BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2012.01950.x
www.brjpharmacol.org

REVIEW

Monoacylglycerol lipase – a target for drug development?

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Keywords
2-arachidonoylglycerol; anandamide; cannabinoid; monoacylglycerol lipase; fatty acid amide hydrolase; pain; cancer

Received
30 November 2011
Revised
17 February 2012
Accepted
12 March 2012

The endocannabinoid (eCB) system is involved in processes as diverse as control of appetite, perception of pain and the limitation of cancer cell growth and invasion. The enzymes responsible for eCB breakdown are attractive pharmacological targets, and fatty acid amide hydrolase inhibitors, which potentiate the levels of the eCB anandamide, are now undergoing pharmaceutical development. 'Drugable' selective inhibitors of monoacylglycerol lipase, a key enzyme regulating the levels of the other main eCB, 2-arachidonoylglycerol, were however not identified until very recently. Their availability has resulted in a large expansion of our knowledge concerning the pharmacological consequences of monoacylglycerol lipase inhibition and hence the role(s) played by the enzyme in the body. In this review, the pharmacology of monoacylglycerol lipase will be discussed, together with an analysis of the therapeutic potential of monoacylglycerol lipase inhibitors as analgesics and anticancer agents.

Abbreviations

Δ^9-THC, Δ^9-tetrahydrocannabinol; 2-AG, 2-arachidonoylglycerol; 2-OG, 2-oleoylglycerol; 2-PG, 2-palmitoylglycerol; 2-SG, 2-stearoylglycerol; ABHD6, α/β-hydrolase domain 6; AEA, anandamide; b.i.d., twice daily; CB, cannabinoid; CCI, chronic constriction injury model of neuropathic pain; CFA, complete Freund's adjuvant model of inflammatory pain; DMSO, dimethylsulphoxide; eCB, endocannabinoid; FAAH, fatty acid amide hydrolase; i.pl., intraplantar; MAFP, methyl arachidonyl fluorophosphonate; MGL, monoacylglycerol lipase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NSAID, non-steroidal anti-inflammatory drug; PAG, periaqueductal grey; PEG, polyethylene glycol; PNL, partial nerve ligation model of neuropathic pain; SNL, spinal nerve ligation model of neuropathic pain; TNBS, trinitrobenzene sulphonic acid; VDM11, *N*-(4-hydroxy-2-methylphenyl) arachidonoyl amide

Introduction

Since antiquity, cannabis extracts have been used both recreationally and for therapeutic purposes. The influential physician Sir John Russell Reynolds, for example, wrote in 1890 that 'In almost all painful maladies I have found Indian hemp by far the most useful of drugs'[1] (Reynolds, 1890; for a comprehensive review over the history of *Cannabis sativa*, see Mechoulam, 1986). The main psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC) produces most of its effects in the body as a result of the activation of two G-protein-coupled receptors, termed CB_1 and CB_2 receptors. Originally, CB_1 receptors were described as being primarily neuronal in localization, while CB_2 receptors were found mainly in cells of immune origin. However, more recent data has suggested that this is an oversimplification, and that both non-neuronal CB_1 receptors are found as well as a limited central nerve system distribution of CB_2 receptors (Atwood and Mackie, 2010). There is evidence for additional CB-like

[1]Russell Reynolds was not uniformly positive as to the beneficial effects of *Cannabis indica* extracts, describing their effects in patients with sciatica as being 'almost useless', and in mania as 'worse than useless' (Reynolds, 1890).

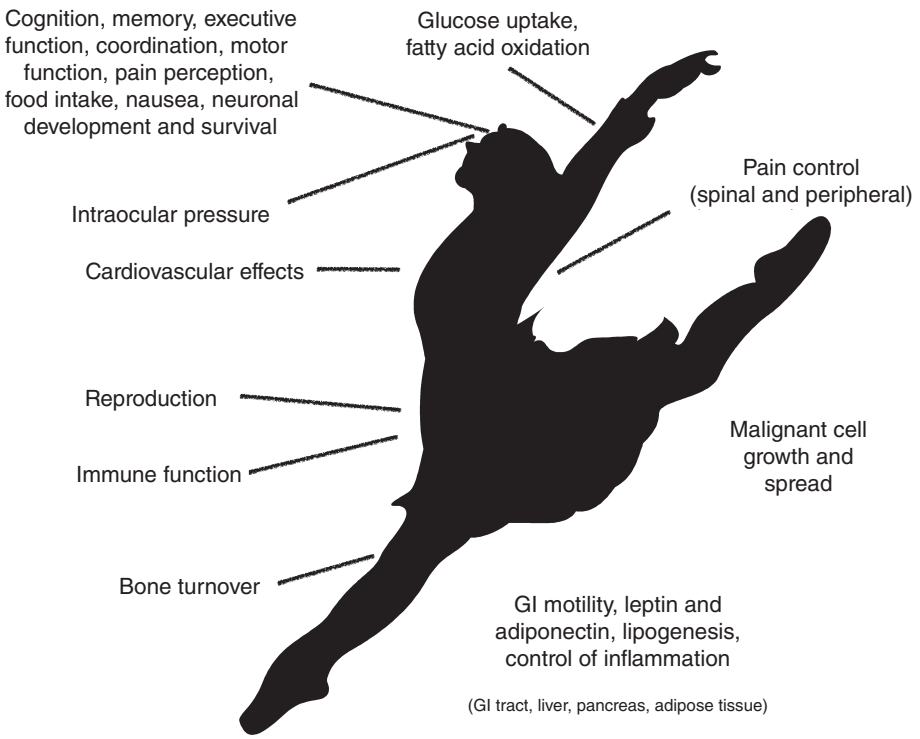

Figure 1

(Patho)physiological processes affected or regulated by CB receptors in the body. For authoritative reviews on these areas, the reader is referred to Bab and Zimmer, 2008; Flygare and Sander, 2008; Katona and Freund, 2008; Yazulla, 2008; Guindon and Hohmann, 2009; Hiley, 2009; Maccarrone, 2009; Idris and Ralston, 2010; Basu and Dittel, 2011; Keimpema *et al.*, 2011; Quarta *et al.*, 2011. It is to be hoped that the ballet dancer shown in silhouette in the figure will recover from the pathologies in question.

receptors, but currently, the evidence is not considered sufficiently strong for them to be formally included in the CB receptor family (Pertwee *et al.*, 2010). Some of the physiological processes affected or regulated by CB receptors are shown in Figure 1.

The demonstration of CB receptors in the body led to the search and thereafter identification of a number of endogenous ligands, mainly (but not exclusively) arachidonic acid derivatives (Devane *et al.*, 1992; Hanus *et al.*, 1993; 2001; Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995). As with the receptors, it is prudent to be restrictive in defining compounds as endogenous cannabinoids or endocannabinoids (eCBs), and in this review, the eCB system is defined as comprising the endogenous ligands 2-arachidonoylglycerol (2-AG, structure, see Figure 2) and anandamide (arachidonoylethanolamide, AEA; structure, see Figure 2), their target CB_1 and CB_2 receptors, and their primary synthetic and degradative enzymes. The life cycle of 2-AG is described in Box 1.

Monoacylglycerol lipase (MGL) is a 33 kDa serine hydrolase that catalyses the hydrolysis of monoacylglycerols to their corresponding fatty acids (Karlsson *et al.*, 1997). The enzyme is found both in the brain and in peripheral tissues such as the kidney, ovary, testis, adrenal gland, adipose tissue and heart (Karlsson *et al.*, 1997). The three-dimensional structure of MGL has been elucidated by X-ray crystallography, and the enzyme has been shown to be a dimeric molecule with amphitropic properties, that is, it can exist both in a soluble form and associated with the membrane lipid bilayers (Bertrand *et al.*, 2010; Labar *et al.*, 2010). Mutagenesis studies have demonstrated the importance of the catalytic triad of ser^{122}, asp^{239} and his^{269} that the enzyme shares with other enzymes in the α/β hydrolase superfamily (Karlsson *et al.*, 1997). The substrate is recruited via a wide hydrophobic tunnel, where it can then interact with the catalytic triad at the end of the tunnel (Bertrand *et al.*, 2010; Labar *et al.*, 2010).

Although MGL can hydrolyse monoacylglycerols with a wide range of acyl chain lengths and degrees of unsaturation (Tornqvist *et al.*, 1974; Nomura *et al.*, 2010), most current interest has emanated from the finding that in the brain, MGL is highly expressed in regions with high CB_1 receptor densities (Dinh *et al.*, 2002) and that in the mouse brain, it is responsible for the bulk (~85%) of the metabolism of 2-AG (Blankman *et al.*, 2007; for details concerning the other metabolic pathways for 2-AG, see Box 1).

Given the roles of the eCB system in the regulation of many processes including pain, appetite and even the control of cancer (Figure 1), enzymes targeting eCB catabolism are potentially important targets for pharmaceutical development. Currently, the main focus has been upon fatty acid amide hydrolase (FAAH), which primarily hydrolyses AEA and related *N*-acylethanolamines to their corresponding fatty acids. FAAH inhibitors are in pharmaceutical development, with pain as a major indication (Ahn *et al.*, 2009). MGL inhibitors have lagged behind, but have a potential

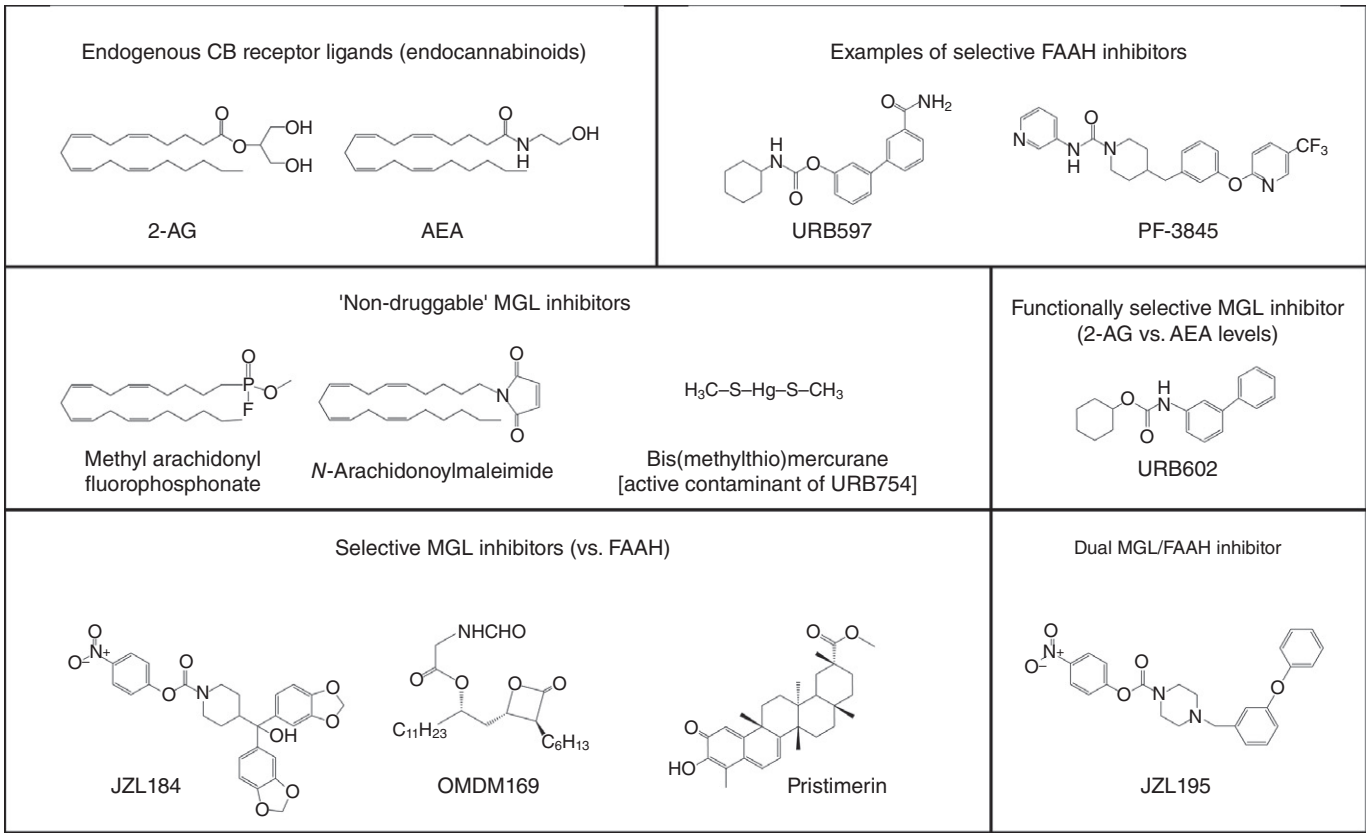

Figure 2

Chemical structures of the main eCBs and of selected MGL inhibitors. The two selective FAAH inhibitors discussed in the review are also shown to aid the reader.

Box 1

The life cycle of 2-AG. 2-AG acts both as an endocannabinoid and as a metabolic intermediary. Without wishing to detract from the importance of the latter role, this review will focus only upon endocannabinoid properties of 2-AG.

Synthesis: 2-AG involved in endocannabinoid signalling is generally thought to be synthesized 'on demand' in response to both physiological and pathological stimuli (for examples, see Stella *et al.*, 1997; Mechoulam *et al.*, 1998; Panikashvili *et al.*, 2001). A major route of 2-AG synthesis from lipids is from its diacylglycerol precursor, which acts as a substrate for diacylglycerol lipase α to form the endocannabinoid; eCB signalling mediated by 2-AG is lost in mice lacking this enzyme (review, see Alger and Kim, 2011).

Targets for 2-AG action: The prime targets for 2-AG are cannabinoid CB_1 and CB_2 receptors, where the ligand acts as a pure agonist. The 2-AG homologues 2-linoleoylglycerol and 2-palmitoylglycerol, which are present in tissues and presumably released together with 2-AG, act as 'entourage compounds' to potentiate the effects of 2-AG at CB receptors without themselves having direct actions upon the receptors in question (Ben-Shabat *et al.*, 1998). 2-AG also has actions upon other receptors: it can, for example, act allosterically upon A_3 adenosine receptors *in vitro* (Lane *et al.*, 2010), and direct actions upon β subunit-containing $GABA_A$ receptors produce hypolocomotor effects in $CB_{1/2}^{-/-}$ mice (Sigel *et al.*, 2011).

Cellular removal and metabolism of 2-AG: After cellular uptake, 2-AG is metabolized by several enzymes in addition to monoacylglycerol lipase (MGL, described in detail in the text). These include the hydrolytic enzymes α/β-hydrolase domain 6 (ABHD6) and fatty acid amide hydrolase (FAAH; Goparaju *et al.*, 1998; Blankman *et al.*, 2007; Marrs *et al.*, 2010), cyclooxygenase-2 (Kozak *et al.*, 2002a), 15-lipoxygenase (Kozak *et al.*, 2002b) and monoacylglycerol acyltransferases/kinases (Simpson *et al.*, 1991). The relative contribution of each enzyme to 2-AG metabolism is of course highly dependent upon the cell in question, but there are examples in the literature where 2-AG signalling and/or levels can be potentiated by FAAH inhibition (e.g. sham-operated rat hind paw, Jhaveri *et al.*, 2006), by COX-2 inhibition (e.g. rat hippocampal pyramidal cells, Kim and Alger, 2004; mouse dorsal root ganglion cells following incubation with inflammatory mediators followed by a calcium ionophore, Duggan *et al.*, 2011), and by ABHD6 inhibition (carbachol- and glutamate-treated mouse brain [minus cerebellum] neurons in primary culture, Marrs *et al.*, 2010). Nonetheless, the ability of selective MGL inhibitors to increase brain 2-AG levels and to produce a variety of effects *in vivo* (Table 1) clearly underlines the central role played by MGL in the metabolism of 2-AG.

advantage over FAAH inhibitors in terms of eCB specificity of action given that the *N*-acylethanolamines are a class of compounds with multiple biological actions (De Petrocellis and Di Marzo, 2009; Hansen, 2010). In this review, the current state of the art with respect to (i) selective MGL inhibitors; (ii) the physiological role of MGL and (iii) the therapeutic potential of MGL inhibitors will be discussed.

Selective MGL inhibitors

MGL is inhibited by a variety of sulfhydryl reagents and disulphide compounds, mercuric compounds, fatty acid trifluoromethylketones and organophosphorus compounds such as methyl arachidonyl fluorophosphonate (MAFP; examples of structures, see Figure 2; Tornqvist and Belfrage, 1976; Di Marzo *et al.*, 1999; Goparaju *et al.*, 1999; Dinh *et al.*, 2002; Ghafouri *et al.*, 2004; Saario *et al.*, 2005; Nithipatikom *et al.*, 2005; Quistad *et al.*, 2006; Labar *et al.*, 2007; Tarzia *et al.*, 2007; Nomura *et al.*, 2008; Matuszak *et al.*, 2009) although the selectivity and/or 'drugability' of these compounds is very limited. Nonetheless, an organophosphorus compound was used *in vivo* to demonstrate that reduction of the 2-AG hydrolytic capacity of the brain is associated with an increased level of 2-AG, with a corresponding decrease in arachidonic acid levels (Nomura *et al.*, 2008). The sulfhydryl reagent *N*-arachidonoylmaleimide (structure, see Figure 2) has also been shown *in vivo* to potentiate the CB_1 receptor-mediated behavioural effects of 2-AG in the tetrad test of cannabinoid function (Burston *et al.*, 2008). Early attempts to design substrate analogues with marked selectivity towards MGL over FAAH (the enzyme responsible for the hydrolysis of the other main eCB AEA) were unsuccessful (Ghafouri *et al.*, 2004; Vandevoorde *et al.*, 2005; Cisneros *et al.*, 2007). A more recent substrate analogue [with respect to 2-oleoylglycerol (2-OG)] (2S,9Z)-octadec-9-ene-1,2-diamine, produced 50% versus 5% inhibition of MGL and FAAH respectively at a concentration of 100 μM (Magrioti *et al.*, 2008). The compound showed analgesic activity in a model of visceral pain, but the potency of the compound towards MGL (K_i value 21.8 μM, Magrioti *et al.*, 2008) is a limiting factor.

The first compound to be reported with purported selectivity for MGL over FAAH was a carbamate compound, URB602 (structure, Figure 2; Hohmann *et al.*, 2005), although this *in vitro* selectivity has been contested (Vandevoorde *et al.*, 2007). The compound, however, has functional selectivity since its administration increases levels of 2-AG but not of AEA in rat forebrain organotypic slice cultures (Hohmann *et al.*, 2005). One possible explanation of this behaviour is that the degree of inhibition of MGL required to increase 2-AG levels is less than that needed towards FAAH to increase AEA levels. There is evidence supporting this assertion in the case of JZL184 (Long *et al.*, 2009b, compound described in detail below; structure, see Figure 2): at a dose of 4 $mg \cdot kg^{-1}$ i.p. of the compound, a 75% inhibition of mouse brain MGL is seen and brain 2-AG levels are increased dramatically 4 h after dosing (although the levels of the oleoyl- and palmitoyl-homologues did not change). In contrast, at a dose of 40 $mg \cdot kg^{-1}$ of the compound, which produced an even larger blockade (~90% inhibition) of FAAH, no increase in AEA levels are seen, although levels of the AEA homologues oleoylethanolamide and palmitoylethanolamide increase by a factor of two (Long *et al.*, 2009b). Regardless as to how URB602 produces its selective increase in 2-AG brain levels, the low potency of the compound is a limitation, although it has been used *in vivo* (Table 1). Nonetheless, the compound has served as an inspiration for the identification and/or design of MGL (and MGL/FAAH) inhibitors (King *et al.*, 2007; Muccioli *et al.*, 2008; Long *et al.*, 2009b; 2010; Bertrand *et al.*, 2010; Szabo *et al.*, 2011; Cisneros *et al.*, 2012). The observation that sulfhydryl reagents are potent MGL inhibitors has also led to identification of octhilinone and *N*-octylbenzisothiazolinone, which inhibit MGL with approximately 20-fold selectivity over FAAH (King *et al.*, 2009a; Matuszak *et al.*, 2011). Replacement of the acyl side chain of the latter compound with the biphenyl moiety found in URB602 resulted in a compound (2-(4-methylbiphenyl)benzo[*d*]isothiazol-3(*2H*)-one) with 73-fold selectivity for MGL over FAAH (Matuszak *et al.*, 2011).

Over the last few years, MGL inhibitors with novel structures have been identified. Starting from the diacylglycerol lipase α (DGLα)/broad specificity lipase inhibitor tetrahydrolipstatin, detailed structure activity relationship studies led to the identification of OMDM169 (structure see Figure 2) as an apparently competitive inhibitor of MGL with IC_{50} values for the inhibition of 2-AG hydrolysis varying from 0.13 μM (rat cerebellar cytosolic fraction) to 10 μM (mouse paw skin). In comparisons for enzymes from the same source, OMDM169 had approximately 10-fold selectivity versus FAAH (rat brain) and versus DGLα (recombinant enzymes expressed in COS-7 cells). However, the compound inhibited recombinant pancreatic lipase with an IC_{50} value of 0.6 μM (Ortar *et al.*, 2008; Bisogno *et al.*, 2009). The thiazolidinedione compound troglitazone, a peroxisome proliferator-activated γ ligand, was found to inhibit MGL with good selectivity versus FAAH, but the potency towards MGL was found to be highly dependent upon the assay used to assess the inhibition (Björklund *et al.*, 2010). Following a screen of a commercial library of compounds, the naturally occurring terpenoid pristimerin (structure, see Figure 2) was identified as a potent reversible inhibitor of MGL (IC_{50} value 93–400 nM, depending upon the enzyme source) with no effect upon FAAH. The compound also inhibited α/β-hydrolase domain 6 (ABHD6, IC_{50} 98 ± 8 nM), and the combined effect upon this enzyme and MGL resulted in increased levels of 2-AG, but not the FAAH substrate palmitoylethanolamide, in rat cortical neurons in primary culture (King *et al.*, 2009b). Whether or not the potentiation of 2-AG signalling contributes to the ability of pristimerin to produce apoptosis in breast and prostate cancer cells (Yang *et al.*, 2008a; see below for discussion of the control of cancer by the eCB system) awaits elucidation. Be that as it may, the reversibility of this compound combined with its good selectivity versus FAAH is a potentially important property, and it is to be hoped that *in vivo* studies with this compound will be forthcoming.

The most extensively studied selective MGL inhibitor is JZL184 (structure, see Figure 2). This compound was designed by an activity-based protein profiling screen of a library of carbamate compounds, followed by chemical optimization of the best compound. JZL184 acts as a potent irreversible inhibitor of MGL with an approximate 300-fold selectivity versus FAAH (see Box 2 for details), and with an estimated

Table 1

In vivo effects of MGL inhibitors, and of genetic deletion of the enzyme. Studies marked with * indicate those where endocannabinoid levels were measured

Treatment (acute unless otherwise stated)	Outcome
URB602	
0.1 nmol into periaqueductal grey (PAG); vehicle; see note a	♂ Adult Sprague-Dawley rats. ↑ Non-opioid stress-induced analgesia, blocked by the CB_1 receptor antagonist/inverse agonist rimonabant (0.2 nmol into PAG), ↑ midbrain levels of 2-AG but not AEA were seen 25 min after the stressor (Hohmann *et al.*, 2005*).
0.1–1000 μg i.pl.; vehicle 1–10% dimethylsulphoxide(DMSO) in saline	♂ Adult Wistar rats, PNL (partial nerve ligation model of neuropathic pain). Peripheral antinociceptive effects seen for ipsi- but not contralateral administration, with ED_{50} values of 127 ± 83 μg (mechanical allodynia) and 86 ± 52 μg (thermal hyperalgesia). Effects of 300 μg of compound were blocked by the CB_1 receptor antagonist/inverse agonist AM251 (80 μg i.pl.) and by the CB_2 receptor antagonist/inverse agonist AM630 (25 μg i.pl.; Desroches *et al.*, 2008).
0.001–600 μg i.pl.; vehicle DMSO : ethanol : cremophor : saline (1:1:1:17) [higher DMSO at high doses]	♂ Adult Sprague-Dawley rats, formalin test of persistent pain. ↓ Nocifensive behaviour in both phases of the test, with ED_{50} values of 120 ± 51 μg (early phase) and 66 ± 24 μg (late phase). Distal and middle, but not proximal hind paw skin levels of 2-AG were increased by 300 μg of the compound during phase 2 of the formalin test. AEA levels were not affected. No effect of 66 μg administration upon formalin-induced paw oedema. No effect of contralateral administration of compound (600 μg; Guindon *et al.*, 2011*).
20 and 40 $mg \cdot kg^{-1}$ i.p.; vehicle 10% DMSO/Tween-80 in saline	♂ 5–6-week-old C57BL/6 mice; ♀ 8-week-old $CB_1^{-/-}$ mice (on C57BL/6 background). 40 $mg \cdot kg^{-1}$ dose ↑ whole gut transit of an Evans blue marker. Not seen in the $CB_1^{-/-}$ mice. No significant effect upon colonic propulsion or upper gastrointestinal transit. 20 $mg \cdot kg^{-1}$ dose had no significant effects in any of the models (Duncan *et al.*, 2008).
OMDM169	
1.25–5 $mg \cdot kg^{-1}$ i.p.; vehicle 10% DMSO in saline	Mice, formalin test of persistent pain. Formalin treatment *per se* ↓ 2-AG levels in the ipsilateral paws. OMDM169 (5 $mg \cdot kg^{-1}$, 15 min before formalin) partially prevented this effect. Dose-dependent ↓ nocifensive behaviour in the second phase of the formalin test. This effect of 5 $mg \cdot kg^{-1}$ OMDM169 was reversed by AM251 (1 $mg \cdot kg^{-1}$ i.p.) and partially reversed by AM630 (1 $mg \cdot kg^{-1}$ i.p.; Bisogno *et al.*, 2009*).
JZL184	
4–40 $mg \cdot kg^{-1}$ i.p; vehicle polyethylene glycol (PEG)300; 4:1 PEG300: Tween-80; or emulphor : ethanol : saline (1:1:18)	♂ C57BL/6J mice. Dose-dependent ↑ brain 2-AG levels; At 16 $mg \cdot kg^{-1}$ dose, the ↑ 2-AG levels were seen at 0.5–8 h after dose, and had returned to baseline at 24 h after dose. Inconsistent effects upon brain 2-palmitoylglycerol (2-PG) and 2-OG levels were reported in the two studies. 16 $mg \cdot kg^{-1}$ ↑ 2-AG levels in liver, kidney, spleen, heart and brown adipose tissue. AEA levels were not affected at this dose. No significant effects upon 2-AG levels in white adipose tissue, lung or testis were seen (Long *et al.*, 2009a*,b*).
40 $mg \cdot kg^{-1}$ i.p.; vehicle emulphor : ethanol : saline (1:1:18)	C57BL/6 mice ↑ brain 2-AG; ↓ brain levels of arachidonic acid, prostaglandins PGD_2, PGE_2, PGF_2 and thromboxan B2. Similar results were seen when the mice had been treated with lipopolysaccharide (LPS), and for PGD_2 and PGE_2 in mice deficient in phospholipase A_2. The effects upon arachidonic acid, PGD_2 and PGE_2 in the LPS-treated mice were not blocked by rimonabant (1 $mg \cdot kg^{-1}$ i.p.) or AM630 (1 $mg \cdot kg^{-1}$ i.p.). ↓ LPS-induced production of brain cytokines IL-1α, IL-1ß, IL-6, TNFα and microglial activation. Effects upon cytokine levels were not blocked by rimonabant or AM630 (Nomura *et al.*, 2011b*).
16 $mg \cdot kg^{-1}$ i.p.; vehicle PEG300; 4:1 PEG300 : Tween-80; or emulphor : ethanol : saline (1:1:18)	C57BL/6 mice. Antinociceptive responses were seen in thermal and visceral pain models, reduced by rimonabant (3 $mg \cdot kg^{-1}$). ↓ Locomotor activity was also reduced by rimonabant. Rimonabant-sensitive hypothermia was seen using the PEG vehicle, but not with the emulphor : ethanol : saline vehicle (Long *et al.*, 2009a*,b*).
16 $mg \cdot kg^{-1}$ i.p.; vehicle 4:1 PEG300 : Tween-80	♂ C57BL/6 mice. Antinociceptive effects were found for both phases of the formalin test of persistent pain, sensitive to rimonabant (3 $mg \cdot kg^{-1}$; Long *et al.*, 2009b*).
0.001–300 μg i.pl.; vehicle 4:1 PEG300 : Tween-80	♂ Adult Sprague-Dawley rats, formalin test of persistent pain. ↓ Nocifensive behaviour in both phases of the test, with ED_{50} values of 0.06 ± 0.028 μg (early phase) and 0.03 ± 0.011 μg (late phase). Effects of 10 μg compound in both phases were blocked by AM251 (80 μg i.pl.) and AM630 (25 μg i.pl.). MGL, but not FAAH activity ↓ in whole paw skin at peak of second phase after 100 μg of compounds. No effect of 0.03 μg administration upon formalin-induced paw oedema. No effect of contralateral administration of compound (300 μg; Guindon *et al.*, 2011*).

Table 1

Continued

Treatment (acute unless otherwise stated)	Outcome
1–100 μg i.pl.; vehicle 4:1 PEG300 : Tween-80	♂ Adult Sprague-Dawley rats, capsaicin-induced nocifensive behaviour and thermal hypersensitivity. Antinociceptive effects of 100 μg of compound were seen; blocked by AM251 (80 μg i.pl.) and AM630 (25 μg i.pl.; Spradley *et al.*, 2010).
4–40 μg i.pl.; vehicle DMSO : Tocrisolve-100 (1:12.5) followed by dilution in saline	♂ Adult C3H/HeNCr MTV$^-$ mice; bone cancer pain model. At dose of 10 μg, compound ↓ tumour (fibrosarcoma into the calcaneous bone)-evoked mechanical hyperalgesia. Effect was blocked by AM630 (4 μg i.pl.) but not AM251 (10 μg i.pl.). 10 μg of compound ↑ hind paw skin 2-AG levels in both naïve and tumour-bearing animals (Khasabova *et al.*, 2011*).
40 mg·kg^{-1} i.p.; vehicle Alkamuls-620 : ethanol : saline (1:1:18)	♂ and ♀ $CB_1^{-/-}$ and $CB_2^{-/-}$ mice on C57BL/6J background + littermate controls. In wild-type animals and $CB_2^{-/-}$ mice, antinociceptive effect of compound was seen for mechanical and cold allodynia in CCI (chronic constriction injury model of neuropathic pain). No effect of compound upon mechanical allodynia and reduced effect on cold allodynia in $CB_1^{-/-}$ mice (Kinsey *et al.*, 2010).
0.25–40 mg·kg^{-1} i.p.; vehicle Alkamuls-620 : ethanol : saline (1:1:18)	♂ C57BL/6 mice; ♂ and ♀ $CB_1^{-/-}$ and $CB_2^{-/-}$ mice on C57BL/6J background + littermate controls. Endpoint: NSAID (diclofenac)-induced gastric haemorrhages. JZL184 was administered 2 h before NSAID (100 mg·kg^{-1} p.o.). JZL184 ≥4 mg·kg^{-1} prevented appearance of gastric haemorrhages. The effect of 4 mg·kg^{-1} of JZL184 was lost in $CB_1^{-/-}$ but not$CB_2^{-/-}$ mice, and was blocked by rimonabant (3 mg·kg^{-1} i.p.) but not by the CB_2 receptor antagonist/inverse agonist SR144528 (3 mg·kg^{-1} i.p.). 4 mg·kg^{-1} dose ↑ stomach 2-AG but not AEA levels for both vehicle- and diclofenac-treated animals, and ↓ the higher levels of IL-1b, IL-6, TNFα, G-CSF and IL-10 seen in the diclofenac-treated animals (no effects in the vehicle-treated animals; Kinsey *et al.*, 2011a*).
16 mg·kg^{-1} i.p. b.i.d.; vehicle Tween-80 : ethanol : saline (1:1:18)	♂ 8–9-week-old C57BL/6 mice. Endpoint: Trinitrobenzene sulphonic acid (TNBS)-induced colitis. JZL184 was given 12 h before TNBS and thereafter b.i.d. until the animals were assessed. Colitis was graded after 3 days. JZL184 ↓ macroscopic colon injury, ↓ plasma markers of endotoxaemia. Effects upon colon morphology were blocked by rimonabant (3 mg·kg^{-1}. i.p. once daily) and by AM630 (10 mg·kg^{-1} i.p. once daily). ↓ mRNA levels of inflammatory markers (IL-6, IL-12, TNFα, MCP-1) accompanied by ↓ colon MGL and FAAH activity and ↑ colon 2-AG levels. Brain and liver mRNA cytokine levels were also reduced (Alhouayek *et al.*, 2011*).
1–8 mg·kg^{-1} i.p.; 8 mg·kg^{-1} i.p. for 6 days; vehicle 20% DMSO/80% emulphor : ethanol : saline (1:1:8)	♂ Adult Sprague-Dawley rats. JZL184 (8 mg·kg^{-1}) gave anxiolytic responses (↑ number of entries and total time in the open arm of the elevated plus maze) under conditions of high- but not low-environmental aversiveness (light intensity). Diazepam was active in both conditions. Effect of JZL184 blocked by rimonabant (1 mg·kg^{-1} i.p.). Anxiolytic effect of JZL184 is retained following repeated treatment. An increased responsiveness to WIN55,212-2 (2.5 mg·kg^{-1} i.p. tail-flick test of thermal nociception) is seen to the same extent following acute and repeated administration of JZL184 (Sciolino *et al.*, 2011).
8 mg·kg^{-1} i.p.; vehicle DMSO	♂ Swiss albino and C57BL/6 mice; ↑ brain 2-AG but not AEA levels. ↑ in the open area of the elevated zero maze and ↑ in the open area of the elevated plus maze. Effect in elevated zero maze not blocked by rimonabant but blocked by SR144528 and AM630 (all doses 1 mg·kg^{-1} i.p.). Effect in elevated maze not seen in $CB_2^{-/-}$ mice. No effects upon memory (object-recognition test; Busquets-Garcia *et al.*, 2011*).
4–40 mg·kg^{-1} i.p.; vehicle Alkamuls-620 : ethanol : saline (1:1:18)	♂ C57BL/6 mice; ↓ marble-burying behaviour of the mice. The effect of 16 mg·kg^{-1} was reduced by pretreatment with rimonabant (0.3 mg·kg^{-1} i.p.). The effect of 40 mg·kg^{-1} was accompanied by an increased immobility of the animals (Kinsey *et al.*, 2011b).
40 mg·kg^{-1} i.p.; vehicle emulphor : ethanol : saline (1:1:18)	♂ ICR mice. ↓ naloxone-precipitated withdrawal signs in morphine-dependent mice. Effects were reversed by rimonabant (3 mg·kg^{-1} i.p. 90 min after JZR184) but not by SR144528 (3 mg·kg^{-1} i.p.). Spontaneous withdrawal signs after removal of morphine were also ↓ by JZL184 (Ramesh *et al.*, 2011*).
40 mg·kg^{-1} i.p.; vehicle PEG200 : Tween-80 (4:1)	♂ and ♀ $FAAH^{+/+}$ and $FAAH^{-/-}$ mice on C57BL/6J background. ↓ rimonabant-precipitated paw tremors, but not head twitches, in both $FAAH^{+/+}$ and $FAAH^{-/-}$ mice treated for 5.5 days with Δ^9-THC (50 mg·kg^{-1} s.c., b.i.d.; Schlosburg *et al.*, 2009).

Table 1

Continued

Treatment (acute unless otherwise stated)	Outcome
16 and 40 mg·kg^{-1} i.p.; vehicle 45% 2-hydroxypropyl-β-cyclodextrin[b]	♂ and ♀ *S. murinus* shrews; ↓ lithium-induced vomiting frequency, prevented by AM251 (5 mg·kg^{-1}). In ♂ Sprague-Dawley rats, JZL184 (40 mg·kg^{-1} i.p.) did not *per se* affect lithium-induced gaping behaviour, but the anti-gaping combination of JZL184 and 2-AG was partially sensitive to AM251, whereas the effect of 2-AG alone was not (Sticht *et al.*, 2012).
16 mg·kg^{-1} i.v.; vehicle DMSO : Tween-20:water (1:1:8)	Wild-type C57BL6/J and $CB_{1/2}^{-/-}$ mice. No significant ($P = 0.08$) effect *per se* upon spontaneous locomotor activity was seen. However, in combination with a threshold dose of a neurosteroid (3α,21-hydroxy-5α-pregnan-20-one, 2 mg·kg^{-1} i.v.), a strong ↓ in spontaneous locomotor activity was seen for both wild-type and $CB_{1/2}^{-/-}$ mice. (Sigel *et al.*, 2011*).
0.7 and 1.4 µmol i.c.v.; vehicle *N,N*-dimethyl formamide	♂ Wistar rats. ↓ bombesin (1 nmol i.c.v)-induced elevations in plasma noradrenaline and adrenaline levels. 1.4 µmol dose was without effect upon basal plasma noradrenaline and adrenaline levels (Shimizu *et al.*, 2011).
40 mg·kg^{-1} i.p. once daily for 6 days; vehicle Alkamuls-620 : ethanol : saline (1:1:18)	♂ C57BL/6 mice; ↑ brain 2-AG levels seen after acute dose retained after repeated dosing, however ↑AEA now seen. Antinociceptive effect upon mechanical allodynia in CCI model of neuropathic pain lost, and effect upon thermal nociception reduced after repeated dosing. Anti-allodynic effects of the CB receptor agonist WIN55,212-2 and FAAH inhibitor PF-3845 in the CCI model lost following repeated dosing of JZL184. JZL184-induced tolerance to the antinociceptive and hypothermic effects of Δ^9-THC also reported, dito the hypothermic effects of WIN55,212-2. Tolerance accompanied by ↓ brain CB_1 receptor density and functionality (agonist-stimulated [^{35}S]GTPγS binding; Schlosburg *et al.*, 2010*).
8 mg·kg^{-1} i.p. once daily for 6 days; vehicle 15% DMSO, 4.25% PEG400, 4.25% Tween-80, 76.5% saline	♂ Swiss albino or C57BL/6 mice. No tolerance to the anxiolytic effect in the elevated plus maze or to the antinociceptive effect in the acetic acid-induced abdominal stretching test. No change in hippocampal CB_1 receptor density (Western blot; Busquets-Garcia *et al.*, 2011).
40 mg·kg^{-1} p.o. once daily for up to 30 days; vehicle PEG300	C.B17 SCID mice; ↓ tumour growth following s.c. injection into the flanks of the mice of either human C8161 melanoma or SKOV3 ovarian cancer cells. Similar results were seen using cancer cells with shRNA-mediated knockdown of their MGL. The effect of MGL knockdown upon C8161 tumour growth was negated by a concomitant high-fat diet (Nomura *et al.*, 2010*).
40 mg·kg^{-1} p.o. once daily for up to 40 days; vehicle PEG300	C.B17 SCID and J/nu mice; In the SCID mice, JZL184 ↓ tumour growth following injection of human PC3 prostate cancer cells into the flanks of the mice. Similar results were seen using cancer cells with shRNA-mediated knockdown of their MGL. The effect of MGL knockdown upon tumour growth was partially negated by either rimonabant (3 mg·kg^{-1} p.o.) or a high-fat diet, and completely negated by a combination of the two. In the J/nu mice, no effect of this dose of JZL184 was seen (37 days of treatment was assessed), despite considerable (albeit less than in the SCID mice) tumour MGL inhibition (Nomura *et al.*, 2011a*).
50 mg·kg^{-1} p.o. every other day for up to 15 days; vehicle PEG300	♂ and ♀ nu/nu nude mice (6–8 weeks); ↓ tumour growth following s.c. injection of human Caco-2 colorectal cancer cells into the backs of the mice. Similar results were seen using cancer cells with siRNA-mediated knockdown of their MGL (Ye *et al.*, 2011).
40 mg·kg^{-1} p.o. once daily for 7 days; vehicle: PEG300	C57BL/6 mice. When first dose given 24 h prior to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), the effects of this dopaminergic neurotoxin upon tyrosine hydroxylase immunoreactivity and dopamine levels in the substantia nigra is mitigated. The protective effect is not blocked by rimonabant/AM630 (10 mg·kg^{-1} p.o., 30 min prior to JZL184). Modest effects of the JZL184 treatment upon striatal dopamine levels (Nomura *et al.*, 2011b*).
$MGL^{-/-}$ mice (consider as sustained complete MGL inhibition)	
$MGL^{-/-}$ mice strains used are given in the notes below the table.	↑ Brain levels of 2-AG, 2-OG but not 2-PG or 2-stearoylglycerol (2-SG). Only small ↑ 2-AG seen in $MGL^{+/-}$ mice. All four lipids ↑ in liver, but no changes in plasma for $MGL^{-/-}$ mice. ↑ spinal cord 2-AG. Total monoacylglycerol levels also ↑ in white adipose tissue. Brain AEA levels not changed (Chanda *et al.*, 2010*; Schlosburg *et al.*, 2010*; Nomura *et al.*, 2011b*; Taschler *et al.*, 2011*).

Table 1

Continued

Treatment (acute unless otherwise stated)	Outcome
	↓ Brain levels of arachidonic acid, prostaglandins PGD_2, PGE_2, PGF_2 and thromboxan B2 versus wild-type mice. Similar results were seen when the mice had been treated with LPS, and for liver and lung arachidonic acid, PGD_2 and PGE_2 levels. No changes in levels of arachidonic acid or PGE_2 in heart, kidney, gut or spleen. ↓ LPS-induced production of brain cytokines IL-1α, IL-1ß, IL-6, TNFα and microglial activation. Similar effects upon lung and liver cytokine levels (Nomura *et al.*, 2011b*).
	Normal weight, lean and fat mass of ♂ mice at age 3 months (Taschler *et al.*, 2011*), but ↓ weight (♂ and ♀) at this age in another study (Chanda *et al.*, 2010*). ↓ Plasma glycerol levels in fed animals (no change for free fatty acid, triglycerol, cholesterol or glucose levels). Fasted mice showed reduced lipolysis (↓ ketone bodies, glycerol and triglycerol). Food intake not affected. ♂ animals fed high-fat diet (54% w/w fat, 12 weeks) had ↑ insulin sensitivity and better performance in glucose tolerance test than wild-type mice, whereas no difference was seen for animals fed a normal chow diet (Taschler *et al.*, 2011*).
	No significant differences in body temperature, locomotor activity, rotor rod, tail flick and hot plate responses for $MGL^{+/+}$, $^{+/-}$and$^{-/-}$ mice (Chanda *et al.*, 2010*; Schlosburg *et al.*, 2010*; Taschler *et al.*, 2011*). No obvious differences between $MGL^{+/+}$, $^{+/-}$ and $^{-/-}$ mice in mechanical allodynia in neuropathic (spinal nerve ligation model of neuropathic pain; SNL) and inflammatory (complete Freund's adjuvant model of inflammatory pain; CFA) pain models (Chanda *et al.*, 2010*).
	↓ MPTP-induced loss of dopaminergic neurons for $MGL^{-/-}$ mice compared with $MGL^{+/+}$ and $MGL^{+/-}$ mice as assessed by tyrosine hydroxylase immunoreactivity in the substantia nigra and dopamine levels in both the striatum and substantia nigra (Nomura *et al.*, 2011b*).
	Mice show tolerance (↑ ED_{50}) to the antinociceptive (thermal) and hypothermic responses to WIN55,212-2 (Chanda *et al.*, 2010*; Schlosburg *et al.*, 2010*) and to the effects of the agonist CP55212,2 (0.15 $mg{\cdot}kg^{-1}$) upon locomotion, food intake and oxygen consumption (Taschler *et al.*, 2011*). Tolerance to WIN55,212-2 not seen in $MGL^{+/-}$ mice (Schlosburg *et al.*, 2010*). Inconsistent results seen for WIN55,212-2-induced catalepsy (Chanda *et al.*, 2010*; Schlosburg *et al.*, 2010*). ↓ brain CB_1 receptor density and functionality (agonist-stimulated [^{35}S]GTPγS binding) (Chanda *et al.*, 2010*; Schlosburg *et al.*, 2010*).

The table has been restricted to compounds that produce selective effects upon MGL versus FAAH (in the case of URB602, this is functional selectivity rather than confirmed enzyme inhibitory selectivity). (2S,9Z)-Octadec-9-ene-1,2-diamine has been shown to be effective in an animal model of visceral pain (Magrioti *et al.*, 2008). However, the methodology used was not described in detail in the paper, and there is limited information with respect to its selectivity for MGL versus FAAH. In consequence, it has not been included in the table.
The $MGL^{-/-}$ mice were either on a C57/Bl6 background (Taschler *et al.*, 2011); a mixed 129SvEv/C57BL/6J background (Schlosburg *et al.*, 2010; Nomura *et al.*, 2011a) or a C57BL6/NTac background (Chanda *et al.*, 2010).
[a]Vehicles are indicated for the MGL inhibitor under study. In the complete study of Hohmann *et al.* (2005), two vehicles were used, emulphor : ethanol : saline (1:1:18) and DMSO, but it is not clear which vehicle was used for the intra-PAG experiments reported here.
[b]Endocannabinoid levels were not measured; however, MGL inhibition was confirmed *ex vivo* using a serine hydrolase-directed FP-rhodamine activity probe.

brain $t^1/_2$ in the mouse of 7 h (Long *et al.*, 2009b). *In vivo*, a dose of 16 $mg{\cdot}kg^{-1}$ i.p. given to mice produces almost complete inhibition of MGL with only partial inhibition of FAAH and no obvious effects on any of the other serine hydrolases investigated using the activity-based profiling technique (Long *et al.*, 2009b). The compound dose dependently increases 2-AG levels in the brain while AEA levels are not affected. At a dose of 16 $mg{\cdot}kg^{-1}$, this increase is seen at the earliest time point measured (0.5 h after dosage) and continues up to 8 h, having returned to baseline at 24 h after dosing (Long *et al.*, 2009b). However, repeated administration to mice of a high dose of JZL184 (40 $mg{\cdot}kg^{-1}$) results in a down-regulation of CB_1 receptors and a tolerance to both the effects of the compound itself and of exogenous cannabinoids *in vivo*, a situation also seen when the enzyme is genetically deleted (Chanda *et al.*, 2010; Schlosburg *et al.*, 2010; Taschler *et al.*, 2011). Tolerance to the context-dependent anxiolytic effects in rats of a lower dose of JZL184 (8 $mg{\cdot}kg^{-1}$ for 6 days) was not, however, seen (Busquets-Garcia *et al.*, 2011; Sciolino *et al.*, 2011), suggesting that the tolerance may require sustained high levels of MGL inhibition to occur.

Probing the (patho)physiological role of MGL

The commercial availability of selective compounds like JZL184, together with the generation of $MGL^{-/-}$ mice, has

Box 2

Calculating the potency and selectivity of JZL184 in vitro. Readers who are not experts in the area of enzyme kinetics are presumably used to interpreting IC_{50} values (and at a stretch, pI_{50} [$-\log_{10}(IC_{50})$] values) of compounds reported in the literature. However, for an irreversible inhibitor, the inhibitory potency is time-dependent, rendering IC_{50} values meaningless unless qualified by the pre-incubation time used. In the case of JZL184, the IC_{50} values following a pre-incubation between enzyme and inhibitor of 30 min are 8 nM and 4 μM for inhibition of mouse brain MGL and FAAH activity respectively (Long *et al.*, 2009b). However, in the absence of pre-incubation, the compound is considerably less potent towards MGL, with IC_{50} values for inhibition of rat brain MGL and FAAH of 350 nM and 6.3 μM respectively (data of Björklund *et al.*, 2010). Given that in the absence of pre-incubation, the selectivity for MGL : FAAH is relatively modest compared to that seen following pre-incubation (Long *et al.*, 2009b; Björklund *et al.*, 2010), the selectivity of the compound is primarily generated by the differences in the rates of irreversible inhibition for the two enzymes. This can be quantified using the unit $k_{obs}/[I]$, where k_{obs} refers to the apparent first order rate constant of loss of enzyme activity at inhibitor concentration [I] due to the irreversible phase of the inhibition. k_{obs} can be calculated from logarithmic plots of the enzyme activity remaining (or in this case, of degree of FP-rhodamine labelling) following different incubation times (Kitz and Wilson, 1962). Using four JZL184 concentrations and five preincubation times ranging from 0–40 min, $k_{obs}/[I]$ values for JZL184 of 4400 ± 300 and 13 ± 3 $M^{-1}.s^{-1}$ for MGL and FAAH respectively, could be calculated, giving a selectivity ratio of 330 (data shown in Suppl. figure 2 of Long *et al.*, 2009b).

resulted in a veritable explosion of our knowledge concerning the consequences of MGL inhibition both *in vitro* and *in vivo* in animal models of physiological function and pathological dysfunction as diverse as colitis, pain, cancer and Parkinson's disease (see Table 1 for a comprehensive list of the *in vivo* effects of MGL inhibitors and of MGL genetic deletion). Rather than repeat the data given in the table, this section will focus on two areas, namely retrograde signalling *in vitro* and the behavioural consequences of selective MGL and FAAH inhibition versus non-selective inhibition of both enzymes *in vivo*.

Teasing out the eCBs involved in retrograde signalling in the brain

In the brain, eCBs act in a retrograde manner, that is, are released post-synaptically and interact with presynaptic CB_1 receptors to modulate neurotransmitter release (Alger and Kim, 2011; see Katona and Freund, 2008 for an elegant animation of retrograde signalling in glutamatergic synapses). In general, retrograde signalling can be investigated electrophysiologically in tissue slices by measurement of processes such as depolarization-induced suppression of excitation (DSE) or inhibition (DSI), with the eCB component being demonstrated by the loss of the response in the presence of a CB_1 receptor antagonist/inverse agonist such as rimonabant, AM251 and AM281 (Kreitzer and Regehr, 2001; Ohno-Shosaku *et al.*, 2001; Wilson and Nicoll, 2001). What was not initially clear, however, was whether AEA and 2-AG played separate roles in retrograde signalling pathways in different brain regions, or whether they acted simply as alternative signalling molecules within the same system. Histochemical studies in the rat hippocampus, cerebellum and amygdala indicated that MGL was mainly presynaptically localized, whereas FAAH was post-synaptic (Gulyas *et al.*, 2004) and a presynaptic localization of MGL in the human hippocampus has also been reported (Ludányi *et al.*, 2011). A presynaptic localization is more logical in terms of termination of the retrograde signalling molecule, and initial evidence favouring 2-AG over AEA was the finding that the selective FAAH inhibitor URB597 (structure, see Figure 2) did not affect DSI in the hippocampus (Kim and Alger, 2004). Blockade of diacylglycerol lipase α with a low-potency compound (RHC-80267) was without effect upon DSI at a concentration of 100 μM, but it did block hippocampal long-term potentiation (Chevaleyre and Castillo, 2003), a finding consistent with data demonstrating that 2-AG is formed in hippocampal slices by high-frequency stimulation of the Schaffer collaterals (Stella *et al.*, 1997).

The availability of MGL (and ABHD6) inhibitors, as well as the use of compounds or genetic deletion strategies to block 2-AG synthesis, have demonstrated convincingly that in rodents, 2-AG is the eCB primarily responsible for retrograde signalling contributing to DSE and DSI in the cerebellum and hippocampus (Szabo *et al.*, 2006; Hashimotodani *et al.*, 2007; Pan *et al.*, 2009; Gao *et al.*, 2010), DSI in the thalamus and prefrontal cortex (Sun *et al.*, 2011; Yoshino *et al.*, 2011) and long-term depression in the prefrontal cortex (Lafourcade *et al.*, 2007; Marrs *et al.*, 2010). This role of 2-AG is not confined to rodents (or even the brain, for that matter), and has been reported for the retrograde inhibition of calcium-activated potassium channels in goldfish retinal cones (Fan and Yazulla, 2007). $MGL^{-/-}$ mice have also been used to confirm 2-AG involvement in hippocampal DSI and cerebellar DSE (Pan *et al.*, 2011; Zhong *et al.*, 2011), although the down-regulation of CB_1 receptors seen in these animals is a complicating factor, as is the ability of cyclooxygenase-2 to contribute to the metabolism of eCBs in preparations used in electrophysiological experiments (Kim and Alger, 2004; Slanina and Schweitzer, 2005; Fan and Yazulla, 2007; Yang *et al.*, 2008b; Straiker and Mackie, 2009).

The predominant role of 2-AG does not mean that AEA is redundant as an eCB signalling molecule in the brain, rather that it has other functions, as has been demonstrated convincingly in many electrophysiological and behavioural experiments using FAAH inhibitors and $FAAH^{-/-}$ mice (e.g. Kathuria *et al.*, 2003; Manwell *et al.*, 2009; Schlosburg *et al.*, 2009; Bambico *et al.*, 2010; Kinsey *et al.*, 2010; Ramesh *et al.*, 2011).

'Cannabis-like' effect of MGL inhibitors?

A concern with the development of molecules affecting eCB metabolism is that they might produce cannabis-like effects, which would limit their usefulness. This concern was greatly reduced when the first selective FAAH inhibitor, URB597, was

found not to produce catalepsy, hypothermia or hyperphagia, responses that are seen with compounds activating central CB_1 receptors (Kathuria *et al.*, 2003), and not to produce reinforcing effects in monkeys trained to self-administer Δ^9-THC (Justinova *et al.*, 2008). A similar lack of 'cannabis-like' behaviour has been seen with other selective FAAH inhibitors, such as PF-3845 (structure, see Figure 2; Long *et al.*, 2009c). With respect to MGL inhibition, JZL184, at a dose of 16 mg·kg^{-1} i.p., was found to produce rimonabant-sensitive thermal hypoalgesia and hypolocomotion in mice, but it did not produce any motor impairments on the rotor rod and catalepsy bar tests, suggesting that at a dose producing a sustained increase in brain 2-AG levels, the full gamut of behavioural responses that characterize a general activation of brain CB_1 receptors is not seen (Schlosburg *et al.*, 2009; Long *et al.*, 2009a,b). A similar pattern was seen at a higher dose (40 mg·kg^{-1} i.p.; Long *et al.*, 2009a), and at a dose of 8 mg·kg^{-1} i.p., JZL184 did not produce catalepsy in the bar test in rats following either a single or a repeated (6-day) dosage regime (Sciolino *et al.*, 2011).

The difference in the pattern of behavioural responses between FAAH and MGL inhibition underlines the point made above that in the brain, AEA and 2-AG have different functions rather than simply being alternate ligands for a given target. However, when both enzymes were inhibited, either by the combination of JZL184 and PF-3845, or by administration of the non-selective MGL and FAAH inhibitor JZL195 (structure, see Figure 2), dramatic effects upon all four measures of the behavioural 'tetrad' (thermal analgesia, catalepsy, locomotor activity and rectal temperature) were seen; effects which in all cases were prevented by rimonabant (Long *et al.*, 2009c). Furthermore, while JZL184 produced only a partial degree of Δ^9-THC-appropriate responding in a drug discrimination assay using $FAAH^{+/+}$ mice, a complete mirror of the Δ^9-THC response was seen with this drug in $FAAH^{-/-}$ mice (Long *et al.*, 2009c). Although these results were obtained at high levels of sustained MGL and FAAH blockade, selectivity of inhibition for MGL versus FAAH (or *vice versa*) appears to be a prerequisite for drug development to avoid overt cannabis-like effects of the drugs.

Clinical potential of MGL inhibitors

Examination of Table 1 would suggest that on the basis of the hitherto undertaken preclinical pharmacology of MGL inhibitors, several different indications can be considered. When the list is restricted to those where several independent studies have supported one another, pain and cancer are the two strongest current potential indications for such compounds. The anxiolytic effects of MGL inhibitors are also of interest, although the context dependency of the effects (i.e. how strong the aversive stimulus is) is a complicating factor in assessing clinical potential. In consequence, the following section is limited to consideration of pain and cancer as potential indications for MGL inhibitors.

Pain

Given that MGL inhibitors represent a novel therapeutic approach, the choice of the initial phase II clinical trial will be a 'best guess' as to which patient group should be targeted. This is critical, given that the outcome of the first trial is likely to affect not only development of the compound in question, but also the willingness of other pharmaceutical companies to invest in MGL inhibitors. In this author's view, three criteria should be fulfilled in order to optimize the 'best guess':

- the pain state(s) under study should have been shown in clinical trials to respond to cannabinoids. This at least indicates that the indication in question is tractable to modulation of eCB signalling. It should be noted that in this respect, cannabinoids refer to 'pure' cannabinoid preparations or drugs such as nabilone, rather than extracts like Sativex, since in the extracts, the presence of cannabidiol may in theory contribute to the observed clinical effects.
- experimental studies should indicate that the eCB system is out of balance in animal models of the disease in question (or preferably in the disease itself), so that the MGL inhibitor has a measurable effect, either to normalize a deficient signal, or to potentiate a protective response. MGL inhibitors might of course work if the system is in balance, but the lack of effect of Δ^9-THC in experimental clinical pain (see below) does not make this author optimistic in this regard.
- for indications requiring repeat dosage regimes, there should be no tolerance to the compound in question.

It can be noted that 'positive effects of the MGL inhibitor in animal models of the indication in question' is not among my criteria. This criterion has been omitted for two reasons: (i) it is extremely unlikely that a clinical trial would be considered in the absence of such data; thus the criterion is redundant; (ii) the criterion assumes that the preclinical data is entirely predictive of the situation in the clinic, which would render the first two of my criteria as irrelevant. However, the preclinical promise of novel analgesic drugs has not resulted in very much clinical success, particularly in the case of drugs directed towards neuropathic pain. Although this is due in part to the heterogeneity of the clinical population targeted in clinical trials (Woolf, 2010), there is a mismatch between the outcome measures used in standard animal models and those used in clinical trials (Rice *et al.*, 2008). Hopefully, the development of animal models which mirror more closely the clinical situation and the use of more homogenous patient populations to target pain indications relevant to the mechanism of action of the drug in question (Woolf, 2010) will remedy this situation.

The potential pain indications for MGL inhibitors discussed below will be considered with respect to these criteria.

Single-/limited-dose pain indications for MGL inhibitors. Single-dose studies are sometimes used in experimental settings as a short cut to seeing if the compound in question is active in man. In the pain field, there have been several studies investigating Δ^9-THC in experimental pain settings (such as warm and cold skin stimulation, cold pressor test, intradermal capsaicin-inducing pain). The studies have been well reviewed elsewhere (Staahl *et al.*, 2009), where the authors concluded that 'The analgesic effect of delta-9- tetrahydro-

Box 3

Persistent pain following surgery – a potential indication for MGL inhibitors? In an ideal world, post-operative pain would be a transient event. However, certain types of surgery are associated with a considerable risk to the patient for residual pain that continues many months after the surgery. Examples of such surgery are amputations, thoracotomy, mastectomy and coronary artery bypass surgery, where 20–50% of patients can suffer persistent pain (Kehlet *et al.*, 2006). Recent experimental data has pointed to a role of the eCB system in the resolution of post-operative pain. When male Sprague-Dawley rats were subjected to paw incision surgery under isoflurane anaesthesia, both ipsi- and contralateral spinal cord AEA levels were decreased on days 1 and 3 following surgery, while 2-AG levels increased ipsilaterally on days 3 and 9, and contralaterally on days 1 and 9 after surgery (Alkaitis *et al.*, 2010). In Balb/c mice anaesthetized with sodium pentobarbital, paw incision surgery resulted in a long-lasting increase in the relative proportion of MGL-positive cells in the paw (Ma *et al.*, 2011), again pointing to a disturbance in the eCB system following surgery. In the rats, the mechanical allodynia produced by the paw incision had resolved by day 12 after surgery. However, when both CB_1 and CB_2 receptors were blocked by treatment with AM251 and AM630 (both 1 $mg \cdot kg^{-1}$ i.p. b.i.d. starting on the day of surgery and continuing for 9 days), there was no resolution of the allodynia (Alkaitis *et al.*, 2010). These data are consistent with the hypothesis that the eCB system is involved in the resolution of pain in this animal model.

Endocannabinoid changes are also seen in man: in 30 patients undergoing cardiac surgery and using isoflurane (with sufentanil) as general anaesthetic, blood levels of AEA decreased following anaesthesia and remained low throughout (the last measurement was taken at the intensive care unit after the operation). In contrast, blood 2-AG levels were not affected by the anaesthesia, but increased during sternotomy before returning to baseline post-cardiopulmonary bypass (Weis *et al.*, 2010). In an earlier study, but this time with patients undergoing a variety of orthopaedic surgical procedures, a similar decrease in blood AEA levels was seen following anaesthesia with etomidate and sevoflurane (2-AG levels were not measured). However, there was no drop in blood AEA levels for patients anaesthetized with propofol-total intravenous anaesthesia (Schelling *et al.*, 2006). Most recently, it was reported that both propofol and thiopental/sevoflurane anaesthesia produced a transient reduction in AEA levels in patients undergoing spinal operations, whereas only the thiopental/sevoflurane anaesthesia affected the 2-AG levels (Jarzimski *et al.*, 2012). Alkaitis *et al.* (2010) concluded that their rodent data 'suggest that therapeutic strategies designed to enhance endocannabinoid signalling may prevent patients from developing persistent or chronic pain states following surgery'. In the present context, this would mean a clinical trial of an MGL inhibitor in a high-risk patient group (such as those undergoing coronary artery bypass surgery) with incidence of residual pain rather than relief of acute post-operative pain as the primary outcome measure. I leave it to clinicians to decide whether or not such a trial is feasible, and to determine the length of time the MGL inhibitor in question would need to be given to the patient.

cannabinol is hard to show in acute experimental pain models', and further that the effects as such could be upon sensory/discriminative rather than affective/motivational components of pain. This should be taken into account if experimental human pain is used to assess the potential of MGL inhibitors.

With respect to alleviation of post-operative pain, the available data does not point to a robust effect of cannabinoids in postoperative pain (indeed, in one case, a hyperalgesic response to a high dose of the cannabinoid nabilone was reported; Beaulieu, 2006). There are issues of sample size, dosing techniques and not least the time after surgery when the cannabinoid was given, which are limiting factors in the interpretation of these clinical trials (for review, see Beaulieu and Ware, 2007). Nonetheless, current data would argue against the use of post-operative pain as an indication with which to test for clinical efficacy of MGL inhibitors, although a more complex study of the effect of MGL inhibition upon the development of persistent pain following surgery may be warranted (see Box 3).

Persistent pain indications for MGL inhibitors. Cannabinoids were given a rather poor write-up in 2001, when they were described as 'no more effective than codeine in controlling pain and have depressant effects on the central nervous system that limit their use' (Campbell *et al.*, 2001). However, since then, several high-quality randomized clinical trials have been conducted, and these have led the first author of Campbell *et al.* (2001) instead to conclude that 'Overall there is evidence that cannabinoids are safe and modestly effective in neuropathic pain with preliminary evidence of efficacy in fibromyalgia and rheumatoid arthritis' (Lynch and Campbell, 2011). Most of these studies used cannabis extracts, that is assessed the effect of CB receptor activation in the presence of cannabidiol, but there were positive outcomes in five placebo-controlled studies using nabilone or dronabinol in patients with chronic pain (Lynch and Campbell, 2011). Cancer pain was not considered in this article, but there are reports of analgesic efficacy of nabilone in advanced cancer patients (Maida *et al.*, 2008) and of a Δ^9-THC : cannabidiol extract (but not of Δ^9-THC alone at the dosing used) in patients with intractable cancer-related pain (Johnson *et al.*, 2010).

From the above, several persistent pain states satisfy the first criterion in that they are responsive to cannabinoids. Less, however, is known about the eCB system itself in human pain states. However, detectable levels of AEA and 2-AG were found in synovial fluid from patients with osteoarthritis and rheumatoid arthritis undergoing total knee arthroplasty, whereas no detectable levels were seen in synovial fluid from normal volunteers (Richardson *et al.*, 2008). The eCBs were suggested to come from immune cells present in the synovial fluid rather than being a reflection of eCB release and turnover in the synovium (Richardson *et al.*, 2008). Increased CB_1 receptor immunoreactivity has also been reported in ventral tendon biopsies from patients with Achilles tendinosis compared to control biopsies[2] (Björklund *et al.*, 2011), and an association between pain scores and tumour CB_1 receptor expression (but not MGL expression) has been reported for patients with pancreatic cancer (Michalski *et al.*, 2008). Whether or not these differences are adaptive changes,

[2]The control biopsies were from the dorsal part of the tendon (rather than ventral, for practical and ethical reasons).

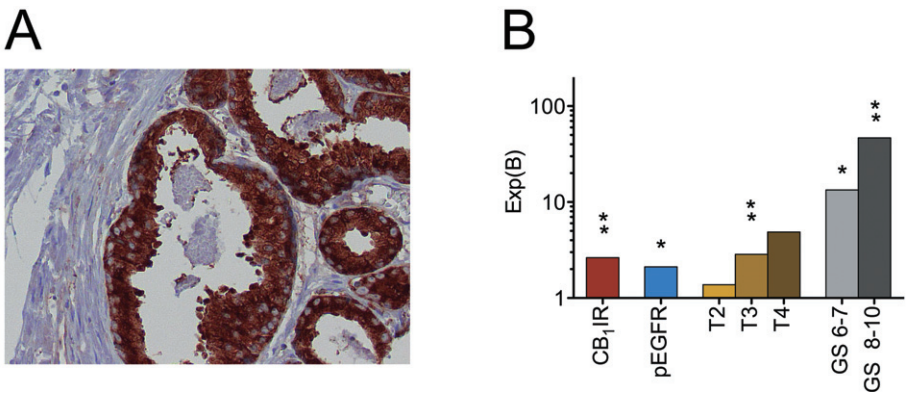

Figure 3

CB_1 receptor expression in prostate cancer. (A) An example of strong CB_1 receptor immunoreactivity (CB_1IR) in the epithelia, but not stroma of a formalin-fixed, paraffin-embedded sample of prostate cancer tissue obtained at transurethral resection for voiding difficulties, when the diagnosis was made (photograph of A. Bergh, S.C. Chung, C.J. Fowler; dataset published in Chung *et al.*, 2009). (B) Influence of CB_1IR with other parameters on disease-specific survival of patients (n = 234) who were followed by watchful waiting after diagnosis until the appearance of metastases. The Exp(B) values, determined by multivariate Cox proportional-hazards regression analysis, refers to the odds ratio, that is the change in odds as a result of an increase in the 'unit' of the predictive variable under study, with a positive value as here indicative of a deleterious influence on disease outcome. For CB_1 receptors, the value Exp(B) is 2.64 (95% confidence limits 1.53–4.58) for the 36 cases with a CB_1IR score above the optimum cut-off value compared to the 198 cases with a score below this cut-off. pEGFR refers to phosphorylated epidermal growth factor receptor expression, T2-T4 the tumour stage and GS the Gleason scores. Significance levels: *$P < 0.05$, **$P < 0.01$. Data from table 1 of Fowler *et al.* (2010a).

responses aimed at mitigating the pain, or alternatively changes that contribute to the disorder requires elucidation, but at least they suggest that the eCB system is changed in pain states, thereby giving some tenuous support to the second criterion. A word of caution, however: potential effects produced by blocking 2-AG metabolism may be mitigated if the 2-AG finds alternative metabolic routes. The obvious example is cyclooxygenase-2 (Duggan *et al.*, 2011), particularly since the metabolic product, prostaglandin glycerol ester, can produce hyperalgesia when given exogenously to experimental animals (Hu *et al.*, 2008)[3]. Currently, data suggesting that cyclooxygenase-2 contributes significantly to eCB metabolism has been from *in vitro* experiments, although there is *in vivo* data suggesting that nimesulide can affect the amount of AEA available for metabolism by FAAH in the mouse brain (Glaser and Kaczocha, 2010).

The third criterion, that of tolerance, may be problematic given the data with JZL184 and with $MGL^{-/-}$ mice (Chanda *et al.*, 2010; Schlosburg *et al.*, 2010; Taschler *et al.*, 2011). However, in these cases, a complete and long-lasting block of MGL produced by the genetic deletion or irreversible inhibition of the enzyme may simply be a case of raising 2-AG levels for too long and too excessively. Certainly, the lack of tolerance to the context-dependent anxiolytic effects of JZL184 in rats and mice and the antinociceptive effects of the compound in a mouse visceral pain model with a lower dose (8 mg·kg^{-1}) of the compound (Busquets-Garcia *et al.*, 2011; Sciolino *et al.*, 2011) are consistent with this conclusion. The field clearly needs to know whether reversible inhibitors (with appropriate pharmacokinetic properties, to avoid long-lasting block of MGL) also produce dose-dependent tolerance, before an informed decision upon whether or not pain is a suitable indication for MGL inhibitors can be made.

The discussion above has considered MGL inhibitors as a stand-alone treatment for pain. However, MGL inhibitors may also be useful in conjunction with non-steroidal anti-inflammatory drugs (NSAIDs), since JZL184 (4 mg·kg^{-1}) provides protection against gastric haemorrhages produced by diclofenac in mice. This protection, which is not seen in the absence of CB_1 receptors, persists (albeit to a slightly lesser extent) even when the animals were pretreated with the MGL inhibitor for 5 days prior to diclofenac treatment (Kinsey *et al.*, 2010). The FAAH inhibitor URB597 shows the same property and also acts synergistically with diclofenac in a model of visceral pain (Naidu *et al.*, 2009). It is not known whether such synergy is also seen with MGL inhibitors, but strategies resulting in lower doses of NSAIDs and with added gastroprotection would clearly be of benefit in the clinic.

Cancer

Ever since the first demonstration that Δ^9-THC and other phytocannabinoids reduced the rate of growth of lung tumour xenografts (Munson *et al.*, 1975), the potential of cannabinoids as anticancer agents has been actively explored and mechanism(s) responsible for their antitumoural effects have been investigated in detail (reviews, see Velasco *et al.*, 2004; Flygare and Sander, 2008; Sarfaraz *et al.*, 2008; Freimuth *et al.*, 2010; Fowler *et al.*, 2010b; Díaz-Laviada, 2011; Malfitano *et al.*, 2011). In many (but by no means all) cancer forms, increased expression of CB receptors is seen in tumours, and in prostate tumours, this increase may have prognostic usefulness in aiding treatment decisions (Fowler *et al.*, 2010a; see Figure 3). It is not clear whether a changed tumour CB_1 expression is a result of malignancy or alternatively a potentially damaging selection factor (by contribut-

[3]It should be noted as a caveat that the glycerol ester used corresponded to 1-AG rather than 2-AG, presumably for reasons of compound stability.

ing to the ability of the tumour to survive in its microenvironment, see Cudaback *et al.*, 2010). Nonetheless, it is indicative of a disturbed local eCB signalling, and thus consideration of the potential of MGL inhibition in cancer is motivated, particularly in the light of the finding that VDM11 (*N*-(4-hydroxy-2-methylphenyl) arachidonoyl amide), which potentiates eCBs by preventing their cellular uptake, reduces the growth of thyroid cancer cells *in vivo* in a xenograft model (Bifulco *et al.*, 2004).

In an elegant study from 2004, the effect of changed endogenous eCB concentrations upon the ability of prostate cancer cell lines was investigated (Nithipatikom *et al.*, 2004). They found that the CB receptor agonist noladin ether reduced the *in vitro* invasivity of androgen-independent human DU145 and PC-3, but not androgen-sensitive LNCaP prostate cancer cells. More importantly in the present context, the authors reported that the 2-AG synthesis inhibitor RHC-80267, at a concentration which greatly reduced 2-AG levels, increased the invasivity of PC-3 and DU-145, but not LNCaP cells in the *in vitro* model (Nithipatikom *et al.*, 2004). The converse was seen for PC-3 cells treated with MAFP, with other compounds found to inhibit 2-AG hydrolysis (including JZL184), and following knockdown of MGL (Nithipatikom *et al.*, 2004; 2005; Nomura *et al.*, 2011a), suggesting that there is an eCB tonus controlling PC-3 invasivity that can be potentiated. Conversely, rimonabant increased the invasive behaviour of PC-3 and DU-145, but not LNCaP cells *in vitro* (Nithipatikom *et al.*, 2004), but it is not clear whether this effect is related to the blockade of an eCB tonus or the inverse agonist properties of this compound. In a xenograft model using PC-3 cells injected into the flanks of immunodeficient mice, reduction of MGL activity either using JZL184 or genetic knockdown of the tumour cell enzyme resulted in a slowed tumour growth that in the latter case was partially blocked by rimonabant (Nomura *et al.*, 2011a; see Table 1).

JZL184 treatment and genetic knockdown of MGL have also been found to reduce the tumour sizes in xenograft models of ovarian, melanoma and colorectal cancers (Nomura *et al.*, 2010; Ye *et al.*, 2011; see Table 1). The potential of MGL inhibitors in other ovarian cancer and melanoma, however, may be related to their ability to reduce the formation of long-chain fatty acids from their corresponding glycerol esters, given the wide substrate specificity of MGL. Thus, (i) the increased MGL activity seen in primary high-grade ovarian tumours compared to benign tumours was accompanied by higher levels of long-chain free fatty acids; (ii) reduction of MGL activity in melanoma cancer cell lines decreased long-chain free fatty acid levels, whereas the converse was seen when MGL was transfected into melanoma cells. The latter also increased tumour growth in a xenograft model; (iii) reduced migration of ovarian and melanoma tumours *in vitro* was produced by reduced MGL activity in a manner overridden by addition of a long-chain free fatty acid; (iv) reduced tumour growth in a xenograft model was seen following knockdown of MGL in the melanoma cells used. The reduced tumour growth was overridden by a high-fat diet (Nomura *et al.*, 2010). Even in the prostate cancer cells, long-chain free fatty acids are important, given that the high-fat diet also partially overrides the effect of tumour MGL disruption upon xenograft tumour growth, with a complete reversal being seen with the combination of rimonabant and a high-fat diet (Nomura *et al.*, 2011a). Although the predictive value of xenografts should not be overestimated (Kerbel, 2003), these results are encouraging, and a non-eCB-mediated mechanism of antitumoural action of MGL inhibitors would reduce the potential importance of tolerance described earlier, the assumption of course being that a down-regulation of eCB signalling due to tolerance is not detrimental in itself.

Conclusions

In just a few years, the availability of selective MGL inhibitors has allowed for the identification of the roles played by 2-AG in the body, and provided preclinical data supporting potential indications for this class of compounds. Within the pain field, issues of tolerance and 'cannabis-like' effects secondary to loss of selectivity *vis á vis* FAAH following repeated high dosing may present obstacles to drug development, but hopefully *in vivo* data with reversible inhibitors will be forthcoming to address these issues. Within the cancer field, it is early days and *in vivo* data with genetic and orthotopic cancer models would be most useful, as would combination studies with standard drug regimes. A separate issue is whether potentiation of eCBs following MGL inhibition may produce unwanted effects upon immune function, given that CB_2 receptor activation can dramatically affect the properties of immune cells (Basu and Dittel, 2011). Nonetheless, when it is remembered that prior to 2005, no selective and 'drugable' MGL inhibitors were available, the rapid progress in the field is impressive, and gives hope that the therapeutic potential of MGL as a target for drug development will be translated into a clinical reality.

Acknowledgements

The author would like to take the opportunity to thank the Swedish Science Council (Grant no. 12158, medicine); the Swedish Cancer Society (Grant no. CAN2010/437); the European Union (Contract no. MTKD-CT-2006-039039, under the FP6 Transfer of Knowledge scheme), the Lions Cancer Research Fund at Umeå University/Cancer Research Fund Norrland and the Research Funds of the Medical Faculty, Umeå University for their financial support of my research into the eCB system and its therapeutic potential.

Conflict of interest

The author reports no conflicts of interest with respect to the compounds discussed in the present article.

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