3 Endocannabinoids and Pain Processing

The anti-nociceptive effects of eCBs are well described. We have demonstrated anti-nociceptive effects of AEA when administered spinally (Harris et al. 2000) and peripherally (Sokal et al. 2003) using extracellular recordings of dorsal horn

neurones in carrageenan-inflamed rats. AEA is also anti-nociceptive in behavioural models of acute and chronic pain (for review see Pertwee 2001). Similarly, 2AG reduces pain behaviour in tail-flick (Mechoulam et al. 1995) and formalin tests (Guindon et al. 2007).

AEA and 2AG are present in key regions involved in the detection, relay and integration of nociceptive inputs, including the skin, DRG, spinal cord, PAG and rostral ventromedial medulla. There is good evidence that eCBs tonically inhibit pain responses and contribute to the setting of nociceptive thresholds. Indeed, spinal administration of selective CB_1 receptor antagonists increases evoked-firing of dorsal horn neurones and thermal hyperalgesia (Chapman 1999). In turn, levels of eCBs are altered under pathological conditions such as inflammation and neuropathic pain. We have demonstrated a significant reduction in levels of AEA and PEA in the hindpaw of rats with a carrageenan-induced inflammation (Jhaveri et al. 2008b). Similarly, levels of AEA, 2AG and PEA were decreased in the hindpaw following intraplantar injection of formalin (Maione et al. 2007). By contrast, Beaulieu et al. reported no significant alteration in levels of AEA, 2AG and PEA in the hindpaw of formalin-treated rats (Beaulieu et al. 2000). In addition to altering levels of eCBs at the site of injury, noxious stimulation such as formalin-evoked hindpaw inflammation increases levels of eCBs at other targets in the nociceptive pathway such as the periaqueductal grey, indicating a role for eCBs in descending control of pain processing (Walker et al. 1999).

Alterations in the levels of eCBs and NAEs under various pathological conditions may occur as a result of either enhanced synthesis or decreased catabolism. ECB levels increased in the spinal cord (Petrosino et al. 2007) and dorsal root ganglia (Mitrirattanakul et al. 2006) following peripheral nerve injury, a model of neuropathic pain. We have shown that levels of AEA are increased, whereas levels of PEA are decreased, in the spinal cord (unpublished observations) in a model of neuropathic pain. These data suggest there is differential synthesis, or catabolism, of AEA and PEA in the spinal cord of neuropathic rats. How these findings relate to the presence of additional cell types, such as activated microglia, which will contribute to the synthesis and catabolism of eCBs, and the emerging evidence for multiple cell related synthesis pathways, is unknown.

4 CB Receptor-Mediated Analgesia

The analgesic effects produced by activation of CB_1 receptors are well described and extensively reviewed (for reviews see Iversen and Chapman 2002; Pertwee 2001; Walker and Huang 2002). Activation of CB_1 receptors in the spinal cord (Hohmann et al. 1998; Kelly and Chapman 2001, 2003) and in the periphery (Kelly et al. 2003) attenuates nociceptive responses of dorsal horn neurones in naive rats. Supra-spinal CB_1 receptors in a number of discrete brain regions also contribute to the anti-nociceptive effects of cannabinoids in models of acute/tonic pain (Finn et al. 2003; Lichtman et al. 1996; Martin et al. 1999; Meng et al. 1998;

Welch et al. 1998; Welch and Stevens 1992). The broad distribution of CB_1 receptors in the brain results in both therapeutic effects, such as analgesia, as well as their side-effects. To avoid these psychoactive side-effects, the analgesic potential of selective activation of peripheral and spinal CB_1 receptors has been studied. Anti-nociceptive effects of CB_1 receptor agonist were substantially reduced in mice with CB_1 receptor gene deletion in the peripheral nociceptors (Agarwal et al. 2007). Thus it appears that CB_1 receptor agonists which do not cross the blood–brain barrier, and thus selectively activate peripheral CB_1 receptors, may be a promising analgesic strategy. This concept is supported by earlier work demonstrating that hindpaw injection of CB_1 receptor agonists produces anti-nociceptive effects in models of inflammatory and chronic pain (Clayton et al. 2002; Elmes et al. 2005; Kelly and Chapman 2002, 2003; Kelly et al. 2003; Richardson et al. 1998; Scott et al. 2004).

Recently, a number of studies have demonstrated analgesic effects of CB_2 agonist receptor agonists in models of acute and chronic pain (reviewed elsewhere by Guindon and Hohmann 2008; Jhaveri et al. 2007b). Administration of CB_2 agonists systemically (Ibrahim et al. 2006; Malan et al. 2001; Valenzano et al. 2005) or locally into the hindpaw (Elmes et al. 2004; Malan et al. 2001) attenuates nociceptive responses in naïve rats. CB_2 receptors are present in the skin and their activation is reported to release endorphins from keratinocytes which act via μ opioid receptors to produce analgesia (Ibrahim et al. 2005). There is little evidence that spinal (Sagar et al. 2005) or supra-spinal (Jhaveri et al. 2008a) CB_2 receptors modulate nociceptive responses in naive rats, despite the description of supra-spinal CB_2 receptors (see earlier). There is, however, evidence for a functional role of CB_2 receptors in the spinal cord (Romero-Sandoval et al. 2008; Sagar et al. 2005; Yamamoto et al. 2008) and thalamus (Jhaveri et al. 2008a) of neuropathic rats. Importantly, CB_2 receptor agonists are devoid of CNS-mediated side-effects (Malan et al. 2003).

5 Attenuation of Endocannabinoid Catabolism Produces Analgesia

As mentioned earlier, the beneficial and analgesic effects of eCBs are hampered by their short duration of action. In order to prolong these effects, research has investigated the effects of inhibiting the breakdown of eCBs. One of the benefits of inhibiting the catabolism of eCBs is that regions with elevated levels of eCBs, for example as a result of noxious stimulation, are targeted as opposed to the global effects of receptor agonists.

The important role of FAAH in metabolism of eCBs has been demonstrated in mice lacking FAAH, which exhibit 15-fold elevated levels of AEA compared to wild-type mice, and display phenotypic hypoalgesia in models of acute and inflammatory pain (Cravatt et al. 2001; Lichtman et al. 2004b), but not neuropathic pain

(Lichtman et al. 2004b). Inhibitors of FAAH, such as URB597 and OL135, are antinociceptive in models of acute and inflammatory pain (Chang et al. 2006; Fegley et al. 2005; Jayamanne et al. 2006; Kathuria et al. 2003; Lichtman et al. 2004a; Russo et al. 2007 (Table 1). A single systemic injection of URB597 significantly reduced thermal allodynia and mechanical hyperalgesia in the Complete Freund's adjuvant (CFA) model of inflammation (Jayamanne et al. 2006). In the carrageenan model of inflammation, we reported that intraplantar injection of URB597 increased levels of AEA and 2AG in hindpaw skin and reduced carrageenan hyperalgesia (Jhaveri et al. 2008b).

The effects of inhibition of FAAH on neuropathic pain behaviour are less consistent than those reported for inflammatory pain states. Acute systemic injection of URB597 (0.3 mg kg^{-1} i.p.) did not alter mechanical allodynia in a model of peripheral neuropathy (Jayamanne et al. 2006). Similarly, a single oral dose of URB597 (10 mg kg^{-1} p.o.) had limited effects on mechanical hyperalgesia in the chronic constriction injury model of peripheral neuropathy (Russo et al. 2007). Repeated oral dosing of URB597 (10 mg kg^{-1} for 4 days p.o.) significantly reduced thermal and mechanical hyperalgesia (Russo et al. 2007) and a far higher dose of OL135 (ED_{50} 9 mg kg^{-1} i.p.) reduced mechanical allodynia (Chang et al. 2006) in neuropathic rodents. These data suggest that there is an alteration in synthesis/metabolism of eCBs and eCB-like compounds or their receptor function following peripheral neuropathy, which is also supported by data from our electrophysiological experiments. Peripheral injection of URB597, at a dose (25 µg in 50 µl) effective in reducing mechanically evoked responses of spinal cord dorsal horn neurones in sham-operated rats, did not alter responses in neuropathic rats (Jhaveri et al. 2006). A fourfold higher dose (100 µg in 50 µl, i.pl.) of URB597 did, however, reduce mechanically evoked responses in these animals (Jhaveri et al. 2006). In the same study, spinal administration of URB597 (10–50 µg in 50 µl) was equi-effective at reducing mechanically evoked responses of dorsal horn neurones in neuropathic and sham-operated rats, suggesting that alteration in the synthesis/metabolism of endocannabinoids and related molecules is focal and not global (Jhaveri et al. 2006).

6 Arthritis – A Therapeutic Target for Cannabinoids?

One of the groups of pain patients in which clinical effectiveness of cannabis-based medicines has been shown is arthritis. Anecdotal evidence indicates the effectiveness of cannabis in arthritis patients (Wright et al. 2006) and the cannabis-based drug Sativex produced significant analgesia in a double-blind multicentre group comparison study of patients with arthritis (Blake et al. 2006) (Table 1).

Recently, we have demonstrated the expression of cannabinoid CB_1 and CB_2 receptors in the synovial tissue of patients with rheumatoid arthritis (RA) and osteoarthritis (OA). In addition, we reported the presence of AEA and 2AG in the synovial fluid of OA and RA patients, neither of which were detected in samples

Table 1 Comparison of effects of FAAH inhibitors in models of neuropathic and inflammatory pain

Species	Route	Neuropathic pain	Inflammatory pain
Rat	Peripheral (i.pl.)	URB597 inhibited mechanically evoked responses following SNL (Jhaveri et al. 2006)	URB597 inhibited carrageenan-evoked changes in weight-bearing (Jhaveri et al. 2008b)
Rat	(i.pl.)		*N*-Arachidonoyl-serotonin attenuates formalin-evoked hyperalgesia (Maione et al. 2007)
Rat	Spinal	URB597 inhibited mechanically evoked responses following SNL (Jhaveri et al. 2006)	
Rat	(i.p.)	*N*-Arachidonoyl-serotonin inhibited thermal hyperalgesia and mechanical allodynia following CCI (Maione et al. 2007)	*N*-Arachidonoyl-serotonin inhibited formalin-evoked hyperalgesia (Maione et al. 2007)
Rat	(i.p.)	URB597 had no effect on mechanical allodynia (Jayamanne et al. 2006)	URB597 reduced CFA-induced allodynia and thermal hyperalgesia (Jayamanne et al. 2006)
Rat	(i.p.)		URB597 decreased carrageenan-evoked paw oedema (Holt et al. 2005)
Mouse	(i.p.)	OL135 inhibited mechanical allodynia (Chang et al. 2006)	
Mouse	(p.o.)	Chronic dosing of URB597 decreased mechanical allodynia following partial nerve ligation (Russo et al. 2007)	

from normal volunteers (Richardson et al. 2008). Whether the peripheral cannabinoid receptors present in the synovium are able to modulate arthritis-induced pain remains unknown. Inhibitory effects of cannabinoids have, however, been demonstrated in animal models of both OA and RA. Systemic administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) was anti-nociceptive in adjuvant-induced arthritis (Cox et al. 2007a, b; Cox and Welch 2004; Smith et al. 1998), an effect mediated by CB_2 receptors (Cox et al. 2007a) and which involved interaction with the opioid receptor system (Cox et al. 2007b; Cox and Welch 2004). Furthermore, application of the CB_1 receptor agonist ACEA onto joint primary afferent fibres decreased arthritis-induced increases in frequency of firing of joint primary afferent fibres in rats (Schuelert and McDougall 2008). AEA also produces analgesia in models of arthritis; these effects did not appear to be CB_1 mediated, but like the effects of Δ^9-THC were attenuated by the opioid receptor antagonist naloxone, implicating a role for the opioid system (Smith et al. 1998). It appears however that the dose of AEA and route of administration used is key, since close arterial injection of AEA produced excitation of nociceptive fibres in knee joints of arthritic rats and normal control rats which was mediated via the transient receptor potential vanilloid 1 (TRPV1) receptor (Gauldie et al. 2001).

A further advantage of cannabinoid-based medicines as candidates for the treatment of rheumatic conditions is their effects on bone metabolism. AEA and 2AG have been identified in bone at levels similar to that in the brain (Bab et al. 2008) and their synthesis is reported in both osteoclasts and osteoblasts in vitro (Tam et al. 2006). The expression of the synthetic enzyme for 2AG, diacylglycerol lipase (DGL), in osteoblasts, osteocytes and bone-lining cells and the presence of FAAH in bone cells (Bab et al. 2008) collectively indicate the role of the cannabinoid system in bone turnover. Indeed, both CB_1 and CB_2 receptors contribute to the regulation of bone mass and the CB_2 receptor is a putative target for osteoporosis and other bone diseases (Bab et al. 2008; Karsak et al. 2005; Ofek et al. 2006).

In conclusion, the analgesic effects of cannabinoid-based medicines acting at CB_1 receptors are well described, but limited by adverse side-effect profiles. The identification of alternative cannabinoid entities, such as the CB_2 receptor and enzymes engaged in the catabolism of eCBs, offers further opportunity for the development of novel cannabinoid based analgesics with an improved side-effect profile.

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