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Targeting CB_2 receptors and the endocannabinoid system for the treatment of pain

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Abstract

The endocannabinoid system consists of the cannabinoid (CB) receptors, CB_1 and CB_2, the endogenous ligands anandamide (AEA, arachidonoylethanolamide) and 2-arachidonoylglycerol (2-AG), and their synthetic and metabolic machinery. The use of cannabis has been described in classical and recent literature for the treatment of pain, but the potential for psychotropic effects as a result of the activation of central CB_1 receptors places a limitation upon its use. There are, however, a number of modern approaches being undertaken to circumvent this problem, and this review represents a concise summary of these approaches, with a particular emphasis upon CB_2 receptor agonists. Selective CB_2 agonists and peripherally restricted CB_1 or CB_1/CB_2 dual agonists are being developed for the treatment of inflammatory and neuropathic pain, as they demonstrate efficacy in a range of pain models. CB_2 receptors were originally described as being restricted to cells of immune origin, but there is evidence for their expression in human primary sensory neurons, and increased levels of CB_2 receptors reported in human peripheral nerves have been seen after injury, particularly in painful neuromas. CB_2 receptor agonists produce antinociceptive effects in models of inflammatory and nociceptive pain, and in some cases these effects involve activation of the opioid system. In addition, CB receptor agonists enhance the effect of μ-opioid receptor agonists in a variety of models of analgesia, and combinations of cannabinoids and opioids may produce synergistic effects. Antinociceptive effects of compounds blocking the metabolism of anandamide have been reported, particularly in models of inflammatory pain. There is also evidence that such compounds increase the analgesic effect of non-steroidal anti-inflammatory drugs (NSAIDs), raising the possibility that a combination of suitable agents could, by reducing the NSAID dose needed, provide an efficacious treatment strategy, while minimizing the potential for NSAID-induced gastrointestinal and cardiovascular disturbances. Other potential “partners” for endocannabinoid modulatory agents include α_2-adrenoceptor modulators, peroxisome proliferator-activated receptor α agonists and TRPV1 antagonists. An extension of the polypharmacological approach is to combine the desired pharmacological properties of the treatment within a single molecule. Hopefully, these approaches

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will yield novel analgesics that do not produce the psychotropic effects that limit the medicinal use of cannabis.

Keywords

Cannabinoid; Anandamide; CB_2 receptor; Fatty acid amide hydrolase; Neuropathic pain; Inflammatory pain

1. Introduction

In 1993 it was reported in Nature that a carbonized material recovered from the abdomen of a young girl who had died in childbirth in the fourth century AD contained traces of a stable constituent of cannabis (Zias et al., 1993). Although this represents a documented early use of cannabis for the presumed treatment of pain, the use of cannabis extracts for this and other medical indications, as well as for recreational uses, has been described in both the ancient world and in more modern times (review, see Mechoulam, 1986). Currently, Sativex™, a buccal formulation of cannabis extract with defined ratios of Δ^9-tetrahydroxannabinol (THC, the main psychoactive ingredient of cannabis) and cannabidiol, is licensed in Canada for the treatment of pain in multiple sclerosis patients (Perez and Ribera, 2008). A major issue, however, for all treatments based on cannabis is the potential for psychotropic effects and concerns about the long-term use of such medications. These concerns place a limitation upon the dosages that can be given and hence the potential level of pain relief. There are, however, a number of approaches that can be taken to circumvent this problem.

2. The endocannabinoid system

The endocannabinoid system consists of the G-protein coupled cannabinoid (CB) receptors, CB_1 and CB_2, the endogenous ligands anandamide (AEA, arachidonoylethanolamide) and 2-arachidonoylglycerol (2-AG), and their synthetic and metabolic machinery. The role of these endocannabinoids and other putative endocannabinoids in pain modulation and pathways for their synthesis and degradation has recently been reviewed (Hohmann and Suplita 2006). CB_1 receptors are primarily neuronal (although they have been found in non-neuronal tissue) and mediate the psychotropic actions of cannabis (see Monory et al. (2007) for a genetic dissection of the neuronal populations involved in the different behavior al and autonomic effects of THC).

CB_1 receptors are expressed in neurons of the CNS (Matsuda et al. 1990), and in DRG neurons (Hohmann and Herkenham 1999, Price et al. 2003), but there is also evidence for the expression of CB1 receptors in non-neural tissue (see e.g. Tokanovic et al., 2007). Similarly, while the CB_2 receptor was originally considered to be expressed primarily in lymphoid tissues in the periphery (Munro et al. 1993, Di Marzo et al. 2004), recent evidence has pointed to a neuronal localization in some regions of the rodent brain (Onaivi et al. 2006), and CB_2 immunoreactivity has been shown in activated microglia in affected regions of multiple sclerosis and amyotrophic lateral sclerosis *post mortem* human spinal cord (Yiangou et al. 2006). A recent study has also presented evidence for the localization of CB_2

receptor-like immunoreactivity in human DRG sensory neurons in vitro (see Fig. 1), in injured nerves including neuromas, and in nerve fibers in human synovium and digit skin (Anand et al. 2008).

Both AEA and 2-AG are synthesized upon demand rather than being pre-stored, and have relatively short durations of action, due to effective metabolic pathways. In the case of AEA, this is brought about by a process of cellular accumulation followed primarily by hydrolysis to arachidonic acid, catalyzed by the enzyme fatty acid amide hydrolase (FAAH) (although it can also act as a substrate for both cyclooxygenase-2 and lipoxygenases). In the case of 2-AG, a similar pattern is seen, although in the brain the enzyme monoacylglycerol lipase is more important. At the outset, it should be pointed out that the exact process whereby cells accumulate AEA is not known and is a current area of controversy; mechanisms ranging from FAAH-gated diffusion to as yet unidentified designated transporter proteins having been postulated (see Fowler, 2008). In addition to the formal components of the endocannabinoid system summarized above, there are additional candidate cannabinoid receptors and endogenous ligands that have been described in the literature (reviews see Bradshaw and Walker, 2005; Brown, 2007). Therefore, endocannabinoid system can and should be regarded as a “work in progress” rather than an absolutely defined entity. The recent report that mice lacking GPR55 receptors (a putative CB receptor) do not show mechanical hyperalgesia following either complete Freund’s adjuvant treatment or partial nerve ligation (Staton et al., 2008) underlines the fact that the detailed characterization of the endocannabinoid system is an important research priority. Notwithstanding, a number of compounds are available that target different components of the currently accepted endocannabinoid system, compounds that have been found to have therapeutic potential in models of inflammatory and in some cases neuropathic pain (see Table 1 for a description of the main compounds forming the core of this review).

3. Targeting the endocannabinoid system for the treatment of pain

3.1. Peripherally-restricted cannabinoid agonists

It has been well established that the ability of cannabinoids to affect pain perception has supra-spinal, spinal and peripheral components (for review, see Hohmann, 2002; Walker and Hohmann, 2005). With respect to the peripheral component, local administration of both synthetic cannabinoids and exogenous anandamide and 2-AG produce antinociceptive effects in the formalin model of inflammatory pain (see e.g. Calignano et al., 1998; Guindon et al., 2007). Recently, Agarwal et al. (2007) reported the effects in pain models of the conditional deletion of CB_1 receptors in nociceptive neurons of the dorsal root ganglia of the mouse. These mice were more sensitive to the effects of intraplantar administration of capsaicin and formalin, whereas their motor performance on a rotorod test was not affected. The hyperalgesic response to administration of complete Freund’s adjuvant was also greater than for wild-type mice, and the ability of a synthetic cannabinoid to alleviate this response was also greatly reduced (Agarwal et al., 2007). These data would suggest that peripherally restricted cannabinoids may have utility in inflammatory pain. In this respect, Fride et al. (2004) have identified analog s of the (+)-enantiomer of cannabidiol that are active towards CB receptors and reduce the pain response in the formalin model of inflammatory pain,

without producing overt signs of central CB_1 receptor activation. Other peripherally restricted CB_1 or CB_1/CB_2 receptor agonists are currently being investigated as potential approaches to the treatment of pain. A good example of this is the study of Dziadulewicz et al. (2007). These authors reported that naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone bound as an agonist to human CB1 and CB2 receptors with IC_{50} values of 15 and 98 nM, respectively, and produced a good separation of effects upon neuropathic pain and catalepsy, consistent with a limited penetration of the compound into the brain.

3.2. CB_2 receptor-selective agonists (see Fig. 2)

Expression of CB_2 receptors in the peripheral and central nervous systems is modulated in a number of rodent pain models. No increase in CB2 receptor mRNA was apparent in the Freund's Complete Adjuvant (FCA) model of inflammatory pain, while upregulation of mRNA and protein were seen in the ipsilateral dorsal horn of the lumbar spinal cord using two models of neuropathic pain, the chronic constriction injury (CCI) (Bennett and Xie, 1988) and spinal nerve ligation (SNL) (Kim and Chung, 1992) models (Zhang et al., 2003). The authors suggest that the increased expression was in microglia as it colocalized with OX-42, a marker of microglia. The upregulation of CB_2 receptor mRNA in spinal cord was confirmed in a subsequent study using the SNL model (Beltramo et al., 2006). Here, the cell type was ambiguous, though in a separate set of experiments the authors did provide evidence for upregulation of CB_2 receptor mRNA in cultured rat spinal microglia. Expression of CB_2 receptors on microglia is supported by additional studies showing interferon γ (IFNγ) induces upregulation (Carlisle et al., 2002) with a distinct subcellular localization at the surface of activated microglia in culture (Walter and Stella, 2004). Upregulation of CB_2 receptors in spinal cord was also demonstrated immunohistochemically following either SNL or axotomy (Wotherspoon et al., 2005). However, in contrast to the earlier studies, colocalization with GAP-43 and galanin in the superficial lamina suggested expression on primary afferent (C fiber) terminals (Wotherspoon et al., 2005). This upregulation was absent in CB_2 receptor knockout mice. Supporting neuronal localization, increased CB_2 receptor immunoreactivity was also seen in nerve sections proximal, but not distal, to the site of ligation (Wotherspoon et al., 2005) (Fig. 2).

In addition to studies demonstrating the presence of CB_2 receptor in components of the pain pathway and alterations in expression level in pain models, agonists both selective and non-selective have been used to investigate the role of CB_2 in nociception. The non-selective endogenous agonists, AEA and PEA, have been combined with the CB_1 receptor-selective antagonists, AM251, AM281 and SR141716A and the CB_2 receptor-selective antagonists, AM630 and SR144528. Administration of AEA locally into the rat hindpaw reverses pain due to both intraplantar carrageenan and formalin (Sokal et al., 2003; Guindon et al., 2006a). While the AEA-induce analgesia following formalin was blocked by the CB_1 receptor antagonist AM251 (Guindon et al., 2006a), both the CB_2 and CB_1 receptor-selective antagonists, SR144528 and SR141716A, respectively, blocked the effect of AEA on carrageenan-induced hypersensitivity of spinal neurons (Sokal et al., 2003). These results imply a contribution of both peripherally expressed CB_1 and CB_2 receptors to the antinociceptive response. Importantly in the study by Sokal et al. (2003), local administration had no effect in non-inflamed tissue. Likewise, systemic administration has

been shown to reverse CFA-induced mechanical hyperalgesia in a non-CB_1 receptor sensitive manner (Smith et al., 1998).

The non-selective synthetic cannabinoid agonists HU-210, nabilone, CP55,940 and WIN55,212-2, have also been combined with CB_1 and CB_2 receptor-selective antagonists to confirm the role of CB_2 receptors in pain transmission. HU-210 reversed carrageenan-induced edema through the CB_2 receptor, as the CB_2 receptor-selective antagonist SR144528 blocked this effect (Clayton et al., 2002). A similar result was found when nabilone was combined with SR144528 (Conti et al., 2002). Studies have also been conducted with CP55,940 and WIN55,212-2. Both compounds are analgesic in assays of acute pain; the effect of CP55,940 is only partially blocked by CB_1 receptor-selective antagonists (Scott et al., 2004) while that of WIN55,212-2 is reduced in CB_2 receptor knockout animals compared to wild type, but remains in CB_1 receptor knockout animals (Ibrahim et al., 2006). CP55,940 administered either systemically or intrathecally has also proven efficacious against neuropathic pain in rats. Interestingly, only the antihyperalgesic effect of systemically administered CP55,940 could be partially blocked by SR144528 (Scott et al., 2004). This result could be due to insufficient CNS penetraton of the antagonist, or may imply that the effects in the spinal cord are due to CB_1 receptor activation alone. In the same study, the dose-dependent catalepsy that was observed was not blocked by a CB_2 receptor antagonist, reinforcing the notion that side-effects track with activation of CB_1 receptors. Finally, WIN55,212-2 has been shown to inhibit carrageenan-induced allodynia and mechanical hyperalgesia and burn-induced thermal and mechanical hyperalgesia after local administration, as well as bone cancer pain and inflammatory muscle pain after systemic administration (Nackley et al., 2003b; Kehl et al., 2003; Johanek and Simone, 2004). All effects, except for muscle pain, were shown to be at least partially blocked by CB2 receptor-selective antagonists.

Concerning studies investigating selective CB_2 agonists, a relatively large number of the pharmacological reports that have been published on the role of CB_2 receptors in pain have been generated using a limited number of selective agonists, namely HU-308, AM1241, GW405833, JWH 015 and JWH 133. HU-308 is a THC derivative that is reported to have an in vitro K_i value of ~23 nM at CB_2 receptors (versus a K_i value that is greater than 10 mM for the CB_1 receptor) (Hanuš et al., 1999). When administered intraperitoneally, it reduced both the inflammation associated with arachidonic acid-induced ear swelling and the late phase of formalin-induced pain behavior in mice, in an SR144528-sensitive manner (Hanuš et al., 1999). In addition, in the plantar incision model of post-surgical pain, it has been shown to reduce allodynia. This effect was blocked by the CB_2 receptor-selective antagonist SR144528 (LaBuda et al., 2005). HU-308 appears to have minimal off target effects as it did not affect acute nociception as measured on a 55 °C hot plate, did not cause catalepsy and did not inhibit ambulation or rearing in the open field (Hanuš et al., 1999).

The small molecule CB_2 receptor-selective compound that has most substantially penetrated the scientific literature is AM1241, that is reported to have an affinity for CB_2 receptors of 2 nM with a high degree of selectivity over CB_1 receptors (95-340 fold) (Malan et al., 2001a). This high degree of selectivity is maintained in membranes prepared from mouse spleen and brain, for CB_2 and CB_1 receptors, respectively (Ibrahim et al., 2003). AM1241 is a racemic

mix; considering the individual enantiomers R,S-AM1241 act as an agonist at human CB_2, but an inverse agonist at rat and mouse CB2 receptors. R-AM1241 binds with more than 40-fold higher affinity than S-AM1241, to human rat and mouse CB_2 receptors and displays a functional profile similar to that of the racemate. In contrast, S-AM1241 is an agonist at human, rat and mouse CB_2 receptors. AM1241 has been described as a protean agonist implying the state of constitutive receptor activity can determine the functional effect of a ligand-receptor interaction (Yao et al., 2006). Behaviorally, AM1241 did not elicit deficits in the rotarod or catalepsy assays (Malan et al., 2001a). AM1241 did however show antinociceptive effects towards an acute thermal stimulus (Hargreaves apparatus) when administered either systemically or locally; this effect could be reversed with the CB_2 receptor-selective antagonist, AM630 (Malan et al., 2001a). AM1241 has also been shown to be anti-inflammatory and efficacious against inflammatory, neuropathic and post-surgical pain, in addition to chemical-(capsaicin and formalin) induced pain and substance P-induced plasma extravasation, when administered either locally or systemically (Malan et al., 2001b; Nackley et al., 2003a; Quartilho et al., 2003; Hohmann et al., 2004; LaBuda et al., 2005). The effects of AM1241 are reversible with selective CB2 receptor antagonists (AM630 and SR144528), were not reversible with selective CB1 receptor antagonists (AM251 and SR141716A), and remained in animals that were null mutants for the CB1 receptor (Ibrahim et al., 2003).

GW405833 (L-768,242) is a CB_2 receptor agonist with a reported in vitro K_i value of 4–12 nM at recombinant human CB_2 receptors (Gallant et al., 1996; Green et al., 1999; Valenzano et al., 2005). At recombinant human CB_1 receptors, GW405833 has reported K_i values ranging from 1900 to 4800 nM, leading to a 160–1200-fold selectivity (Gallant et al., 1996; Valenzano et al., 2005). GW405833 has shown agonist properties in both direct cAMP accumulation assays (Valenzano et al., 2005) and a cAMP reporter system. Against native rat CB_2 receptors the K_i value is comparable to human, but the compound is more potent at CB_1 receptors compared to the human ortholog leading to reduced selectivity (80-fold) (Valenzano et al., 2005). In vivo, GW405833 reduces edema and inhibits hypersensitivity associated with intraplantar injection of carrageenan (Clayton et al., 2002). These effects were inhibited by SR144528, providing evidence that the effect of GW405833 is mediated by CB2 receptors (Clayton et al., 2002). The compound (up to 30 mg/kg) elicits potent and efficacious antihyperalgesic effects in the rat SNL and PSN (Seltzer et al., 1990) models of neuropathic pain, as well as incisional, chemical-induced (formalin) and inflammatory (CFA) pain (Valenzano et al., 2005; LaBuda et al., 2005; Beltramo et al., 2006). Analgesia, sedation and catalepsy were not seen in this dose range, but were apparent at 100 mg/kg (Valenzano et al., 2005). Comparable effects were seen in mouse models of inflammatory and neuropathic pain but were absent in CB_2 receptor knockout mice. The side effects and frank analgesia remained in these knockout animals suggesting a CB_1 receptor-related mechanism of action at these high doses (Whiteside et al., 2005).

JWH 015 is a synthetic cannabinoid agonist with literature K_i values of 14–54 nM at recombinant human CB_2 receptors and 380 nM at recombinant human CB_1 receptors (Showalter et al., 1996; Aung et al., 2000; Mukherjee et al., 2004). At recombinant rat CB_2 receptors, the compound is reported to be less potent, with a K_i value of 150 nM (Mukherjee

et al., 2004). Additionally, JWH 015 decreases FSK-mediated cAMP accumulation in vitro (Mukherjee et al., 2004). It is perhaps due to this limited selectivity for CB_2 over CB_1 receptors that this pharmacological tool has not been as extensively profiled *in vivo* compared to those compounds discussed earlier. Importantly however, when administered systemically it can reduce microglial activation following infection with TMEV (Theiler's murine encephalomyelitis virus) in mice (Arévalo-Martin et al., 2003), an effect that may indicate the mechanism of action of CB_2 receptor agonists in neuropathic pain. Lastly, JWH 133 is a published CB_2 receptor-selective compound with a reported K_i value against recombinant human CB_2 receptors of ~3 nM and against native rat CB_1 receptors of ~670 nM (Huffman et al., 1999). No in vitro functional data have been published for JWH 133. When administered systemically, JWH 133 can inhibit citric acid-induced cough in conscious guinea pigs, possibly indicating a neuronal mechanism of action (Patel et al., 2003). In support of this conclusion JWH 133 has been shown to decrease capsaicin-induced depolarizations of guinea pig and human vagus nerve (Patel et al., 2003). In addition, it has been shown to reverse hyperalgesia due to intraplantar injection of carrageenan with a concurrent reduction of edema (Elmes et al., 2005). Importantly, JWH 133 has been shown to reduce the responses of wide dynamic range dorsal horn neurons to both innocuous and noxious intensities of mechanical stimuli. This effect was seen in models of inflammatory and neuropathic pain, in addition to sham operated animals and was partially blocked by the CB2 receptor antagonist SR144528 (Elmes et al., 2004). A comparable effect was observed following direct spinal application of JWH 133 (Sagar et al., 2005).

Functional studies in cultured human sensory neurons demonstrated CB_2 agonists, GW842166 and GW833972, produced inhibition of capsaicin-induced Ca^{2+} influx. Further mechanistic studies showed CB_2 agonist inhibition of capsaicin responses was reversed in the presence of the CB_2 antagonist, GW818646, and exogenous 8-bromo cAMP, but was unaffected in the presence of the opioid blocker, naloxone, or the CB_1 receptor antagonist SR141716A. Studies demonstrating the inhibition of sensory nerve activity by CB_2 receptor activation in guinea pigs (Patel et al. 2003), and rat models of acute and chronic pain (Ross et al. 2001, Nackley et al. 2004, Elmes et al. 2004, Sagar et al. 2005) also suggest direct action on sensory neurons. In addition, there is evidence for the expression of CB_2 receptors in keratinocytes, which in the presence of CB_2 agonists, release endogenous β-endorphins to activate opioid receptors in the peripheral nerve terminals of sensory neurons (Ibrahim et al. 2005).

Taken together these studies summarized above imply both a peripheral and spinal site of actions at different cell types. Regarding clinical trials, GlaxoSmithKline has undertaken a CB_2 agonist Phase II trial for inflammatory pain. Pharmos is still considering clinical trials for their CB_2-selective agonist PRS-639058 (structure not disclosed) as is Glenmark with their most advanced candidate GRC 10693 (structure not disclosed).

3.3. Inhibitors of endocannabinoid metabolism

The finding that formalin administration to the hindpaw of rats produced a release of AEA into the periaqueductal gray (Walker et al., 1999) led those authors to suggest that compounds preventing the metabolism of this endocannabinoid may be useful agents for the

treatment of pain. This has subsequently been shown to be the case, in particular for inflammatory pain. Thus, selective inhibitors of fatty-acid amide hydrolase such as URB597 (structure see Table 1) and OL-135 produce beneficial effects in a variety of models of inflammatory pain, whereas their effects upon neuropathic pain are less clear (Lichtman et al., 2004; Jayamanne et al., 2006; Chang et al., 2006; Russo et al., 2007a,b). Inhibitors of FAAH and MGL also enhance endocannabinoid-mediated analgesia that is induced by exposure to environmental stressors and increase the bioavailability of anandamide and/or 2-AG (Hohmann et al. 2005; Suplita et al., 2005). Importantly, FAAH inhibitors do not produce behaviors associated with a central activation of CB_1 receptors (Kathuria et al., 2003; Jayamanne et al., 2006) and do not appear to behave like THC in drug-discrimination tests (Gobbi et al., 2005), although there may be some issues with respect to alcohol consumption (Vinod et al., 2008; but see Cippitelli et al., 2008).

It should be emphasized that the notion that FAAH inhibitors produce all their effects via a potentiation of endocannabinoid signaling may be an oversimplification. FAAH inhibitors also increase the bioavailability of endogenous fatty-acid amides that are biologically active, but that do not bind to cannabinoid receptors, such as palmitoylethanolamide (PEA). PEA blocks both phases of the formalin response in rats and mice, in addition to carrageenan-induced hyperalgesia in rats, when applied locally or systemically (Calignano et al., 1998, 2001; Jaggar et al., 1998; Conti et al., 2002); an effect that was blocked by SR144528 (Calignano et al., 1998). PEA has also been shown to block acetic acid-induced visceral pain (Calignano et al., 2001), nerve growth factor (NGF)-induced hyperalgesia, and accumulation of immune cells at the site of NGF injection; CB_2 receptor-, but not CB_1 receptor-selective antagonists blocked both effects (Farquhar-Smith and Rice, 2003). Given that PEA lacks direct effects upon CB_1 and CB_2 receptors, it is possible that the effects of the compound are either indirect (via entourage effects, that increase the bioavailability of anandamide by competing with the same enzymes for hydrolysis), or via CB_2-like receptor (review, see Lambert et al., 2002). However, recent data has suggested that peroxisome-proliferator activated receptor α (PPARα) may mediate at least some of the effects of PEA, and that the ability of SR144528 to block PEA may be related to an off-target action of this compound upon PPARα (LoVerme et al., 2006). In this respect, Sagar et al. (in press) recently reported that the responsiveness of wide dynamic range neurons following carrageenan-injection into the hind paws is attenuated by local injection of URB597 in a manner blocked by concomitant local injection of a PPAR-α, but not a CB_1 receptor antagonist. Indeed, the observation that the effects of URB597 in the CFA model are partially blocked by rimonabant and SR144528 (the combination producing a complete block) (Jayamanne et al., 2006) may reflect involvement of PPAR-α rather than CB_2 receptors in addition to CB_1 receptors.

Inhibitors of endocannabinoid uptake are also effective in models of inflammatory and neuropathic pain (see Maione et al., in press, for a recent article investigating the efficacy and degree of CB-receptor involvement in a series of compounds with different relative potencies towards FAAH and AEA uptake). The most potent compound so far described is LY2318912, which produces good effects in the formalin model (Moore et al., 2005). This compound, however, is a potent FAAH inhibitor (Alexander and Cravatt, 2006), and so it is unclear as to whether its effects in the formalin test are due to blockade of AEA

accumulation or its subsequent metabolism. Indeed, the uncertainty concerning the mechanism(s) by which AEA is accumulated into cells remains an important issue that in the worst case could prevent development of a potentially useful class of drugs.

4. Endocannabinoid modulating agents as a component of new pharmacotherapies for pain

The approaches outlined in section 3 have all been considered per se, but a useful approach may be the combination of these actions with other drugs, with the aim of either improving efficacy or providing a better safety profile than that seen with currently available analgesics. An obvious combination is that of cannabinoids and opioids, given that some of the antinociceptive effects of cannabinoids involve activation of the opioid system and vice versa (Ibrahim et al., 2005; da Fonseca Pacheco et al., 2008). Moreover, CB receptor agonists enhance the effect of μ-opioid receptor agonists in a variety of models of analgesia (Reche et al., 1996; Yesilyurt et al., 2003; Finn et al., 2004; Tham et al., 2005; Cox et al., 2007). Activation of CB receptors either directly or indirectly also increases the analgesic effect of non-steroidal anti-inflammatory drugs (NSAIDs) (Guindon et al., 2006a,b; Ulugöl et al., 2006; Naidu and Lichtman, 2007), raising the possibility that a combination of suitable agents could, by reducing the NSAID dose needed, provide an efficacious treatment strategy while minimizing the potential for NSAID-induced gastrointestinal and cardiovascular disturbances. Other potential “partners” for endocannabinoid modulatory agents include α_2-adrenoceptor, peroxisome proliferator-activated receptor α agonists and TRPV1 antagonists (see e.g. Yoon and Choi, 2003; Tham et al., 2005; Russo et al., 2007a,b).

An extension of the polypharmacological approach above is to combine the desired pharmacological properties of the treatment within a single molecule. Although this lacks the flexibility of dosing that is possible when separate drugs are given, a major advantage is that the potential for variability of response due to inter-individual variations in the metabolism of the two components relative to each other is eliminated (for a review of the concept of “designed multiple ligands” see Morphy and Rankovic, 2005). Such compounds are beginning to appear in the literature. For example, *N*-arachidonoylserotonin is a TRPV1 (transient receptor potential vanilloid type 1) antagonist with FAAH inhibitory properties that is active in a number of models of inflammatory and neuropathic pain (Maione et al., 2007). The ibuprofen analog *N*-(3-methylpyridin-2-yl)-2-(4′-isobutylphenyl)propionamide, a compound active in models of visceral pain (Cocco et al., 2003), is 2-3 orders of magnitude more potent an inhibitor of FAAH than ibuprofen, while retaining its cyclooxygenase-inhibitory potency (Holt et al., 2007) and may be useful as a template for the design of potent FAAH/cyclooxygenase-2 inhibitors. Pravadoline, which has both cyclooxygenase inhibitory and cannabinoid receptor agonist properties (D’Ambra et al., 1992), has greater analgesic efficacy than NSAIDs such as zomepirac in several different tests for analgesia (Haubrich et al., 1990).

5. Conclusions

The aim of this review has been to highlight different approaches whereby the endocannabinoid system can be harnessed to produce novel analgesic drugs that lack the psychotropic effects that place a limit upon the usefulness of THC. Lead discovery and lead optimization activities at numerous industrial and academic pharmacology/chemistry laboratories has led to the identification of a number of novel, selective and peripherally restricted modulators of the endocannabinoid system highlighted here. The preclinical profile of a number of these proof-of-concept compounds highlighted here is extremely encouraging, and it is hoped that these potent, selective and efficacious compounds will translate to the clinic.

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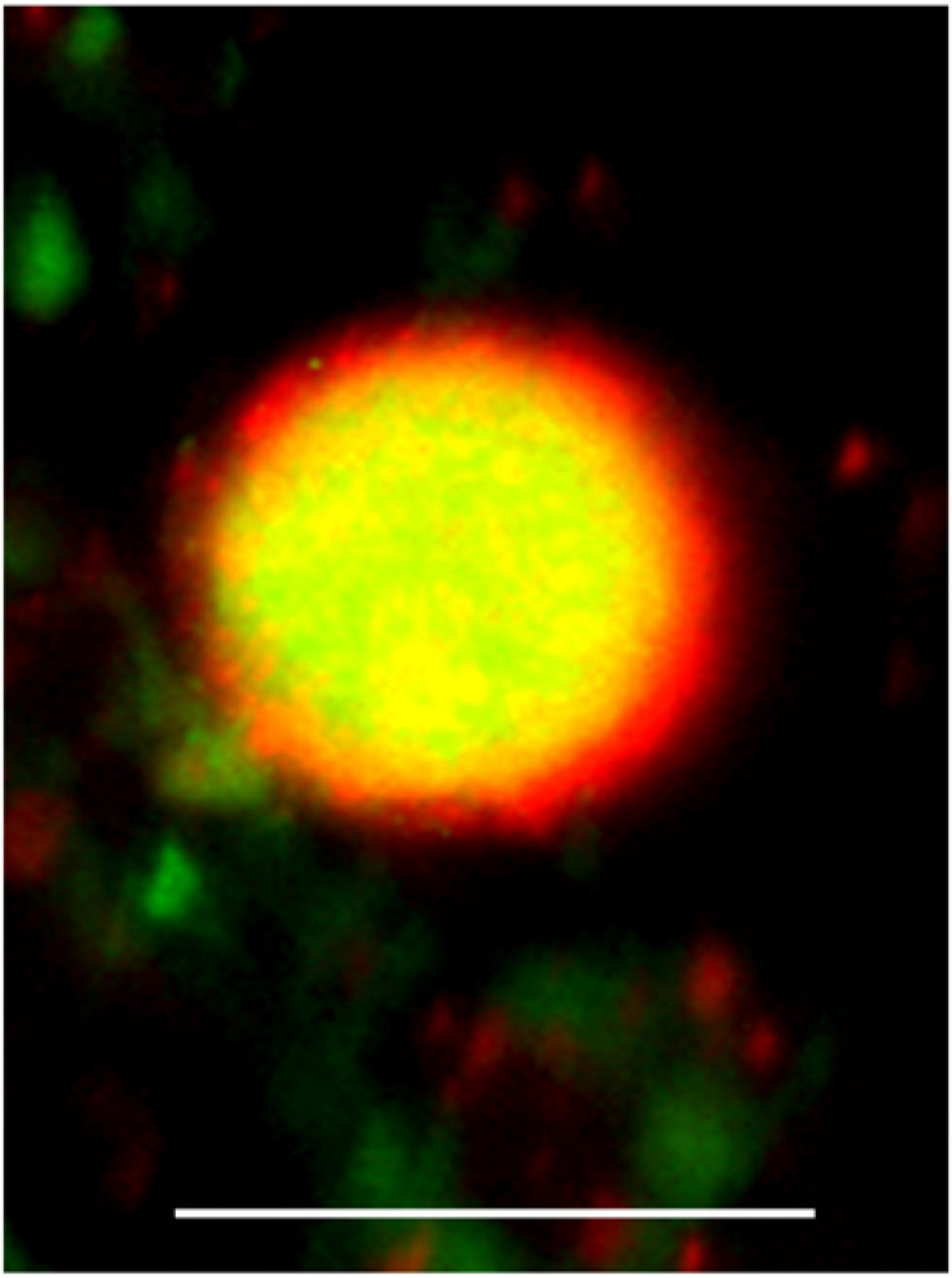


Fig. 1.

Membrane bound CB_2 receptor (red) and cytoplasmic Gap43 (green) immunostaining in a human DRG small neuron *in vitro*. Bar=50 μm.

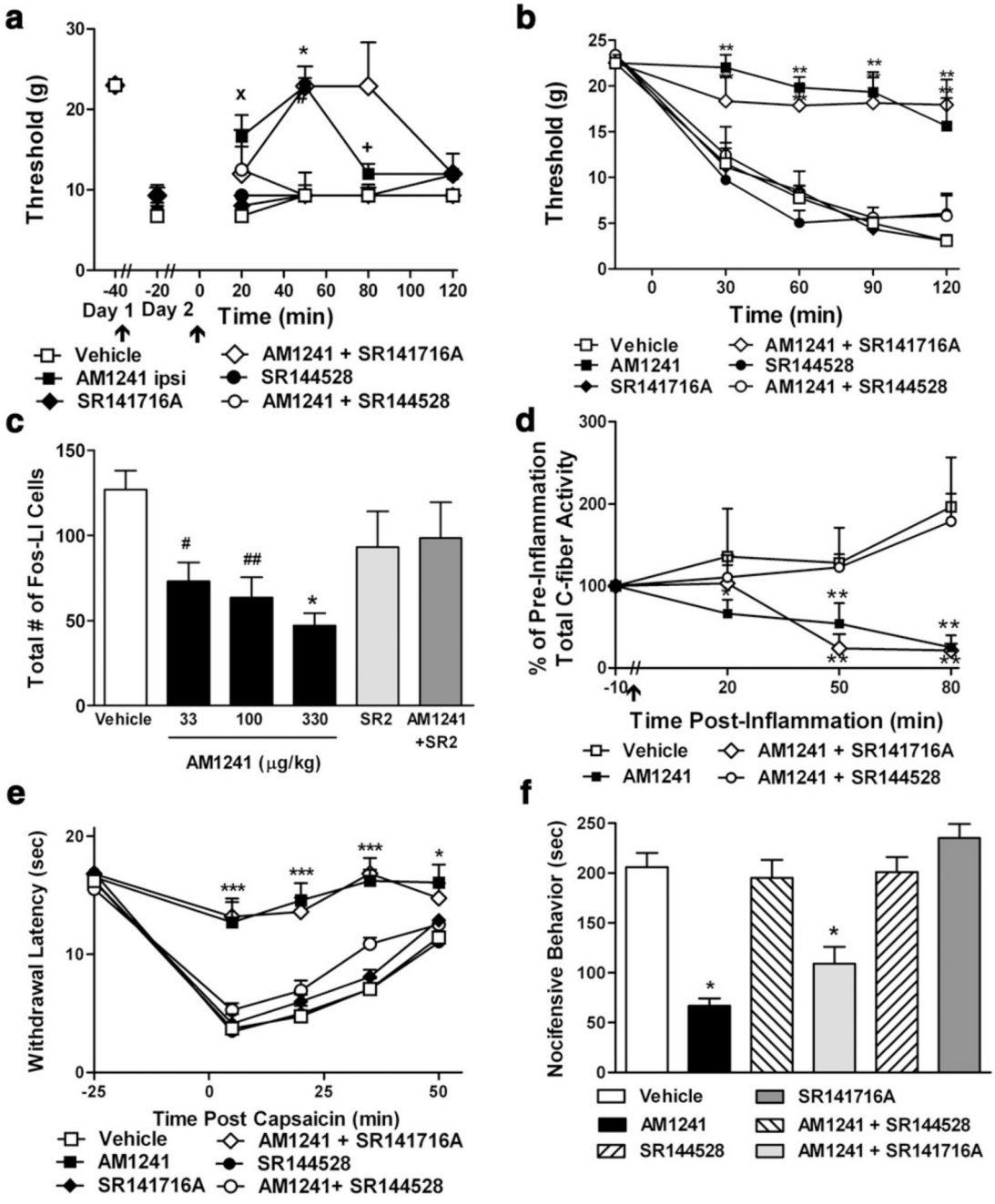
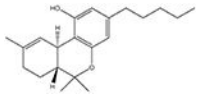
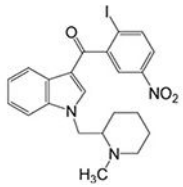
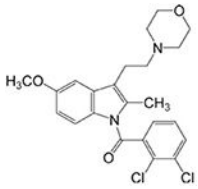
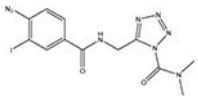
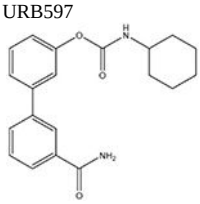
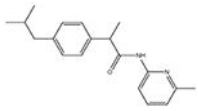
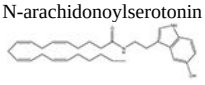


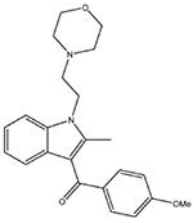
Fig. 2.

Activation of cannabinoid CB_2 receptors suppresses the development and maintenance of inflammatory nociception in behavioral, electrophysiological and neurochemical studies. (a) AM1241 (33 μg/kg i.p.), administered locally in the inflamed paw, suppresses established carrageenan-evoked mechanical allodynia. Effects were blocked by the CB_2 antagonist SR144528 (33 μg/kg i.p.), but not by SR141716A (33 μg/kg i.p.). (b) AM1241 (330 μg/kg i.p.) induces a CB2-mediated suppression of the development of carrageenan-evoked (b) mechanical allodynia and (c) spinal Fos protein expression. (d) AM1241 (330 μg/kg, i.v.)

suppressed total C-fiber-mediated neuronal excitability in spinal wide dynamic range neurons through a CB2-specific mechanism. AM1241 (330 μg/kg i.p.) also suppresses capsaicin-evoked (e) thermal hyperalgesia and (f) nocifensive behavior. (b–f) Effects were completely blocked by SR144528 (1 mg/kg), but not by SR141716A (1 mg/kg). Data (Mean +SEM). Sources: (a) Gutierrez et al. (2007); (b–c) Nackley et al. (2003a), (d) Nackley et al. (2004); (e–f) Hohmann et al. (2004).

Table 1
The pharmacology of the endocannabinoid system–selected compounds

Compound	Mechanism of action	Effect in pain
THC 	Primarily activation of CB receptors, although has off-target actions (e.g. Barann et al., 2002)	Inflammatory: + Neuropathic: +
AM1241 	CB_2-receptor selective ligand; acts as a “protean” agonist *in vitro* (Yao et al., 2006) and CB2 agonist *in vivo*	Inflammatory: + Neuropathic: +
GW405833 	CB_2-receptor selective ligand (efficacy dependent upon assay used, see Yao et al., 2008)	Inflammatory: + Neuropathic: +
LY2318912 	Blocks the accumulation and metabolism of AEA (Moore et al., 2005). Acts primarily as a potent FAAH inhibitor, but with many off-target actions (Alexander and Cravatt, 2006)	Inflammatory: +
URB597 	Selective FAAH inhibitor (Kathuria et al., 2003). Some off-target actions have been reported, but their importance is unclear	Inflammatory: + Visceral: + Neuropathic: +/−
ibu-am5 	Dual COX- and FAAH-inhibitory compound (Holt et al., 2007).	Visceral: +
N-arachidonoylserotonin 	Dual TRPV1 antagonist/FAAH-inhibitory compound (Maione et al., 2007).	Inflammatory: + Neuropathic: +

Compound	Mechanism of action	Effect in pain
Pravadoline 	Dual CB agonist/COX-inhibitory compound (D'Ambra et al., 1992).	Inflammatory: + Visceral: +

The compounds shown in this table have been selected since they are those principally discussed in this review."+" indicates efficacy in a preclinical model of this pain type (for details, see text). For a more complete list, see Fowler (2008).