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REVIEW

Endocannabinoid metabolism and uptake: novel targets for neuropathic and inflammatory pain

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Cannabinoid CB_1 and CB_2 receptors are located at key sites involved in the relaying and processing of noxious inputs. Both CB_1 and CB_2 receptor agonists have analgesic effects in a range of models of inflammatory and neuropathic pain. Importantly, clinical trials of cannabis-based medicines indicate that the pre-clinical effects of cannabinoid agonists may translate into therapeutic potential in humans. One of the areas of concern with this pharmacological approach is that CB_1 receptors have a widespread distribution in the brain and that global activation of CB_1 receptors is associated with adverse side effects. Studies of the endogenous cannabinoids (endocannabinoids) have demonstrated that they are present in most tissues and that in some pain states, such as neuropathic pain, levels of endocannabinoids are elevated at key sites involved in pain processing. An alternative approach that can be used to harness the potential therapeutic effects of cannabinoids is to maximise the effects of the endocannabinoids, the actions of which are terminated by re-uptake and metabolism by various enzymes, including fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL) and cyclooxygenase type 2 (COX2). Preventing the metabolism, or uptake, of endocannabinoids elevates levels of these lipid compounds in tissue and produces behavioural analgesia in models of acute pain. Herein we review recent studies of the effects of inhibition of metabolism of endocannabinoids versus uptake of endocannabinoids on nociceptive processing in models of inflammatory and neuropathic pain.
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Abbreviations: AEA, anandamide; 2AG, 2-arachidonoylglycerol; CB_1, cannabinoid type 1; CB_2, cannabinoid type 2; CNS, central nervous system; COX2, cyclooxygenase type 2; EC, endocannabinoid; FAAH, fatty acid amide hydrolase; LOX, lipoxygenase; MAGL, monoacylglycerol lipase; PEA, *N*-palmitoylethanolamine

Introduction

The analgesic effects of the cannabinoid agonists acting at both cannabinoid type 1 (CB_1) and CB_2 receptors have been widely described and reviewed (Pertwee, 2001; Walker and Huang, 2002; Hohmann and Suplita, 2006). One of the major drawbacks of this approach to analgesia is the widespread distribution of the CB_1 receptor in the brain and the associated side effects such as sedation, dependence, cognitive impairment and psychosis (Thomas, 1996; Kalant, 2004; Pacher *et al*., 2006) arising from their global activation. The recent report that CB_1 receptors present on nociceptors play a major role in cannabinoid-induced anti-nociceptive effects suggests that peripherally acting CB_1 receptor agonists have potential therapeutic effects (Agarwal *et al*., 2007). Studies describing anti-nociceptive effects of CB_2 receptor agonists in models of inflammatory (Clayton *et al*., 2002; Quartilho *et al*., 2003; Nackley *et al*., 2003a, b; Elmes *et al*., 2004, 2005; Valenzano *et al*., 2005; Whiteside *et al*., 2005) and neuropathic pain (Malan *et al*., 2001; Ibrahim *et al*., 2003; Elmes *et al*., 2004; Scott *et al*., 2004; Sagar *et al*., 2005; Valenzano *et al*., 2005; Whiteside *et al*., 2005) support the further investigation of this receptor as a potential analgesic target.

The endocannabinoids (ECs), anandamide (AEA; Devane *et al*., 1992) and 2-arachidonoylglycerol (2AG; Mechoulam *et al*., 1995; Sugiura *et al*., 1995) and *N*-arachidonoyl dopamine (Bisogno *et al*., 2000; Huang *et al*., 2002) act at CB_1 and CB_2 receptors to modulate physiological responses, including nociception (for review see Cravatt and Lichtman, 2004a). Recent studies have investigated the targeting of the ECs, rather than the receptors, as an alternative approach to achieving analgesia in the absence of side effects. This approach should have the benefit of cannabinoid receptor activation at sites of high EC turnover, as opposed to global CB_1 receptor activation that can result in side effects. Increased neuronal activity such as that associated

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with noxious stimulation of pain pathways is believed to drive the synthesis of ECs (Figure 1). There are multiple targets within peripheral tissue at which increased ECs may act including CB_1, and potentially CB_2, receptors as well as pro-nociceptive transient receptor potential vanilloid subfamily member 1 (TRPV1) receptors present on peripheral nerves and immune cells (Figure 1). The rapid termination of the biological actions of the ECs, by metabolism and transport, however, limits the level of analgesia that is achieved by ECs.

AEA is predominantly metabolised by fatty acid amide hydrolase (FAAH), whereas 2AG is predominantly metabolised by monoacylglycerol lipase (MAGL), nevertheless there is evidence that 2AG is also metabolised by FAAH and both AEA and 2AG can be metabolised by cyclooxygenase type 2 (COX2) (Yu *et al*., 1997; Kozak *et al*., 2000, 2001, 2003, 2004), see also review by Fowler in this themed issue. In addition, both AEA and 2AG are believed to be transported into cells (Day *et al*., 2001; Fegley *et al*., 2004; Ligresti *et al*., 2004; Ortega-Gutierrez *et al*., 2004; McFarland and Barker, 2004; Hillard and Jarrahian, 2005; Hermann *et al*., 2006), which also contributes to the termination of the biological activity of ECs. Here, we review recent studies of the impact of noxious stimulation on levels of ECs in peripheral and central nervous system (CNS) tissue involved in nociceptive processing and the ability of inhibition of metabolism, or transport of ECs to produce anti-nociceptive effects in models of inflammatory and neuropathic pain.

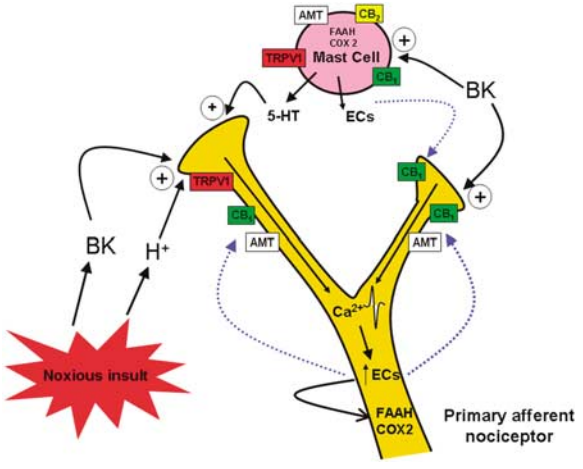

Figure 1 Tissue injury results in the generation of proinflammatory substances such as bradykinin (BK) and protons that activate primary afferent nociceptors resulting in the relay of action potentials from the periphery to the spinal cord. In addition, proinflammatory substances stimulate immune cells, such as mast cells, which also release proinflammatory substances including serotonin (5-HT). Increases in intracellular calcium (Ca^{2+}) drive the synthesis of endocannabinoids (ECs), which act at inhibitory CB_1 and CB_2 receptors to decrease excitability of primary afferent nociceptors and the release of proinflammatory substances from mast cells. The biological effects of the ECs are terminated by their uptake into cells by the putative AEA transport process (AMT) and metabolism by fatty acid amide hydrolase (FAAH) and cyclooxygenase type 2 (COX2). AEA, anandamide; CB_1, cannabinoid type 1.

The relationship between nociceptive processing and levels of ECs

The impact of noxious stimulation on levels of ECs has been investigated in different models of persistent pain. ECs are present in the rat paw skin (Calignano *et al*., 1998; Beaulieu *et al*., 2000; Guindon *et al*., 2006; Maione *et al*., 2007). There are, however, variable reports on the changes in levels of ECs in the paw skin following inflammation induced by intraplantar injection of formalin (Beaulieu *et al*., 2000; Maione *et al*., 2007). Beaulieu *et al*. (2000) reported that the levels of AEA, 2AG and *N*-palmitoylethanolamine (PEA) in the paw skin were not altered 1 h after intraplantar injection of formalin in rats. By contrast, levels of AEA, 2AG and PEA have been reported to be reduced in the hindpaw of both mice and rats, 1 h following intraplantar injection of formalin (Maione *et al*., 2007). We have shown that levels of AEA (Richardson *et al*., 2007) and PEA (unpublished observation) are significantly reduced in the hindpaw of carrageenan-inflamed rats compared with vehicle control, corroborating the work by Maione *et al*. (2007). Hindpaw inflammation induced by intraplantar injection of formalin has also been shown to increase levels of AEA in the periaquaductal grey matter (PAG) (Walker *et al*., 1999), an important area involved in the descending modulation of nociceptive processing (Fields *et al*., 2006).

There have been a small number of studies in patients on the effects of inflammation on ECs. Increased levels of AEA, but not 2AG, have been reported in inflamed tissue in the colon of patients suffering from ulcerative colitis, a finding replicated in a mouse model of colonic inflammation (D'Argenio *et al*., 2006). The converse was described in a model of allergic inflammation, which was associated with increased levels of 2AG, but not AEA (Oka *et al*., 2005).

Overall, levels of ECs and related compounds are differentially altered depending on the nature of inflammation and the tissue under investigation. These differences may arise due to the impact of the pathological condition on levels of the enzymes contributing to the metabolism of ECs and the differential role of these enzymes to the metabolism of different members of the EC and related family of lipids. Thus, levels of FAAH, COX and lipoxygenase (LOX) may be differentially altered in a manner that is specific to the pathological condition. It is also feasible that changes in levels of cannabinoid receptors, which are known to be modulated in pathological conditions, may also impact on levels of ECs. The presence of additional cell types during inflammatory responses may provide a further source of EC synthesis and metabolism, which will impact on levels of ECs present in tissue. An obvious cell type that may contribute to changes in levels of ECs is the mast cell; however, the presence of the EC system in these cells is currently unclear. Although CB_1 and CB_2 mRNA and protein have been identified in mast cells *in vitro* and *ex vivo* (Samson *et al*., 2003; Stander *et al*., 2005), functional evidence for cannabinoid receptors was not found in two further studies using a human mast cell line (Maccarrone *et al*., 2000a) or rat peritoneal mast cells (Lau and Chow, 2003). Another study showed the presence of CB_2 receptors on mast cells (Facci *et al*., 1995); however, when AEA and PEA were tested, only

PEA, which displays little or no activity at cannabinoid receptors, produced a functional response. Furthermore, there have been no reports of EC biosynthesis and release from mast cells, although there is one report of an active membrane transport uptake mechanism present in a human mast cell line which was inhibited by the transport inhibitor AM404 (Maccarrone *et al*., 2000a). Interestingly, this study also showed FAAH-mediated metabolism of AEA in the presence of a 5-LOX inhibitor, but not COX inhibitors, which was subsequently inhibited by PEA (Maccarrone *et al*., 2000a).

In neuropathic pain states, changes in the levels of ECs and related compounds have been reported in several regions of ascending and descending pain pathways. In general, the effects of neuropathic pain on levels of ECs are more consistent between studies. We have shown that the levels of AEA and *N*-oleoylethanolamine, but not 2AG, were higher in the ipsilateral hindpaw of neuropathic rats, compared to sham-operated controls (Figure 2a). When considering the peripheral nerves involved in the relay of nociceptive inputs to the spinal cord, increased levels of AEA and 2AG have been reported in the L5 dorsal root ganglion of neuropathic rats (Mitrirattanakul *et al*., 2006). Similarly, levels of AEA and 2AG, but not PEA, were increased in the spinal cord, PAG and rostral ventromedial medulla in neuropathic rats (Petrosino *et al*., 2007). In the dorsal raphe nucleus, which has reciprocal projections to the PAG (Jansen *et al*., 1998), levels of AEA, but not 2AG, were increased in the chronic constriction injury model of neuropathic pain (Palazzo *et al*., 2006; Petrosino *et al*., 2007). Collectively, these studies suggest that the impact of inflammation on levels of ECs is variable, depending on the nature of the inflammatory stimulus and experimental protocol. By contrast, in models of neuropathic pain, levels of ECs are consistently reported to be increased at multiple sites involved in the nociceptive processing. It is currently unclear whether these changes in levels of ECs reflect changes in synthesis and/or metabolism. Studies using selective inhibitors of the various catabolic enzymes in models of persistent pain have provided further insight into this question.

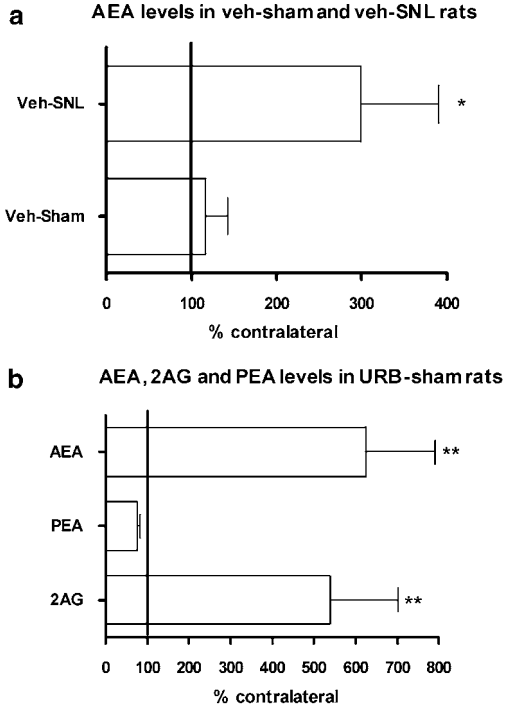

Figure 2 (**a**) Effects of spinal nerve ligation (SNL) versus sham-surgery on levels of AEA in the hindpaw skin of vehicle-treated rats. (**b**) Effects of intraplantar injection of URB597 (URB, 25 μg in 50 μl) on levels of AEA, 2AG and PEA in the hindpaw skin of sham-operated rats. Data were analysed using Mann–Whitney non-parametric test and are expressed as a percentage of the contralateral value + s.e.mean. ($n = 6$ rats per group). *$P < 0.05$, **$P < 0.01$ ipsilateral versus contralateral hindpaw. AEA, anandamide; 2AG, 2-arachidonoylglycerol; PEA, palmitoyl ethanolamide. Modified from Jhaveri *et al*. (2006). Copyright © 2006 Society for Neuroscience.

Functional effects of inhibition of FAAH in models of pain

A number of studies have shown that FAAH is the predominant enzyme responsible for the hydrolysis of AEA (Cravatt *et al*., 2001; Kathuria *et al*., 2003; Lichtman *et al*., 2004; Cravatt and Lichtman, 2004a; Fegley *et al*., 2005). Indeed, levels of AEA in FAAH knockout mice are 15-fold higher than levels in wild-type mice and FAAH knockout mice have reduced sensitivity to pain in models of acute and tonic inflammatory pain (Cravatt *et al*., 2001). There appears to be a complex relationship between the different enzymes involved in the metabolism of ECs in particular under pathological conditions, when LOX is upregulated (reviewed in Phillis *et al*., 2006). FAAH metabolism of AEA and 2AG has been shown to be limited by LOX-mediated production of hydroperoxides from ECs, which are competitive inhibitors of FAAH in human mast cells (Maccarrone *et al*., 2000b), although, to date, this has not been shown to be physiologically relevant in other systems.

A tissue-specific FAAH knockout mouse with deletion of the gene coding for FAAH in peripheral tissues (CNS-spared) has provided further insight into the role of peripheral versus central FAAH in nociceptive processing (Cravatt *et al*., 2004b). Comparison of the CNS-spared FAAH knockout with complete FAAH knockout mice, has shown that CNS-spared FAAH knockout mice do not show hypoalgesia in the tail immersion test but do show a reduction in carrageenan-induced inflammation similar to that seen with the complete knockout. These data suggest that there is an important role of peripheral FAAH in modulating inflammatory nociceptive processing (Cravatt *et al*., 2004b).

Inhibition of FAAH using pharmacological agents such as URB597 or OL135 produces analgesia and reduces inflammation in models of acute-inflammatory pain (Holt *et al*., 2005; Chang *et al*., 2006; Jayamanne *et al*., 2006). We have studied the influence of FAAH inhibition on the levels of ECs and related compounds in the carrageenan model of inflammatory pain. Intraplantar injection of URB597 (25, 100 μg in 50 μl) dose relatedly increased the levels of AEA and 2AG in the ipsilateral hindpaw of carrageenan-inflamed rats (Richardson *et al*., 2007). By contrast, the effects of URB597 on inflammatory hyperalgesia were not dose-related

(Robinson *et al.*, 2006). The lower dose of URB597 (25 μg in 50 μl) attenuated hyperalgesia, whereas the higher dose of URB597 (100 μg in 50 μl) produced a transient pro-nociceptive effect, despite increasing levels of AEA and 2AG in the hindpaw. These data show that increased levels of ECs do not always translate into anti-nociceptive effects. Studies using a FAAH inhibitor with combined TRPV1 antagonist activity, *N*-arachidonoyl-serotonin, have indicated anti-nociceptive effects in the formalin test, mediated by activation of CB_1 receptors and blockade of TRPV1 receptors (Maione *et al.*, 2007).

Studies of the effects of FAAH inhibitors on aberrant pain behaviour in models of neuropathic pain have produced conflicting reports. To date, inhibition of FAAH has been reported to produce no effect (Jayamanne *et al.*, 2006) or anti-nociceptive effects (Chang *et al.*, 2006; Russo *et al.*, 2007) in models of neuropathic pain. Given this disparity in the literature, it is worthwhile to consider these studies in more detail. In the study by Jayamanne *et al.* (2006), a single intraperitoneal injection of URB597 (0.3 mg kg^{-1}) was ineffective at reducing mechanical allodynia in the partial sciatic nerve ligation model of neuropathic pain in rats. This same dose of URB597 did, however, attenuate mechanical allodynia in the Complete Freund's Adjuvant model of inflammatory pain, suggesting that there is a shift in the sensitivity to URB597 in neuropathic rats (Jayamanne *et al.*, 2006). A single higher dose (10 mg kg^{-1} po) of URB597 or repeated oral dosing of URB597 (1–50 mg kg^{-1} po for 4 days) at the peak of neuropathic pain behaviour dose dependently reduced mechanical hyperalgesia in the mouse chronic constriction injury model (Russo *et al.*, 2007). On the basis of antagonist selectivity, the effects of URB597 were suggested to be mediated by both CB_1 and CB_2 receptors (Russo *et al.*, 2007). Similarly, intraperitoneal injection of another FAAH inhibitor, OL135, dose dependently (ED_{50}, 5.3 mg kg^{-1}) inhibited mechanical allodynia in the spinal nerve ligation model of neuropathic pain in rats (Chang *et al.*, 2006). *N*-Arachidonoyl-serotonin has also been shown to attenuate pain behaviour in the chronic constriction injury model of neuropathic pain in rats (Maione *et al.*, 2007).

Given the differential role of peripheral and spinal FAAH in controlling nociceptive processing, identified by the study using FAAH CNS-sparing knockout mice (Cravatt *et al.*, 2004b), we compared the effects of peripheral versus spinal inhibition of FAAH on nociceptive processing in spinal nerve ligation model of neuropathic rats. We reported that a fourfold higher dose of URB597 into the hindpaw was required to inhibit mechanically evoked responses of wide dynamic range spinal cord dorsal horn neurons in neuropathic rats, compared with sham-operated controls (Jhaveri *et al.*, 2006). In this study, intraplantar injection of 100 μg (in 50 μl), but not 25 μg (in 50 μl), URB597 significantly inhibited both innocuous and noxious mechanically evoked responses of wide dynamic range neurons in neuropathic rats. In contrast, intraplantar injection of the lower dose of URB597 significantly attenuated mechanically evoked responses of wide dynamic range neurons in sham-operated rats. Nevertheless, the degree of inhibition produced by the higher dose (100 μg in 50 μl) of URB597 in neuropathic rats was comparable with that produced by the lower dose (25 μg in 50 μl) in sham-operated rats. The functional effects of URB597 in sham-operated rats were associated with significant increases in levels of AEA, 2AG and *N*-oleoylethanolamine in the hindpaw (Figure 2b). By contrast, neither dose of URB597 increased levels of ECs in the hindpaw of neuropathic rats (Jhaveri *et al.*, 2006). The effects of inhibition of spinal FAAH on neuronal responses in neuropathic rats were also investigated. In this case, spinal administration of URB597 increased levels of ECs and attenuated mechanically evoked responses of spinal neurons to a similar extent in both neuropathic and sham-operated rats (Jhaveri *et al.*, 2006). Thus, despite the reported increased levels of ECs in the spinal cord of neuropathic rats (see earlier), inhibition of FAAH did not produce any greater effect on responses of wide dynamic range neurons in neuropathic rats, compared with sham-operated rats. Our data suggest that there are differential effects of nerve injury on the contribution of FAAH to the metabolism of ECs at peripheral and spinal sites of nociceptive processing. These differences may arise as a result of the presence of immune cells, changes in pH (Paylor *et al.*, 2006) in areas of tissue damage and changes in the expression of FAAH (Lever *et al.*, 2006).

Effects of inhibition of MAGL in models of pain

The predominant route of catabolism of 2AG is described as being via MAGL, which has a localised distribution in the brain (Dinh *et al.*, 2002). There is, however, mounting *in vitro* (Cravatt *et al.*, 1996; Bisogno *et al.*, 1997; Goparaju *et al.*, 1998, 1999; Ueda *et al.*, 1998; Lang *et al.*, 1999; Fowler *et al.*, 2001; Ueda, 2002) and *in vivo* (Jhaveri *et al.*, 2006; Maione *et al.*, 2006) evidence suggesting the involvement of FAAH in the metabolism of 2AG. To date, there have been far fewer studies of the effects of inhibitors for MAGL on physiological responses, including nociceptive processing, compared with inhibitors of FAAH because of a lack of selective pharmacological agents. URB602 has been described as a selective, but low potency, inhibitor of MAGL, which does not alter the catabolism of AEA (Hohmann *et al.*, 2005; see however Vandevoorde *et al.*, 2007). Local administration of URB602 into the periaqueductal grey area has been shown to increase levels of 2AG, but not AEA, in the midbrain and enhance non-opioid-mediated stress-induced analgesia (Hohmann *et al.*, 2005). The effects of MAGL inhibition are not limited to central sites of action as local hindpaw administration of URB602 attenuates the late phase of the formalin response, a model of inflammatory pain, with these effects blocked by a CB_2 receptor antagonist (Guindon *et al.*, 2007). Although the analgesic effects associated with inhibition of MAGL suggests that this approach has therapeutic potential for the development of novel analgesics, the selectivity of URB602 has recently been questioned. Despite initial work reporting that URB602 inhibited MAGL, but not FAAH (Hohmann *et al.*, 2005), URB602 has subsequently been reported to inhibit both MAGL and FAAH *in vitro* (Muccioli *et al.*, 2007; Vandevoorde *et al.*, 2007). Thus, whether increasing levels of 2AG produce anti-nociceptive effects remains to be determined once selective inhibitors of MAGL are available.

COX2 metabolism of ECs: increased importance in models of inflammatory pain?

Although hydrolysis of AEA and 2AG by FAAH and MAGL has been most extensively studied, AEA and 2AG can also be oxidised by cyclooxygenase that may have increased importance under conditions associated with an upregulation of the mainly inducible isoform COX2 (reviewed by Fowler in this themed issue). AEA and 2AG interact with the conserved side pocket of the COX2 isoform of the enzyme to produce a range of AEA and 2AG metabolites, such as the prostaglandin ethanolamides and prostaglandin glycerol esters, respectively (Kozak *et al*., 2001, 2003). COX2 is constitutively expressed in the spinal cord (Ghilardi *et al*., 2004) but is upregulated both peripherally and spinally in inflammatory and neuropathic pain states (for a review see Svensson and Yaksh, 2002). The analgesic and anti-inflammatory effects of COX2 inhibitors such as meloxicam, SC-58125 and celecoxib in models of inflammatory (Zhang *et al*., 1997; Yaksh *et al*., 2001; Francischi *et al*., 2002) and neuropathic pain (Bingham *et al*., 2005; Takahashi *et al*., 2005; Matsunaga *et al*., 2007) are well described.

The contribution of the cyclooxygenase system to inflammatory and neuropathic hyperalgesia, coupled with the limited literature on the effects of inhibition of COX2 on levels of ECs *in vivo*, led us to examine the role of COX2 metabolism of ECs under conditions of inflammation. We have shown that intraplantar administration of the COX2 inhibitor nimesulide increased levels of AEA and PEA in carrageenan-inflamed hindpaw, alongside a significant decrease in carrageenan-induced changes in weight bearing an index of hyperalgesia (Robinson *et al*., 2006, 2007). Although a number of COX inhibitors also inhibit FAAH, previous studies have shown that this is not the case for COX2-selective nimesulide (Fowler *et al*., 2003). Our data suggest that there is a contribution of COX2 to the metabolism of AEA and, indirectly, PEA under conditions of inflammation *in vivo* and that blocking this effect can produce analgesia. The effects of another COX2 inhibitor, rofecoxib, which is also a weak inhibitor of FAAH, on levels of ECs has also been reported (Guindon *et al*., 2006). In this study, peripheral injection of rofecoxib into the rat hindpaw did not alter levels of AEA, *N*-oleoylethanolamine or PEA in the formalin model of persistent pain. This discrepancy may be due to differences in the importance of COX2- and FAAH-mediated mechanisms and the extent of inflammation induced in the models used. It is clear from these initial studies that further studies of the role of COX2 in the metabolism of ECs *in vivo* are warranted.

Although the majority of the evidence for COX2 metabolism of AEA and 2AG to prostaglandin ethanolamides and the corresponding prostaglandin glycerol esters has been shown *in vitro* (Yu *et al*., 1997; Kozak *et al*., 2002a), there is evidence that this route of metabolism is relevant *in vivo* under certain conditions. To date, there have been no reports of detection of AEA or 2AG oxidative metabolites in naive tissue. However, oxidative metabolites of AEA were detected following exogenous administration of AEA in FAAH knockout mice (Weber *et al*., 2004). This suggests that, although FAAH appears to be the predominant metabolic enzyme of the ECs, there is an enhanced role of alternative metabolic pathways when the EC system is activated while FAAH activity is compromised. Consistent with the idea of functionally relevant EC metabolism by COX2, bimatoprost a structural analogue of prostaglandin-$F_{2\alpha}$ ethanolamide, and prostaglandin-E2 glycerol ester have been shown to produce pharmacological effects *in vivo* (Woodward *et al*., 2004; Sang *et al*., 2006). It has been suggested that bimatoprast, has therapeutic potential as an antiglaucoma agent due to its effects on ocular hypotension (Woodward *et al*., 2004). Prostaglandin-E_2 glycerol ester has been shown to modulate inhibitory synaptic transmission in primary hippocampal neurons (Sang *et al*., 2006). Although the receptors mediating the effects of these COX2 metabolites of ECs are unknown, it is likely that the role of COX2 *in vivo* is greater than just terminating the actions of ECs.

Despite little direct evidence in terms of changes in levels of ECs to support their role in COX2-mediated analgesia, links between the two systems are now well established (reviewed by Fowler in this themed issue). Synergistic analgesic actions of ECs and COX2 inhibitors have been shown (Guindon *et al*., 2006), and although not COX2-specific, several non-selective cyclooxygenase inhibitors inhibit the activity of FAAH in a pH-dependent manner (Holt *et al*., 2001; Fowler *et al*., 2003) highlighting the importance of the balance and interactions between metabolic pathways. There is some evidence that ECs are able to modulate COX2 activity, which would provide an interesting feedback control of COX2 metabolism of these compounds. Both AEA and 2AG have been shown to increase cyclooxygenase and 5-LOX activity in intact human neuroblastoma CHP100 cells (Maccarrone *et al*., 2000b). Similarly, AEA produced a concentration-dependent increase in the expression of COX2 in cerebral microvascular endothelium cells (Chen *et al*., 2005). By contrast, PEA has been shown to reduce carrageenan inflammation-induced increases in COX activity, which would be expected to produce anti-inflammatory effects (Costa *et al*., 2002). Although current work continues to focus on the interactions between COX2- and FAAH-mediated mechanisms involved in the analgesic effects of ECs, it is interesting to speculate as to the effects of joint COX/LOX inhibitors on the EC system. On the basis of *in vitro* evidence that both COX2 and LOX metabolise AEA and 2AG (Yu *et al*., 1997; Moody *et al*., 2001; Kozak *et al*., 2002a, b), it would be predicted that dual inhibition of these enzymes will alter levels of ECs and metabolites. This may involve increasing levels of ECs, or pushing them towards a FAAH-mediated route of metabolism, dependent on the availability and efficiency of FAAH under the pathophysiological conditions.

An area that has been overlooked to date is the impact of the various metabolic pathways on other ECs, such as *N*-arachidonoyl dopamine and the associated fatty acid amides PEA and *N*-oleoylethanolamine. Nevertheless, it is clear that the interactions of ECs with these associated compounds have profound effects on the EC system, and therefore, metabolism of these compounds must also be considered alongside AEA and 2AG.

Targeting the cellular uptake of AEA: anti-nociceptive effects *in vivo*?

In addition to metabolism, removal of synaptic ECs, which involves trans-membrane transport into cells followed by metabolism, regulates their effects. Thus, an alternative approach to increasing levels of ECs is to block their transport. Although the identity of the AEA transporter is still debated (Glaser *et al.*, 2003, 2005), a number of inhibitors of the putative AEA transport process are available, including arvanil, AM404, VDM11, UCM707, OMDM-1 and OMDM-2. UCM707 (Lopez-Rodriguez *et al.*, 2001), unlike its predecessors, has little affinity for TRPV1 channels (Lopez-Rodriguez *et al.*, 2001, 2003) and CB_1 receptors (de Lago *et al.*, 2002). This compound does, however, bind to the CB_2 receptor (Lopez-Rodriguez *et al.*, 2003), which might compromise interpretations of its effects. The effects of selective inhibitors of EC uptake on nociceptive processing have been investigated in some models of pain. In general terms, the anti-nociceptive effects of inhibitors of the putative AEA transport process are not as robust as the effects of FAAH inhibitors. For example, systemic administration of UCM707 alone did not alter acute nociception in the hot-plate test. It did, however, potentiate the effects of exogenously administered AEA (de Lago *et al.*, 2002). Inhibitors of the putative AEA transport process do appear to have greater effects in models of inflammatory/neuropathic pain. For example, UCM707 significantly inhibited formalin-evoked pain behaviour (La Rana *et al.*, 2006). Systemic administration of AM404, which is a TRPV1 agonist (Rawls *et al.*, 2006), a FAAH inhibitor (Jarrahian *et al.*, 2000) and an inhibitor of the putative AEA transport process, attenuates thermal and mechanical hyperalgesia in models of acute, inflammatory and neuropathic pain (Costa *et al.*, 2006; La Rana *et al.*, 2006) and spinal expression of Fos-immunoreactivity in neuropathic rats (Rodella *et al.*, 2005). AM404 also binds to CB_1 and CB_2 receptors, albeit with low affinity (Khanolkar *et al.*, 1996). Indeed, the inhibitory effects of AM404 have been shown to be partially mediated via the CB_1, CB_2 and TRPV1 receptors, with administration of a combination of all three antagonists producing a complete block of the effects of AM404 (Costa *et al.*, 2006). AM404 is also reported to inhibit COX activity *in vitro* (Hogestatt *et al.*, 2005), which may contribute to the reported functional effects. Other more recently developed inhibitors of the putative AEA transport process, such as OMDM-2, have been reported to be more efficacious in increasing levels of ECs, compared to UCM707 (Ortar *et al.*, 2003; de Lago *et al.*, 2005). The effects of these compounds on nociceptive processing, however, remain to be determined.

In conclusion, despite the multiple enzymes involved in the metabolism of ECs, selective inhibition of FAAH increases levels of ECs that are associated with CB_1 and CB_2 receptor-mediated anti-nociceptive effects in models of acute and inflammatory pain. Importantly, increase in levels of ECs has not been associated with the adverse side effects associated with direct agonists. The impact of persistent pain states on levels of ECs and the enzymes responsible for their metabolism has yet to be fully addressed. This is particularly relevant in models of inflammatory pain that are associated with an upregulation of COX2. The consensus of studies in neuropathic rats is that levels of ECs are increased at multiple levels following this type of injury, but the effects of inhibition of FAAH are less clear-cut. Further studies of the effects of inhibitors of MAGL and transport mechanisms in models of persistent pain are required to identify their potential therapeutic effect.

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Conflict of interest

The authors state no conflict of interest.

References

Agarwal N, Pacher P, Tegeder I, Amaya F, Constantin CE, Brenner GJ *et al.* (2007). Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat Neurosci* **10**: 870–879.

Beaulieu P, Bisogno T, Punwar S, Farquhar-Smith WP, Ambrosino G, Di Marzo V *et al.* (2000). Role of the endogenous cannabinoid system in the formalin test of persistent pain in the rat. *Eur J Pharmacol* **396**: 85–92.

Bingham S, Beswick PJ, Bountra C, Brown T, Campbell IB, Chessell IP *et al.* (2005). The cyclooxygenase-2 inhibitor GW406381X [2-(4-ethoxyphenyl)-3-[4-(methylsulfonyl)phenyl]-pyrazolo[1,5-b]pyridazine] is effective in animal models of neuropathic pain and central sensitization. *J Pharmacol Exp Ther* **312**: 1161–1169.

Bisogno T, Melck D, Bobrov M, Gretskaya NM, Bezuglov VV, De Petrocellis L *et al.* (2000). *N*-Acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity *in vitro* and *in vivo*. *Biochem J* **351** (Part 3): 817–824.

Bisogno T, Sepe N, Melck D, Maurelli S, De Petrocellis L, Di Marzo V (1997). Biosynthesis, release and degradation of the novel endogenous cannabimimetic metabolite 2-arachidonoylglycerol in mouse neuroblastoma cells. *Biochem J* **322** (Part 2): 671–677.

Calignano A, La Rana G, Giuffrida A, Piomelli D (1998). Control of pain initiation by endogenous cannabinoids. *Nature* **394**: 277–281.

Chang L, Luo L, Palmer JA, Sutton S, Wilson SJ, Barbier AJ *et al.* (2006). Inhibition of fatty acid amide hydrolase produces analgesia by multiple mechanisms. *Br J Pharmacol* **148**: 102–113.

Chen P, Hu S, Yao J, Moore SA, Spector AA, Fang X (2005). Induction of cyclooxygenase-2 by anandamide in cerebral microvascular endothelium. *Microvasc Res* **69**: 28–35.

Clayton N, Marshall FH, Bountra C, O'Shaughnessy CT (2002). CB_1 and CB_2 cannabinoid receptors are implicated in inflammatory pain. *Pain* **96**: 253–260.

Costa B, Conti S, Giagnoni G, Colleoni M (2002). Therapeutic effect of the endogenous fatty acid amide, palmitoylethanolamide, in rat acute inflammation: inhibition of nitric oxide and cyclo-oxygenase systems. *Br J Pharmacol* **137**: 413–420.

Costa B, Siniscalco D, Trovato AE, Comelli F, Sotgiu ML, Colleoni M *et al.* (2006). AM404, an inhibitor of anandamide uptake, prevents pain behaviour and modulates cytokine and apoptotic pathways in a rat model of neuropathic pain. *Br J Pharmacol* **148**: 1022–1032.

Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR *et al.* (2001). Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci USA* **98**: 9371–9376.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**: 83–87.

Cravatt BF, Lichtman AH (2004a). The endogenous cannabinoid system and its role in nociceptive behavior. *J Neurobiol* **61**: 149–160.

Cravatt BF, Saghatelian A, Hawkins EG, Clement AB, Bracey MH, Lichtman AH (2004b). Functional disassociation of the central and peripheral fatty acid amide signaling systems. *Proc Natl Acad Sci USA* **101**: 10821–10826.

D'Argenio G, Valenti M, Scaglione G, Cosenza V, Sorrentini I, Di Marzo V (2006). Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation. *FASEB J* **20**: 568–570.

Day TA, Rakhshan F, Deutsch DG, Barker EL (2001). Role of fatty acid amide hydrolase in the transport of the endogenous cannabinoid anandamide. *Mol Pharmacol* **59**: 1369–1375.

de Lago E, Fernandez-Ruiz J, Ortega-Gutierrez S, Viso A, Lopez-Rodriguez ML, Ramos JA (2002). UCM707, a potent and selective inhibitor of endocannabinoid uptake, potentiates hypokinetic and antinociceptive effects of anandamide. *Eur J Pharmacol* **449**: 99–103.

de Lago E, Petrosino S, Valenti M, Morera E, Ortega-Gutierrez S, Fernandez-Ruiz J *et al*. (2005). Effect of repeated systemic administration of selective inhibitors of endocannabinoid inactivation on rat brain endocannabinoid levels. *Biochem Pharmacol* **70**: 446–452.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G *et al*. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**: 1946–1949.

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL *et al*. (2002). Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci USA* **99**: 10819–10824.

Elmes SJ, Jhaveri MD, Smart D, Kendall DA, Chapman V (2004). Cannabinoid CB_2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naive rats and in rat models of inflammatory and neuropathic pain. *Eur J Neurosci* **20**: 2311–2320.

Elmes SJ, Winyard LA, Medhurst SJ, Clayton NM, Wilson AW, Kendall DA *et al*. (2005). Activation of CB1 and CB2 receptors attenuates the induction and maintenance of inflammatory pain in the rat. *Pain* **118**: 327–335.

Facci L, Dal Toso R, Romanello S, Buriani A, Skaper SD, Leon A (1995). Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc Natl Acad Sci USA* **92**: 3376–3380.

Fegley D, Gaetani S, Duranti A, Tontini A, Mor M, Tarzia G *et al*. (2005). Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleoylethanolamide deactivation. *J Pharmacol Exp Ther* **313**: 352–358.

Fegley D, Kathuria S, Mercier R, Li C, Goutopoulos A, Makriyannis A *et al*. (2004). Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. *Proc Natl Acad Sci USA* **101**: 8756–8761.

Fields HL, Basbaum AI, Heinricher MM (2006). Central nervous system mechanisms of pain modulation. In: McMahon SB, Koltzenburg M (eds). *Textbook of Pain*. Elsevier Limited: Philadelphia, pp 125–142.

Fowler CJ, Holt S, Tiger G (2003). Acidic nonsteroidal anti-inflammatory drugs inhibit rat brain fatty acid amide hydrolase in a pH-dependent manner. *J Enzyme Inhib Med Chem* **18**: 55–58.

Fowler CJ, Jonsson KO, Tiger G (2001). Fatty acid amide hydrolase: biochemistry, pharmacology, and therapeutic possibilities for an enzyme hydrolyzing anandamide, 2-arachidonoylglycerol, palmitoylethanolamide, and oleamide. *Biochem Pharmacol* **62**: 517–526.

Francischi JN, Chaves CT, Moura AC, Lima AS, Rocha OA, Ferreira-Alves DL *et al*. (2002). Selective inhibitors of cyclo-oxygenase-2 (COX-2) induce hypoalgesia in a rat paw model of inflammation. *Br J Pharmacol* **137**: 837–844.

Ghilardi JR, Svensson CI, Rogers SD, Yaksh TL, Mantyh PW (2004). Constitutive spinal cyclooxygenase-2 participates in the initiation of tissue injury-induced hyperalgesia. *J Neurosci* **24**: 2727–2732.

Glaser ST, Abumrad NA, Fatade F, Kaczocha M, Studholme KM, Deutsch DG (2003). Evidence against the presence of an anandamide transporter. *Proc Natl Acad Sci USA* **100**: 4269–4274.

Glaser ST, Kaczocha M, Deutsch DG (2005). Anandamide transport: a critical review. *Life Sci* **77**: 1584–1604.

Goparaju SK, Ueda N, Taniguchi K, Yamamoto S (1999). Enzymes of porcine brain hydrolyzing 2-arachidonoylglycerol, an endogenous ligand of cannabinoid receptors. *Biochem Pharmacol* **57**: 417–423.

Goparaju SK, Ueda N, Yamaguchi H, Yamamoto S (1998). Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. *FEBS Lett* **422**: 69–73.

Guindon J, Desroches J, Beaulieu P (2007). The antinociceptive effects of intraplantar injections of 2-arachidonoyl glycerol are mediated by cannabinoid CB2 receptors. *Br J Pharmacol* **150**: 693–701.

Guindon J, LoVerme J, De Lean A, Piomelli D, Beaulieu P (2006). Synergistic antinociceptive effects of anandamide, an endocannabinoid, and nonsteroidal anti-inflammatory drugs in peripheral tissue: a role for endogenous fatty-acid ethanolamides? *Eur J Pharmacol* **550**: 68–77.

Hermann A, Kaczocha M, Deutsch DG (2006). 2-Arachidonoylglycerol (2-AG) membrane transport: history and outlook. *AAPS J* **8**: E409–412.

Hillard CJ, Jarrahian A (2005). Accumulation of anandamide: evidence for cellular diversity. *Neuropharmacology* **48**: 1072–1078.

Hogestatt ED, Jonsson BA, Ermund A, Andersson DA, Bjork H, Alexander JP *et al*. (2005). Conversion of acetaminophen to the bioactive *N*-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *J Biol Chem* **280**: 31405–31412.

Hohmann AG, Suplita II RL (2006). Endocannabinoid mechanisms of pain modulation. *AAPS J* **8**: E693–708.

Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R *et al*. (2005). An endocannabinoid mechanism for stress-induced analgesia. *Nature* **435**: 1108–1112.

Holt S, Comelli F, Costa B, Fowler CJ (2005). Inhibitors of fatty acid amide hydrolase reduce carrageenan-induced hind paw inflammation in pentobarbital-treated mice: comparison with indomethacin and possible involvement of cannabinoid receptors. *Br J Pharmacol* **146**: 467–476.

Holt S, Nilsson J, Omeir R, Tiger G, Fowler CJ (2001). Effects of pH on the inhibition of fatty acid amidohydrolase by ibuprofen. *Br J Pharmacol* **133**: 513–520.

Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F *et al*. (2002). An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci USA* **99**: 8400–8405.

Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP *et al*. (2003). Activation of CB_2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: Pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci USA* **100**: 10529–10533.

Jansen AS, Farkas E, Mac Sams J, Loewy AD (1998). Local connections between the columns of the periaqueductal gray matter: a case for intrinsic neuromodulation. *Brain Res* **784**: 329–336.

Jarrahian A, Manna S, Edgemond WS, Campbell WB, Hillard CJ (2000). Structure–activity relationships among *N*-arachidonylethanolamine (Anandamide) head group analogues for the anandamide transporter. *J Neurochem* **74**: 2597–2606.

Jayamanne A, Greenwood R, Mitchell VA, Aslan S, Piomelli D, Vaughan CW (2006). Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models. *Br J Pharmacol* **147**: 281–288.

Jhaveri MD, Richardson D, Kendall DA, Barrett DA, Chapman V (2006). Analgesic effects of fatty acid amide hydrolase inhibition in a rat model of neuropathic pain. *J Neurosci* **26**: 13318–13327.

Kalant H (2004). Adverse effects of cannabis on health: an update of the literature since 1996. *Prog Neuropsychopharmacol Biol Psychiatry* **28**: 849–863.

Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A *et al*. (2003). Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med* **9**: 76–81.

Khanolkar AD, Abadji V, Lin S, Hill WA, Taha G, Abouzid K *et al*. (1996). Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. *J Med Chem* **39**: 4515–4519.

Kozak KR, Crews BC, Morrow JD, Wang LH, Ma YH, Weinander R *et al*. (2002a). Metabolism of the endocannabinoids, 2-arachidonyl-

glycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. *J Biol Chem* **277**: 44877–44885.

Kozak KR, Gupta RA, Moody JS, Ji C, Boeglin WE, DuBois RN *et al.* (2002b). 15-Lipoxygenase metabolism of 2-arachidonylglycerol. Generation of a peroxisome proliferator-activated receptor alpha agonist. *J Biol Chem* **277**: 23278–23286.

Kozak KR, Prusakiewicz JJ, Marnett LJ (2004). Oxidative metabolism of endocannabinoids by COX-2. *Curr Pharm Des* **10**: 659–667.

Kozak KR, Prusakiewicz JJ, Rowlinson SW, Prudhomme DR, Marnett LJ (2003). Amino acid determinants in cyclooxygenase-2 oxygenation of the endocannabinoid anandamide. *Biochemistry* **42**: 9041–9049.

Kozak KR, Prusakiewicz JJ, Rowlinson SW, Schneider C, Marnett LJ (2001). Amino acid determinants in cyclooxygenase-2 oxygenation of the endocannabinoid 2-arachidonylglycerol. *J Biol Chem* **276**: 30072–30077.

Kozak KR, Rowlinson SW, Marnett LJ (2000). Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. *J Biol Chem* **275**: 33744–33749.

La Rana G, Russo R, Campolongo P, Bortolato M, Mangieri RA, Cuomo V *et al.* (2006). Modulation of neuropathic and inflammatory pain by the endocannabinoid transport inhibitor AM404 [*N*-(4-hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide]. *J Pharmacol Exp Ther* **317**: 1365–1371.

Lang W, Qin C, Lin S, Khanolkar AD, Goutopoulos A, Fan P *et al.* (1999). Substrate specificity and stereoselectivity of rat brain microsomal anandamide amidohydrolase. *J Med Chem* **42**: 896–902.

Lau AH, Chow SS (2003). Effects of cannabinoid receptor agonists on immunologically induced histamine release from rat peritoneal mast cells. *Eur J Pharmacol* **464**: 229–235.

Lever IJ, Masuda K, Cravatt BF, Rice ASC (2006). Immunohistochemical localisation of anandamide degrading enzyme: fatty acid amide hydrolase (FAAH) in rat dorsal root ganglion and spinal cord tissue. *16th Annual Symposium of International Cannabinoid Research Society*, http://cannabinoidsociety.org/symposium.2006/2006.ICRS.Program.and.Abstracts.pdf.

Lichtman AH, Leung D, Shelton CC, Saghatelian A, Hardouin C, Boger DL *et al.* (2004). Reversible inhibitors of fatty acid amide hydrolase that promote analgesia: evidence for an unprecedented combination of potency and selectivity. *J Pharmacol Exp Ther* **311**: 441–448.

Ligresti A, Morera E, Van Der Stelt M, Monory K, Lutz B, Ortar G *et al.* (2004). Further evidence for the existence of a specific process for the membrane transport of anandamide. *Biochem J* **380**: 265–272.

Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, Fowler CJ, Tiger G, de Lago E *et al.* (2003). Design, synthesis, and biological evaluation of new inhibitors of the endocannabinoid uptake: comparison with effects on fatty acid amidohydrolase. *J Med Chem* **46**: 1512–1522.

Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, Lastres-Becker I, Gonzalez S, Fernandez-Ruiz J *et al.* (2001). Design, synthesis and biological evaluation of novel arachidonic acid derivatives as highly potent and selective endocannabinoid transporter inhibitors. *J Med Chem* **44**: 4505–4508.

Maccarrone M, Fiorucci L, Erba F, Bari M, Finazzi-Agro A, Ascoli F (2000a). Human mast cells take up and hydrolyze anandamide under the control of 5-lipoxygenase and do not express cannabinoid receptors. *FEBS Lett* **468**: 176–180.

Maccarrone M, Salvati S, Bari M, Finazzi A (2000b). Anandamide and 2-arachidonoylglycerol inhibit fatty acid amide hydrolase by activating the lipoxygenase pathway of the arachidonate cascade. *Biochem Biophys Res Commun* **278**: 576–583.

Maione S, Bisogno T, de Novellis V, Palazzo E, Cristino L, Valenti M *et al.* (2006). Elevation of endocannabinoid levels in the ventrolateral periaqueductal grey through inhibition of fatty acid amide hydrolase affects descending nociceptive pathways via both cannabinoid receptor Type 1 and transient receptor potential vanilloid Type-1 receptors. *J Pharmacol Exp Ther* **316**: 969–982.

Maione S, de Petrocellis L, de Novellis V, Moriello AS, Petrosino S, Palazzo E *et al.* (2007). Analgesic actions of *N*-arachidonoylserotonin, a fatty acid amide hydrolase inhibitor with antagonistic activity at vanilloid TRPV1 receptors. *Br J Pharmacol* **150**: 766–781.

Malan Jr TP, Ibrahim MM, Deng H, Liu Q, Mata HP, Vanderah T *et al.* (2001). CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain* **93**: 239–245.

Matsunaga A, Kawamoto M, Shiraishi S, Yasuda T, Kajiyama S, Kurita S *et al.* (2007). Intrathecally administered COX-2 but not COX-1 or COX-3 inhibitors attenuate streptozotocin-induced mechanical hyperalgesia in rats. *Eur J Pharmacol* **554**: 12–17.

McFarland MJ, Barker EL (2004). Anandamide transport. *Pharmacol Ther* **104**: 117–135.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR *et al.* (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**: 83–90.

Mitrirattanakul S, Ramakul N, Guerrero AV, Matsuka Y, Ono T, Iwase H *et al.* (2006). Site-specific increases in peripheral cannabinoid receptors and their endogenous ligands in a model of neuropathic pain. *Pain* **126**: 102–114.

Moody JS, Kozak KR, Ji C, Marnett LJ (2001). Selective oxygenation of the endocannabinoid 2-arachidonylglycerol by leukocyte-type 12-lipoxygenase. *Biochemistry* **40**: 861–866.

Muccioli GG, Xu C, Odah E, Cudaback E, Cisneros JA, Lambert DM *et al.* (2007). Identification of a novel endocannabinoid-hydrolyzing enzyme expressed by microglial cells. *J Neurosci* **27**: 2883–2889.

Nackley AG, Makriyannis A, Hohmann AG (2003a). Selective activation of cannabinoid CB(2) receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* **119**: 747–757.

Nackley AG, Suplita II RL, Hohmann AG (2003b). A peripheral cannabinoid mechanism suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* **117**: 659–670.

Oka S, Yanagimoto S, Ikeda S, Gokoh M, Kishimoto S, Waku K *et al.* (2005). Evidence for the involvement of the cannabinoid CB2 receptor and its endogenous ligand 2-arachidonoylglycerol in 12-*O*-tetradecanoylphorbol-13-acetate-induced acute inflammation in mouse ear. *J Biol Chem* **280**: 18488–18497.

Ortar G, Ligresti A, De Petrocellis L, Morera E, Di Marzo V (2003). Novel selective and metabolically stable inhibitors of anandamide cellular uptake. *Biochem Pharmacol* **65**: 1473–1481.

Ortega-Gutierrez S, Hawkins EG, Viso A, Lopez-Rodriguez ML, Cravatt BF (2004). Comparison of anandamide transport in FAAH wild-type and knockout neurons: evidence for contributions by both FAAH and the CB1 receptor to anandamide uptake. *Biochemistry* **43**: 8184–81890.

Pacher P, Batkai S, Kunos G (2006). The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* **58**: 389–462.

Palazzo E, de Novellis V, Petrosino S, Marabese I, Vita D, Giordano C *et al.* (2006). Neuropathic pain and the endocannabinoid system in the dorsal raphe: pharmacological treatment and interactions with the serotonergic system. *Eur J Neurosci* **24**: 2011–2020.

Paylor B, Holt S, Fowler CJ (2006). The potency of the fatty acid amide hydrolase inhibitor URB597 is dependent upon the assay pH. *Pharmacol Res* **54**: 481–485.

Pertwee RG (2001). Cannabinoid receptors and pain. *Prog Neurobiol* **63**: 569–611.

Petrosino S, Palazzo E, de Novellis V, Bisogno T, Rossi F, Maione S *et al.* (2007). Changes in spinal and supraspinal endocannabinoid levels in neuropathic rats. *Neuropharmacology* **52**: 415–422.

Phillis JW, Horrocks LA, Farooqui AA (2006). Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders. *Brain Res Rev* **52**: 201–243.

Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A *et al.* (2003). Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. *Anesthesiology* **99**: 955–960.

Rawls SM, Ding Z, Cowan A (2006). Role of TRPV1 and cannabinoid CB1 receptors in AM 404-evoked hypothermia in rats. *Pharmacol Biochem Behav* **83**: 508–516.

Richardson D, Jhaveri MD, Kendall DA, Barrett DA, Chapman V (2007). Effects of inhibition of FAAH on levels of endocannabinoids in the hindpaw and spinal cord of rats with a carrageenan-

induced inflamed hindpaw. *British Pharmacological Society and 3rd European Workshop on Cannabinoid Research PO33*.

Robinson I, Richardson D, Jhaveri MD, Barrett DA, Kendall DA, Chapman V (2006). Effects of fatty acid amide hydrolase and cyclooxygenase-2 inhibitors on nociceptive behaviour in the carrageenan model of inflammatory pain in rats. *Proceedings of British Pharmacological Society Meeting Dec 2006*, http://www.pa2online.org/abstracts/Vol4Issue2abst093P.pdf.

Robinson I, Richardson D, Jhaveri MD, Barrett DA, Kendall DA, Chapman V (2007). Effects of inhibition of cyclooxygenase 2 on levels of endocannabinoids in the carrageenan model of inflammatory pain. *British Pharmacological Society and 3rd European Workshop on Cannabinoid Research PO34*.

Rodella LF, Borsani E, Rezzani R, Ricci F, Buffoli B, Bianchi R (2005). AM404, an inhibitor of anandamide reuptake decreases Fos-immunoreactivity in the spinal cord of neuropathic rats after non-noxious stimulation. *Eur J Pharmacol* **508**: 139–146.

Russo R, Loverme J, La Rana G, Compton T, Parrot J, Duranti A *et al*. (2007). The fatty-acid amide hydrolase inhibitor URB597 (cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester) reduces neuropathic pain after oral administration in mice. *J Pharmacol Exp Ther* **322**: 236–242.

Sagar DR, Kelly S, Millns PJ, O'Shaughnessey CT, Kendall DA, Chapman V (2005). Inhibitory effects of CB1 and CB2 receptor agonists on responses of DRG neurons and dorsal horn neurons in neuropathic rats. *Eur J Neurosci* **22**: 371–379.

Samson MT, Small-Howard A, Shimoda LM, Koblan-Huberson M, Stokes AJ, Turner H (2003). Differential roles of CB1 and CB2 cannabinoid receptors in mast cells. *J Immunol* **170**: 4953–4962.

Sang N, Zhang J, Chen C (2006). PGE2 glycerol ester, a COX-2 oxidative metabolite of 2-arachidonoyl glycerol, modulates inhibitory synaptic transmission in mouse hippocampal neurons. *J Physiol* **572**: 735–745.

Scott DA, Wright CE, Angus JA (2004). Evidence that CB-1 and CB-2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat. *Pain* **109**: 124–131.

Stander S, Schmelz M, Metze D, Luger T, Rukwied R (2005). Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) on sensory nerve fibers and adnexal structures in human skin. *J Dermatol Sci* **38**: 177–188.

Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K *et al*. (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* **215**: 89–97.

Svensson CI, Yaksh TL (2002). The spinal phospholipase-cyclooxygenase-prostanoid cascade in nociceptive processing. *Annu Rev Pharmacol Toxicol* **42**: 553–583.

Takahashi M, Kawaguchi M, Shimada K, Nakashima T, Furuya H (2005). Systemic meloxicam reduces tactile allodynia development after L5 single spinal nerve injury in rats. *Reg Anesth Pain Med* **30**: 351–355.

Thomas H (1996). A community survey of adverse effects of cannabis use. *Drug Alcohol Depend* **42**: 201–207.

Ueda N (2002). Endocannabinoid hydrolases. *Prostaglandins Other Lipid Mediat* **68–69**: 521–534.

Ueda N, Goparaju SK, Katayama K, Kurahashi Y, Suzuki H, Yamamoto S (1998). A hydrolase enzyme inactivating endogenous ligands for cannabinoid receptors. *J Med Invest* **45**: 27–36.

Valenzano KJ, Tafesse L, Lee G, Harrison JE, Boulet JM, Gottshall SL *et al*. (2005). Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. *Neuropharmacology* **48**: 658–672.

Vandevoorde S, Jonsson KO, Labar G, Persson E, Lambert DM, Fowler CJ (2007). Lack of selectivity of URB602 for 2-oleoylglycerol compared to anandamide hydrolysis *in vitro*. *Br J Pharmacol* **150**: 186–191.

Walker JM, Huang SM (2002). Cannabinoid analgesia. *Pharmacol Ther* **95**: 127–135.

Walker JM, Huang SM, Strangman NM, Tsou K, Sanudo-Pena MC (1999). Pain modulation by release of the endogenous cannabinoid anandamide. *Proc Natl Acad Sci USA* **96**: 12198–12203.

Weber A, Ni J, Ling KH, Acheampong A, Tang-Liu DD, Burk R *et al*. (2004). Formation of prostamides from anandamide in FAAH knockout mice analyzed by HPLC with tandem mass spectrometry. *J Lipid Res* **45**: 757–763.

Whiteside GT, Gottshall SL, Boulet JM, Chaffer SM, Harrison JE, Pearson MS *et al*. (2005). A role for cannabinoid receptors, but not endogenous opioids, in the antinociceptive activity of the CB2-selective agonist, GW405833. *Eur J Pharmacol* **528**: 65–72.

Woodward DF, Phelps RL, Krauss AH, Weber A, Short B, Chen J *et al*. (2004). Bimatoprost: a novel antiglaucoma agent. *Cardiovasc Drug Rev* **22**: 103–120.

Yaksh TL, Dirig DM, Conway CM, Svensson C, Luo ZD, Isakson PC (2001). The acute antihyperalgesic action of nonsteroidal, anti-inflammatory drugs and release of spinal prostaglandin E2 is mediated by the inhibition of constitutive spinal cyclooxygenase-2 (COX-2) but not COX-1. *J Neurosci* **21**: 5847–5853.

Yu M, Ives D, Ramesha CS (1997). Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. *J Biol Chem* **272**: 21181–21186.

Zhang Y, Shaffer A, Portanova J, Seibert K, Isakson PC (1997). Inhibition of cyclooxygenase-2 rapidly reverses inflammatory hyperalgesia and prostaglandin E2 production. *J Pharmacol Exp Ther* **283**: 1069–1075.