Review

The endocannabinoid signaling system: Pharmacological and therapeutic aspects


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Abstract

Since the discovery of anandamide in 1992, our knowledge of the endocannabinoid system and its physiological effects has increased greatly, not the least as a result of the availability of compounds affecting endocannabinoid function. In the present review, the pharmacology of the endocannabinoid system is discussed. At present, there are no compounds selectively inhibiting the synthesis of anandamide, and the mechanisms by which anandamide release and reuptake are blocked are a matter for current debate. In contrast, selective agonists and inverse agonists at the CB1 and CB2 receptors have been well characterised, as have inhibitors of the metabolism of anandamide by fatty acid amide hydrolase. Accumulating evidence has suggested that such compounds may be useful for the treatment of a number of disorders. With respect to the treatment of pain, topical CB1 agonists and CB2 agonists may prove therapeutically useful, and there is evidence that the non-steroidal inflammatory agent indomethacin produces effects secondary to activation of the endocannabinoid system. Modulation of the endocannabinoid system may also produce neuroprotective effects, although present data would suggest that the observed effects are highly dependent upon the nature of the neurotoxic insult.

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Keywords: Cannabinoid; Anandamide; 2-arachidonoylglycerol; Pain; Neuroprotection

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1. Introduction

Although historical references to the use of cannabinoids for medicinal purposes date back over four thousand
years (see Adams and Martin, 1996), the structures of the (at cannabinoid receptors) inactive compounds cannabidiol and cannabidinol, and the active compounds Δ⁶-tetrahydrocannabinol and Δ⁶-tetrahydrocannabivarin were not identified until the middle of the last century (see Adams et al., 1940a,b; Gaoni and Mechoulam, 1964; Hively et al., 1966; Mechoulam and Hanus, 2000). Detailed structure–activity relationship studies (Razdan, 1986) together with radioligand binding studies (Devane et al., 1988) suggested the presence of a cannabinoid (CB) receptor. CB₁ and CB₂ receptors were cloned during the early 1990s (Matsuda et al., 1990; Munro et al., 1993; review, see Howlett et al., 2002), and shortly after anandamide (AEA) and 2-arachidonoylglycerol (2-AG) were identified as endogenous cannabinoid (endocannabinoid) compounds (see Devane et al., 1992; Mechoulam et al., 1995; Sugiyara et al., 1995; Mechoulam and Hanus, 2000). Since then, our knowledge of the endocannabinoid system and its physiological roles have expanded enormously (for recent reviews, see Piomelli, 2003; Gerdes and Lovinger, 2003; De Petrocellis et al., 2004).

A key to the elucidation of the roles played by endocannabinoids in the body have been the development of pharmacological agents that affect their function (see Fig. 1). In the present review, the pharmacology of the endocannabinoid system is discussed.

2. Endocannabinoid synthesis and release

It is now well established that endocannabinoids are synthesised and released “on demand” and that this process can be regulated both physiologically and under pathological conditions (reviews see Piomelli, 2003; Fowler, 2003). However, pharmacological agents selectively affecting the synthetic enzymes are lacking, although tetrahydrocannabinol is a potent inhibitor of diacylglycerol lipases and has as such been used to identify the physiological processes involving 2-AG in the brain (Bisogno et al., 2003; Melis et al., 2004). With respect to the release of AEA, there is some debate as to its nature. Initially, it was suggested that the release was simply the uptake process acting in reverse (Hillard et al., 1997). However, Kathuria et al. (2003) reported that the release of AEA into the medium from prelabelled rat cortical neurons in primary culture was not blocked by the putative reuptake inhibitor AM404 and suggested that the release was by passive diffusion rather than reverse transport. The fact that [³H]AEA can bind to, and be released by a temperature-dependent first order process from cell culture wells (Karlsson et al., 2004) does not make the situation easier. However, Maccarrone et al. (2002) have reported that estrogen-stimulated AEA release from prelabelled human endothelial (HUVEC) cells could be blocked by AM404. More recently, Ligresti et al. (2004) reported that the release of de novo synthesised AEA from thapsigargin-stimulated HEK293 cells was blocked by the putative reuptake inhibitor VDM11.

This latter finding has interesting implications for studies investigating endocannabinoid tone: should a compound blocking a bidirectional carrier prevent endocannabinoid effects (by preventing the release of newly synthesised endocannabinoids) or potentiate it (by preventing the reuptake of released endocannabinoids)? In this respect, Ronesi et al. (2004) reported that intracellular application of VDM11 and AM404 to brain slices via a patch pipette were able to prevent striatal long term depression produced by high frequency stimulation. In contrast, extracellularly applied VDM11 was without effect. Taken together, the data summarised above would suggest that the release of AEA can be modulated pharmacologically, although the mechanism for such a modulation remains unclear, as indeed, is the case for...
3. Interaction with receptors

As it is clear from their name, endocannabinoids activate CB receptors, although with different levels of efficacy (see Sugiura et al., 1999) and with different rates of induction of receptor desensitization (Luk et al., 2004). In addition, endocannabinoids, in particular anandamide, have been shown to have effects upon other systems, especially TRPV1 (vanilloid 1) receptors, either by direct mechanisms (Zygmunt et al., 1999; see also Pacher et al., 2004, for a recent study investigating the haemodynamic effects of AEA in TRPV1−/− mice) or as a result of lipoxygenase-derived metabolites (Kagaya et al., 2002) (review, see Ross, 2003). A key to our understanding of the cannabinoid-mediated processes in the body have been the development of a variety of selective agonists and antagonists for CB1 and CB2 receptors (see Fig. 1 for examples). With respect to agonists, most of the available information has been obtained using non-selective “standard” compounds such as CP 55,940 and WIN 55,212-2 (for a detailed review of the antinociceptive effects of these compounds, see Pertwee, 2001). CB1 and CB2 selective agonists are now available, and have provided vital information as to the role of these agents in fields as diverse as pain processing (see Section 6.1 below) and control of cell proliferation (see Guzmán, 2003).

With respect to blockade of cannabinoid receptors, compounds such as rimonabant (SR141716A) (Rinaldi-Carmona et al., 1994), the closely related AM251 (designed to allow radioiodination) (Gatley et al., 1996) (structures, see Fig. 2) and SR144528 (Rinaldi-Carmona et al., 1998) have played central roles in determining the contribution of CB1 and CB2 receptors to endocannabinoid effects, and rimonabant is now in phase III clinical trials for weight reduction and an aid to smoking cessation. Rimonabant and AM251 are generally described as inverse agonists rather than pure antagonists. Whether or not this is of importance in vivo remains to be determined, since whilst constitutive activity can clearly be demonstrated in heterologous expression systems (see e.g. Nie and Lewis, 2001 for detailed molecular studies), there is some debate as to whether native CB1 receptors are constitutively active. In physiological systems, a situation whereby, for example, a CB1 receptor agonist produces a response and rimonabant alone produces an opposite effect may be interpreted either by suggesting the presence of constitutively active receptors, or alternatively the presence of an endocannabinoid tone. An example of this is the opposite effects of AEA and rimonabant upon the sleep-waking cycle in rodents, accompanied by opposite changes in the brain levels of adenosine (Santucci et al., 1996; Murillo-Rodríguez et al., 2003). More important, at least in terms of development of CB1 receptor antagonists/inverse agonists as possible anti-obesity agents (see Verty et al., 2004, and references therein), is the evidence of endocannabinoid tone controlling food intake (Di Marzo et al., 2001). However, in contrast, Wade et al. (2004) demonstrated that whilst the CB receptor agonist WIN 55,212-2 decreased both basal and forskolin-stimulated striatal extracellular levels of cAMP in awake rats after local administration in a manner blocked by rimonabant, the antagonist alone had no effect, arguing against either a general endocannabinoid tone (or constitutive receptors) in vivo.

1 Relatively little clinical data has so far been published for rimonabant, although it is clear from information in the public domain that the developers of this drug place high hopes (and considerable investment) upon its therapeutic usefulness.
Table 1

<table>
<thead>
<tr>
<th>Compound and dose</th>
<th>Finding</th>
<th>Reference</th>
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<tbody>
<tr>
<td>AM404</td>
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<tr>
<td>10 mg/kg i.v. C57/B6 mice</td>
<td>No effect on the hot plate test per se at 20, 30 and 60 min after administration, but potentiates and prolongs the increased latency produced by 20 mg/kg i.v. AEA</td>
<td>Beltramo et al. (1997)</td>
</tr>
<tr>
<td>10 mg/kg i.v. guinea pigs</td>
<td>Modest effect on blood pressure per se, potentiates the ↓ systemic blood pressure produced by 5 mg/kg i.v. AEA in vagotomised and pancuronium-treated animals</td>
<td>Calignano et al. (1997)</td>
</tr>
<tr>
<td>10 mg/kg i.p. C57/B6 mice</td>
<td>↓ plasma prolactin but not luteinizing hormone levels; ↑ inactivity and ↓ ambulation but not exploration or frequency of stereotypy in open field test</td>
<td>González et al. (1999)</td>
</tr>
<tr>
<td>10 mg/kg s.c. repeated injections</td>
<td>↑ AEA but not PEA levels in plasma; ↑ immobility and ↓ locomotion; not seen in rats pretreated with 0.5 mg/kg i.p. rimonabant</td>
<td>Giuffrida et al. (2000)</td>
</tr>
<tr>
<td>10 µg i.c.v. C57/B6 mice</td>
<td>↑ immobility, not seen in rats pretreated with 1 mg/kg i.p. rimonabant. No effects on behaviours like grooming, oral movements, sniffing and hotplate jumping</td>
<td>Beltramo et al. (2000)</td>
</tr>
<tr>
<td>2 µg i.c.v. C57/B6 mice</td>
<td>↓ apomorphine induced yawning, not seen in rats pretreated with 0.2 mg/kg i.v. rimonabant.</td>
<td>Beltramo et al. (2000)</td>
</tr>
<tr>
<td>10 mg/kg i.v. Biozzi ABH mice</td>
<td>↓ spasticity in mice induced to display chronic relapsing experimental allergic encephalomyelitis</td>
<td>Baker et al. (2001)</td>
</tr>
<tr>
<td>62.5 µg topically (eye) normotensive C57/B6 and Swiss mice</td>
<td>↓ intraocular pressure when administered in 2-hydroxy-β-cyclodextrin; initial ↓ when administered in propylene glycol</td>
<td>Laine et al. (2001)</td>
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<tr>
<td>5 nmol i.t. C57/B6 mice</td>
<td>↓ pain related behaviour in the formalin test to the level seen with 3 nmol i.t. AEA. Not additive with AEA. Blocks pronociceptive effect of NO-donor RE-2047 (45 mg/kg i.p.)+</td>
<td>Gühring et al. (2002)</td>
</tr>
<tr>
<td>10 mg/kg i.p. C57/B6 mice</td>
<td>↓ jumping behaviour due to spontaneous withdrawal after repeated morphine treatment. Less marked effects with 2 mg/kg i.p. AM404. No significant effects upon naloxone-induced withdrawal</td>
<td>Del Arco et al. (2002)</td>
</tr>
<tr>
<td>10 mg/kg i.p. Sprague–Dawley rats</td>
<td>↓ ambulatory activity in 3-nitropropionic acid-lesioned rats; effect not blocked by rimonabant (3 mg/kg i.p.), but blocked by capsazepine (10 mg/kg i.p.)</td>
<td>Lastres-Becker et al. (2002, 2003b)</td>
</tr>
<tr>
<td>1 mg/kg s.c. repeated injections (prenatal days E11–E20), C57/B6 and Swiss mice</td>
<td>↓ activity in novelty situations on postnatal day 60, as assessed by the Lát-maze.</td>
<td>Viggiano et al. (2003)</td>
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<tr>
<td>0.1–1 mg/kg i.p.</td>
<td>No effect on motor activity produced by L-DOPA (150 mg/kg i.p.)+ benserazide (50 mg/kg i.p.) in reserpine-treated animals</td>
<td>Segovia et al. (2003)</td>
</tr>
<tr>
<td>1 and 5 mg/kg i.p.; 1 and 5 µg intrastriatally, C57/B6 rats</td>
<td>↓ amphetamine-induced turning, blocked by 1:1 cotreatment with AM251, in 6-hydroxydopamine unilaterally nigral lesioned animals. i.p. AM404 also reduced the sensorimotor deficit seen in the lesioned animals. Significant interaction between intrastriatal AM404 and either quinpirone or agents interacting with 5-HT1B receptors</td>
<td>Fernandez-Espejo et al. (2004)</td>
</tr>
<tr>
<td>5 mg/kg i.p. C57/B6 mice</td>
<td>↓ prepulse inhibition (PPI) after both acute or chronic administration; effect blocked by rimonabant (1 mg/kg)</td>
<td>Fernandez-Espejo and Galan-Rodriguez (2004)</td>
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<tr>
<td>10 mg/kg i.p. FAAH+/+ mice</td>
<td>No effect on body temperature alone. ↑ AEA (5 mg/kg i.p.) hyperthermic effects.</td>
<td>Fegley et al. (2004)</td>
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<tr>
<td>10 mg/kg i.p. FAAH−/− mice</td>
<td>Small decrease in body temperature alone. Powerful increase in the hypothermic effects of AEA (2 mg/kg i.p.). Effect blocked by rimonabant (0.3 mg/kg i.p.) but not by capsazepine (30 mg/kg i.p.)</td>
<td>Fegley et al. (2004)</td>
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<td>VDMII</td>
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<tr>
<td>10 mg/kg i.v. Biozzi ABH mice</td>
<td>↓ spasticity in mice induced to display chronic relapsing experimental allergic encephalomyelitis</td>
<td>Baker et al. (2001)</td>
</tr>
<tr>
<td>10 mg/kg i.p. C57/B6 mice</td>
<td>Potentiates the reduction of intestinal transit produced by i.p. administration of acetic acid. Effect blocked by 1 mg/kg i.p. rimonabant</td>
<td>Mascolo et al. (2002)</td>
</tr>
<tr>
<td>5 mg/kg i.p. Sprague–Dawley rats</td>
<td>No effect on ambulatory activity in 3-nitropropionic acid-lesioned rats</td>
<td>Lastres-Becker et al. (2003b)</td>
</tr>
<tr>
<td>10 mg/kg i.p. C57/B6 mice</td>
<td>Blocks intestinal fluid accumulation produced by cholera toxin; effect antagonised by 0.3 mg/kg rimonabant</td>
<td>Izzo et al. (2003)</td>
</tr>
<tr>
<td>5 mg/kg i.p. Wistar rats</td>
<td>↑ latency on hot plate test (not seen with 1 or 10 mg/kg doses); potentiates effect of 2 mg/kg i.p. AEA in this test. No effect of either compound at these doses either separately or combined on locomotor activity</td>
<td>de Lago et al. (2004)</td>
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</table>
In vitro, rimonabant at micromolar concentrations produces the opposite effects upon brain G-protein function (measured by effects upon either $[^{35}S]$GTPγS binding or forskolin-stimulated cAMP accumulation) to those seen with CB1 receptor agonists (Sim-Selley et al., 2001; Mato et al., 2002). However, Savinainen et al. (2003) recently reported that the decreased binding of $[^{35}S]$GTPγS to rat cerebellar membranes produced by 10 μM concentrations of rimonabant and AM251 was blocked by the A1 adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine. Similarly, rimonabant and AM251 produced a small, but statistically significant rightward shift in the dose–response curve for the adenosine receptor agonist 2-chloroadenosine with respect to its ability to stimulate $[^{35}S]$GTPγS binding. In contrast, binding stimulated by carbachol and baclofen were not affected by rimonabant and AM251 (Savinainen et al., 2003). These data would suggest that the inhibition of $[^{35}S]$GTPγS binding at 1 and 10 μM concentrations of rimonabant and AM251 is an indication of an influence upon adenosine A1 receptor function rather than a demonstration of an inverse agonist property at CB1 receptors.

The interaction with A1 receptors is by no means a unique effect of these compounds at μM concentrations, since non-CB1 receptor mediated effects upon ERK phosphorylation in JB6 P+ cells (Berdyshev et al., 2001), TRPV1 receptors expressed in hVR1-HEK cells (De Petrocellis et al., 2001), Ca2+-induced relaxation of mesenteric branch arteries from CB1 receptor knockout (CB1−/−) mice (Bukoski et al., 2002), and sodium channel function in mouse brain synaptic preparations (Liao et al., 2004) have also been reported. Rimonabant can also produce pharmacological and behavioural effects in CB1+/− mice (Fride et al., 2003; Bátkai et al., 2004). This lack of selectivity at high concentrations is an important caveat in the interpretation of data with CB1 antagonists, and an obvious recommendation would be the use of several compounds from different chemical classes, such as, for example, LY320135 (Felder et al., 1998) and O-2654 (Thomas et al., 2004) (structures, see Fig. 2).

From the above discussion, it remains unclear whether opposite effects of cannabinoid agonists and inverse agonists in vivo reflect the presence of constitutively active receptors, endocannabinoid tone, or both. Hopefully, this dilemma will be aided by the development of “neutral” CB1 receptor antagonists (as opposed to inverse agonists), such as has been suggested to be the case for O-2654 (Thomas et al., 2004). An alternative approach would be the use of compounds selectively affecting the levels of extracellular endocannabinoids, since such compounds would be expected to enhance endocannabinoid tone without affecting constitutive activity. Such compounds are discussed below.

### 4. AEA uptake inhibitors

Most of our knowledge concerning the reuptake of endocannabinoids has been with respect to AEA, and this section is in consequence confined to this endocannabinoid. There is at present considerable debate as to the nature, or even existence, of an AEA transporter protein, current thinking ranging from facilitated transport and/or endocytic

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**Table 1 (continued)**

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<tr>
<th>Compound and dose</th>
<th>Finding</th>
<th>Reference</th>
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<tr>
<td>UCM707</td>
<td>↑ time spent in inactivity (10 mg/kg); no effects on hot plate latency, exploratory, or stereotypic activity. No effects on locomotor activity, but significant ↓ambulation and exploratory activity in combination with AEA. No effects of these parameters with AEA alone. The enantiomer OMDM-1 produced no significant changes in these tests</td>
<td>de Lago et al. (2004)</td>
</tr>
<tr>
<td>OMDM-2</td>
<td>↑ latency on hot plate test (not seen with 1 or 10 mg/kg doses); no effect in presence of 2 mg/kg i.p. AEA in this test. No effect of the compound per se on locomotor activity, but significant ↓ambulation and exploratory activity in combination with AEA. No effects of these parameters with AEA alone. The enantiomer OMDM-1 produced no significant changes in these tests</td>
<td>de Lago et al. (2004)</td>
</tr>
<tr>
<td>5 mg/kg i.v. Biozotti ABH mice (gender not given)</td>
<td>↓ spasticity in mice induced to display chronic relapsing experimental allergic encephalomyelitis. Also seen with OMDM-1 at this dose</td>
<td>de Lago et al. (2004)</td>
</tr>
</tbody>
</table>
uptake to a passive diffusion process driven to a varying extent by the AEA metabolising enzyme fatty acid amidase hydrolase (FAAH) (Glaser et al., 2003; Hillard and Jarrahian, 2003; McFarland and Barker, 2004; see also Ronesi et al., 2004; Ligresti et al., 2004; Fegley et al., 2004; Ortega-Gutiérrez et al., 2004; McFarland et al., 2004). One argument in favour of the existence of a transport process is that it can be inhibited pharmacologically by arachidonoyl-based compounds such as AM404 (Beltramo et al., 1997), VDM11 (De Petrocellis et al., 2000) and UCM707 (López-Rodríguez et al., 2001), and by the oleoyl-based enantio-meric pair OMDM-1 and OMDM-2 (Ortar et al., 2003).

Why the compounds act as inhibitors, in the case of AM404 in a competitive manner (Rakshan et al., 2000) is not without controversy (see e.g. Patricelli and Cravatt, 2001)—indeed the compounds can prevent the adsorption of AEA to plastic cell culture wells at similar concentrations (Karlsson et al., 2004; Fowler et al., 2004; Ortega-Gutiérrez et al., 2004)—but it is clear that they are biologically active and can potentiate the effects of AEA both in vitro (Beltramo et al., 1997) and in vivo (Table 1), as well as prevent effects of exogenous AEA upon TRPV1 receptors (which require intracellular transport since the binding site for this molecule is on the intracellular face of the receptor) in vitro (De Petrocellis et al., 2001; Andersson et al., 2002; Jonsson et al., 2003). In addition, the compounds produce effects per se both in vitro (Gubellini et al., 2002; Trettel and Levine, 2003; Ronesi et al., 2004) and in vivo (Table 1).

Most of the data have been obtained using AM404, which, however, shows little selectivity for the uptake process over FAAH and indeed acts as a substrate for FAAH (Jarrahian et al., 2000; Fegley et al., 2004) and in addition interacts with TRPV1 receptors (Zygmun et al., 2000) as a partial agonist (Roberts et al., 2002). This compound can also produce effects in vitro at low micromolar concentrations (i.e. similar to those used to block uptake) that are not prevented by either CB1 receptor antagonists or TRPV1 receptor antagonists (Jonsson et al., 2003; Kelley and Thayer, 2004). Nevertheless, the report that AM404 can potentiate the hypothermic effects of AEA in FAAH−/− mice in a manner blocked by rimonabant but not capsazepine (Fegley et al., 2004) does support an action of this compound in vivo that can be distinguished from effects upon FAAH and TRPV1 receptors. UCM707, OMDM-1 and OMDM-2 show little effect on FAAH and TRPV1 receptors (López-Rodríguez et al., 2003; Ortar et al., 2003) and are now generally available for experimental work, so it is likely that our knowledge in this area will increase.

5. Inhibitors of endocannabinoid metabolism

Effective metabolism of endogenous signaling molecules is a prerequisite for their action, and it is now well established that both AEA and 2-AG are rapidly metabolised (see e.g. Wiley et al., 2000; Járai et al., 2000). In the case of AEA, the key enzyme for metabolism is FAAH, and mice lacking this enzyme show raised levels of AEA in the brain (Cravatt et al., 2001). Similarly, selective inhibition of FAAH produces an increased level of AEA, but not 2-AG in the brain (Kathuria et al., 2003). PMSF was discovered fortuitously to inhibit FAAH (Deutsch and Chin, 1993) and thereafter shown at a dose of 30 mg/kg i.p. to potentiate the actions of AEA in vivo (Compton and Martin, 1997; Wiley et al., 2000) without producing deleterious actions secondary to inhibition of acetylcholinesterase (Quistad et al., 2002). A variety of FAAH inhibitors have since been identified (review, see Fowler, 2004a). Perhaps the compounds that have received the most attention are the carbamate derivatives URB532 and URB597, in view of the finding that they have positive effects in an animal model for anxiety (Kathuria et al., 2003), a finding consistent with the role of CB1 receptors in the regulation of anxious behaviour (Urigüen et al., 2004; Haller et al., 2004). However, other compounds as diverse as 1-(2-benzoxazolyl)-1-oxo-9(Z)-octadecene, AM374, OL-135, profopfol and the NSAIDs indomethacin and flurbiprofen inhibit FAAH in vitro (Paria et al., 1996; Deutsch et al., 1997; Boger et al., 2000; Patel et al., 2003; Fowler et al., 2003; Lichtman et al., 2004b), a property that may contribute to their pharmacological effects in vivo (Baker et al., 2001; Fedorova et al., 2001; Gühring et al., 2002; Ates et al., 2003; Patel et al., 2003; Arizzii et al., 2004; Holt et al., 2004; Lichtman et al., 2004b).

In addition to FAAH, AEA is a substrate for cyclooxygenase-2 and lipoxygenases (see Kozak and Marnett, 2002; Maccarrone, 2004) in vitro. Weber et al. (2004) treated male Swiss Webster mice with AEA (50 mg/kg i.v.) and measured the liver, kidney, lung and small intestine levels of AEA and cyclooxygenase-2 derived metabolites (prostaglandin F2α, prostamide E2+D2) 30 min later. For normal mice, there was little or no detectable prostamide formation. However, when the experiment was repeated in FAAH−/− mice, detectable levels of prostamides (together with raised levels of AEA) were seen (Weber et al., 2004). Another study has reported in an abstract the detection of prostamide F2α in both brain and peripheral tissues from FAAH−/− and FAAH+/− mice (Woodward et al., 2004). With respect to the brain, evidence that cyclooxygenase-2 may be physiologically important for endocannabinoid metabolism has been suggested by the finding that a cyclooxygenase-2 inhibitor, but not an FAAH inhibitor, potentiated depolarization-induced suppression of inhibition in hippocampal slices (a process mediated by endocannabinoids) (Kim and Alger, 2004). In contrast, in the amygdala, endocannabinoid-mediated long-term depression of inhibitory GABAergic synaptic transmission is enhanced in FAAH−/− mice (Azad et al., 2004), whereas in other systems, 2-AG may be more important (Melis et al., 2004; Makara et al., 2004).

In the case of 2-AG, multiple metabolic pathways are also possible, since this endocannabinoid is a substrate for
FAAH, monoacylglycerol lipase (MAGL), lipoxygenases and cyclooxygenases (review, see Fowler, 2004a). In the brain, however, MAGL appears to be the dominant enzyme (Dinh et al., 2002; Saario et al., 2004), although additional cytosolic 2-AG metabolising enzymes may be present (Dinh et al., 2004). MAGL has been cloned (Karlsson et al., 1997; Dinh et al., 2002), and is presynaptically located in the hippocampus, in contrast to FAAH, which is found postsynaptically (Gulyas et al., 2004). To our knowledge no potent (i.e. IC\textsubscript{50} values <1 μM) MAGL-selective inhibitors or genetically modified animals have yet been reported in the literature. “Standard” FAAH inhibitors like PMSF and acyl trifluoromethyl ketones are in fact less potent towards MAGL than FAAH (Bisogno et al., 1997; Goparaju et al., 1999; Di Marzo et al., 1999; Dinh et al., 2002; Saario et al., 2004; Ghafoori et al., 2004). However, compounds that have equal or greater selectivity for MAGL than FAAH in vitro (albeit with IC\textsubscript{50} values >10 μM) have been reported (Ghafoori et al., 2004; Casicco et al., 2004; Makara et al., 2004), so it is to be hoped that further investigations will identify potent selective MAGL inhibitors.

6. Modulation of the endocannabinoid system as a therapeutic approach

It is clear that the multitude of physiological (and pathological) processes involving cannabinoid receptors raise a number of therapeutic targets. This subject has been the topic of extensive recent reviews (see e.g. Guzmán, 2003; Baker et al., 2003; Lastres-Becker et al., 2003a; Harrold and Williams, 2003) and so the present article will confine itself to two therapeutic areas, pain processing and neuroprotection. As pointed out in the introduction, cannabinoids have been used for medicinal purposes for a very long time and the debate concerning whether or not “medicinal marijuana” should be an acceptable form of treatment continues (see e.g. Wingerchuk, 2004). Anecdotal evidence for the usefulness of cannabis extracts encompasses a wide variety of ailments, although its possible utility for the treatment of pain has been a recurring theme (see Reynolds, 1890). The commercial development of cannabis extracts such as Sativex\textsuperscript{TM} and their clinical evaluation in properly controlled tests have started to provide crucial information as to the therapeutic usefulness of cannabinoids, and some double-blind placebo-controlled studies have now been reported in the literature with respect to the alleviation of pain either per se or as a symptom of multiple sclerosis (Wade et al., 2003; Zajicek et al., 2003; Neef et al., 2003; Berman et al., 2004; see also Svendsen et al. (2004) for a recent study with dronabinol). A key issue, of course, will always be the presence of unwanted psychotropic effects of centrally acting cannabinoids (Huestis et al., 2001; D’Souza et al., 2004) which can be minimised by the use of carefully controlled formulations, but never removed (thereby placing a limit on possible efficacy via limitation of possible dosages), unless, of course, cannabinoids lacking psychotropic effects can be identified. One such compound may be ajulemic acid (CT-3) which has been the subject of a preliminary placebo-controlled clinical trial as an analgesic (Karst et al., 2003; see Burstein et al., 2004).

An alternative approach has been made possible by the identification of the different components of the endocannabinoid system. Thus, it may be possible to target receptors that are not present in the brain (by the topical application of CB\textsubscript{1} receptor agonists or the use of CB\textsubscript{2} receptor agonists). An alternative approach would be to bolster up existing cannabinoid signals rather than overlaying new signals (by the use of inhibitors of endocannabinoid uptake and metabolism). These possibilities, which have not as yet been tested clinically, are discussed below.

6.1. The endocannabinoid system and pain processing

A large body of evidence now supports the contention that the endocannabinoid system is involved in pain processing, and that antinoceptive effects of cannabinoids involve supraspinal, spinal and peripheral CB\textsubscript{1} receptors, as well as peripheral CB\textsubscript{2} receptors (see Pertwee, 2001; Rice, 2001; Walker and Huang, 2002; Scott et al., 2004). In addition, endogenous compounds related to AEA, such as arachidonoyl glycine and palmitoyl ethanolamide have antinoceptive actions (Jaggar et al., 1998; Calignano et al., 1998; Huang et al., 2001). The case of palmitoyl ethanolamide is particularly interesting, since the antinoceptive effects of this compound are blocked by SR144528 (Jaggar et al., 1998; Calignano et al., 1998), although palmitoyl ethanolamide itself has no affinity for CB\textsubscript{2} receptors (Lambert et al., 1999), raising the possibility that an as yet unidentified receptor sensitive to SR144528 is involved. There is also evidence for other “CB-like receptors”, often based upon residual activities of endocannabinoids in CB\textsubscript{1}\textsuperscript{−/−} mice (see e.g. Di Marzo et al., 2000; Baskfield et al., 2004). As yet, these additional receptors have not been cloned, and a detailed discussion of their activities is beyond the scope of the present review.

The involvement of the endocannabinoid system in pain processing suggests a number of potential therapeutic targets, that can be summarised briefly below.

6.1.1. Selective activation of peripheral CB\textsubscript{1} receptors

The peripheral component of CB\textsubscript{1} receptors in pain processing would suggest that local administration of cannabinoids may produce beneficial effects without the problems of unwanted psychotropic effects. There is certainly good evidence in experimental animals that either intraplantar or topical administration of cannabinoids can produce antinoceptive effects in a manner blocked by rimonabant or AM251 (Richardson et al., 1998; Fox et al., 2001; Nackley et al., 2003b; Dogrul et al., 2003). Topical cannabinoids may also act synergistically with topically
applied morphine (Yesilyurt et al., 2003). The selective CB1 receptor agonist ACEA has also been demonstrated to possess peripherally mediated effects upon noxious somatosensory processing that are blocked by rimonabant (Kelly et al., 2003). This compound has more recently been found in vitro also to produce effects mediated by TRPV1 receptors, although it lacks the pungency associated with TRPV1 agonists such as capsaicin (Price et al., 2004).

### 6.1.2. CB2 receptor agonists

The lack of central CB2 receptors (other than on activated microglia) make them an attractive target for drug development. Initially, it was demonstrated that the CB2 receptor agonist HU308 (structure shown in Fig. 3) was efficacious in the formalin model in a manner blocked by SR144528 (Hanus et al., 1999). Subsequent studies have demonstrated that the CB2 receptor agonists AM1241 (structure shown in Fig. 3) and GW405833 (1-(2,3-dichlorobenzoyl)-5-methoxy-2-methyl-(2-(morpholin-4-yl)ethyl)-1H-indole) have antinociceptive effects in a number of models of inflammatory and, in the case of AM1241, neuropathic pain (Malan et al., 2001; Clayton et al., 2002; Quartilho et al., 2003; Ibrahim et al., 2003; Nackley et al., 2003a; Hohmann et al., 2004).

### 6.1.3. Modulation of endocannabinoid levels

The finding that intraplantar injection of formalin produces a release of anandamide in the periaqueductal grey region (Walker et al., 1999) would suggest that compounds preventing the breakdown of anandamide may be useful. Unwanted central effects would be unlikely, since the levels of AEA in other areas of the brain would be predicted to remain low. This contention is supported by the finding that mice lacking FAAH do not show overt signs of central CB1 receptor activation, but do have a reduced pain sensitivity in models of thermal and inflammatory pain, but not in the chronic constriction injury model of neuropathic pain (Cravatt et al., 2001; Lichtman et al., 2004a). Interestingly, animals lacking peripheral, but not central, FAAH (“FAAH-NS mice”) show greater sensitivities to thermal nociception than FAAH−/− mice, but retain the reduced oedema response to intraplantally administered carrageenan that is seen in the FAAH−/− mice (Cravatt et al., 2004). FAAH inhibitors have modest effects per se in models of thermal nociception, although they of course potentiate the antinociceptive effects of exogenous AEA (Compton and Martin, 1997; Kathuria et al., 2003, Lichtman et al., 2004b). With respect to the formalin test of inflammatory pain, Lichtman et al. (2004b) found that the selective FAAH inhibitor OL-135 dose dependently reduced both phase 1 and 2 pain behaviours in a manner that was blocked by rimonabant but not by SR144528. The findings that the antinociceptive effects of the NSAIDs flurbiprofen and indomethacin, when spinaly administered, in the formalin test involve CB1 receptors (Gühring et al., 2002; Ates et al., 2003; for review, see Fowler, 2004b) are consistent with an activation of the endocannabinoid system in the actions of these compounds. In addition, we have found that the ability of indomethacin to reduce carrageenan-induced inflammation of the mouse paw can be blocked by SR144528 (Holt et al., 2004). Whether or not FAAH inhibition is involved in these effects awaits elucidation.

### 6.2. The endocannabinoid system and neuroprotection

The role of endocannabinoids in neuroprotection has been reviewed in detail elsewhere (Fowler, 2003), and so will only be discussed briefly here. In essence, several independent lines of evidence suggest that under certain conditions, compounds modulating the endocannabinoid system may have useful neuroprotective actions.

- Neurotoxic insult almost invariably produces an increase in the levels of AEA and related N-acyl ethanolamines. Recent examples of this include the massive increase in the levels of AEA following permanent middle artery occlusion in rats (Berger et al., 2004), the finding of an increased level of AEA in the microdialysate from a stroke patient (Schäbitz et al., 2002) and the increased levels of AEA following excitotoxic insults (Hansen et al., 2001; Marsicano et al., 2003). AEA levels are also increased in the striatum following unilateral lesion of rats with 6-OHDA (Gubellini et al., 2002). The notion of a localized increase in endocannabinoid levels raises the obvious possibility that compounds preventing endocannabinoid breakdown may be useful: in unaffected regions, the increased endocannabinoid levels secondary to block of breakdown will not be sufficient to activate local CB1 receptors to a degree resulting in unwanted effects, whereas in the affected region, CB1 receptors will be activated (see Fig. 4 for a schematic representation). There are case reports of cannabis smokers suffering from cerebrovascular events which may (or of course may not) be secondary to cardiovascular effects of cannabis (Finsterer et al., 2004). If this is the case, then a local activation of CB1 receptors in the affected region would be more preferable to a generalised effect on all CB1 receptors. Whether or not a local potentiation of AEA is therapeutically desirable, however, is a matter of some contention (see below).

![Fig. 3. Chemical structures of two CB2 receptor agonists. Note that the structure of AM1241 is that shown in the study of Ibrahim et al. (2003).](image-url)
• Middle cerebral artery occlusion in rats increases the level of CB$_1$ receptor expression (Jin et al., 2000). Mice with genetically deleted CB$_1$ receptors show more severe damage following ischaemic and excitotoxic insult (Parmentier-Batteur et al., 2002; Marsicano et al., 2003).

• Modulation of cannabinoid receptor tone affects the outcome following neurotoxic insult. The resultant response appears to be dependent upon a number of factors, since in some cases the cannabinoid receptor agonists show neuroprotective effects (see e.g. Nagayama et al., 1999; Panikashvili et al., 2001; van der Stelt et al., 2001a; Mauler et al., 2002; Martinez-Orgado et al., 2003), whereas in other studies it is rimonabant that is neuroprotective (Hansen et al., 2002; Berger et al., 2004; Muthian et al., 2004). The ability of AEA to activate TRPV1 receptors may also be a complicating factor. Thus, AEA given i.c.v. produces an increased hippocampal calpain activity and cell loss 24 h after injection, a cerebral oedema at 24 h to 7 days, and an impaired performance in the Morris water maze at 17 to 20 days (Cernak et al., 2004). The neuron loss and cognitive deficits were antagonised by capsazepine and the calpain inhibitor SJA6017 but not by AM251 (Cernak et al., 2004). These authors, in their title, referred to a "dark side" of endocannabinoids. Similarly, genetic deletion of FAAH increases the seizure sensitivity to high doses of kainate (Clement et al., 2003). However, the situation is by no means simple, since AEA (in a manner antagonised by rimonabant but not capsazepine), capsaicin and the combined CB$_1$/TRPV1 agonist arvanil reduce the lesion volume 7 days after intracerebral ouabain administration (van der Stelt et al., 2001b; Veldhuis et al., 2003).

The above discussion has mainly considered potentiation of endocannabinoids, the levels of which are increased by the toxic insult. However, a situation where prevention of a decreased endocannabinoid tone may be useful has been suggested by the recent study of Maccarrone et al. (2004). These authors found that repeated i.c.v. administration of the HIV-1 coat glycoprotein gp120 (100 ng) increased cortical FAAH activity and decreased cortical AEA levels. The cortical apoptosis produced by this treatment was reduced by concomitant i.c.v. treatment with the FAAH inhibitor methylarachidonoyl fluorophosphonate (0.2 μg), whereas VDM11, rimonabant, SR144528 and capsazepine (all 2 μg) were without effect (Maccarrone et al., 2004). The authors concluded on the basis of this and other studies that AEA can either induce, or prevent apoptosis, depending upon the experimental situation.

Another important aspect of neuroprotection is the involvement of neuroinflammation. Post-ischemic neuroinflammation is postulated to be of importance for cell death in the penumbra following a stroke (for review, see Dimagl et al., 1999). As previously mentioned, the levels of many endocannabinoids increase following a neurotoxic insult. Some of the endocannabinoids, e.g AEA and 2-AG, can promote motility in vitro in microglial cells that express both CB$_1$ and CB$_2$ receptors upon activation (Walter et al., 2003). This group have also shown that PEA, which is increased after experimental focal cerebral ischaemia, can potentiate anandamide-induced microglial motility (Franklin et al., 2003). Leukocytes infiltrating the brain following an ischaemic episode may exacerbate the inflammatory response and reducing this infiltration could be beneficial as seen in neutropenic animals subjected to experimental stroke (Connolly et al., 1996). Preliminary studies show that AEA can inhibit fMLP-induced neutrophil migration in a concentration-dependent manner (McHugh and Ross, 2004).

The notion that cannabinoids may be useful in neuroinflammation has been particularly well studied experimentally in animal models of multiple sclerosis (for reviews see Baker et al., 2003; Walter and Stella, 2004). Thus, for example, Croxford and Miller (2003) have shown that in mice infected with Theilers murine encephalomyelitis virus (TMEV), the levels of mRNA for proinflammatory cytokines were decreased by WIN 55,212-2. This compound also affects leukocyte function and neurological signs in mice with experimental autoimmune encephalomyelitis (Baker et al., 2000; Ni et al., 2004), although different CB receptors may be involved in the different effects. With respect to endocannabinoids, spinal and brain levels of AEA are increased in animals showing spasticity in an animal model of multiple sclerosis (chronic relapsing experimental allergic encephalomyelitis in mice), and the spasticity could be reduced by...
AM404, VDM11 and the FAAH inhibitor AM374 (Baker et al., 2001). CB1−/− mice were more susceptible to injury in this model than wild-type animals (Pryce et al., 2003), suggesting a protective endocannabinoid tone. Similarly, three-dimensional mouse aggregate brain cultures from CB1−/− mice were more susceptible to the deleterious effects of interferon-gamma (Jackson et al., 2004). The recent case report of an individual developing multiple sclerosis after starting treatment with rimonabant (van Oosten et al., 2004) would also be an alarming result of such a protective role played by the endocannabinoid system. However, as pointed out by the authors of that report, this occurrence of multiple sclerosis may be purely coincidental.

Taken together, the above studies suggest that potentiation of endocannabinoids will affect neuronal survival and function. In their study, Berger et al. (2004) concluded that “It remains unclear by what mechanism NAEs [N-acyl ethanolamines], including anandamide, accumulate under ischemic conditions and whether this accumulation has any beneficial or adverse effects”. In line with this, Clement et al. (2003) suggested that “The context-dependent effects that cannabinoids exhibit on neural circuits, in combination with the broad distribution of the CB1 receptor in the CNS, make it difficult to predict the net impact of CB1 activation on complex pathological events such as seizure and neurotoxicity”. Nevertheless, in the right context, enhancement of endocannabinoid tone may be a useful neuroprotective strategy.

7. Conclusions

The present review has attempted to present the pharmacology of the endocannabinoid system, and to give two examples where this system may provide a useful therapeutic target. Although the current arsenal of compounds has enabled considerable information to be obtained with respect to the physiological roles played by the endocannabinoid system, we still lack compounds selectively interfering with the synthesis of AEA, and with the MAG lipase catalysed breakdown of 2-AG. It is to be hoped that such compounds will be found, and allow the elucidation of the roles played by the individual endocannabinoids.

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