Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 81 (2005) 248 – 262

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Review

The endocannabinoid signaling system: Pharmacological and therapeutic aspects

Christopher J. Fowler*, Sandra Holt, Olov Nilsson, Kent-Olov Jonsson, Gunnar Tiger, Stig O.P. Jacobsson

Department of Pharmacology and Clinical Neuroscience, Umeå University, SE901 87 Umeå, Sweden

Received 8 June 2004; received in revised form 8 December 2004; accepted 16 January 2005

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 CB_1 and CB_2 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 CB_1 agonists and CB_2 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 endocannabionid 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.

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

Contents

1. Introduction

Although historical references to the use of cannabinoids for medicinal purposes date back over four thousand

* Corresponding author. Tel.: +46 90 7851510; fax: +46 90 7852752.
E-mail address: cf@pharm.umu.se (C.J. Fowler).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.01.023

years (see Adams and Martin, 1996), the structures of the (at cannabinoid receptors) inactive compounds cannabinol and cannabidiol, and the active compounds Δ^9-tetrahydrocannabinol and Δ^8-tetrahydrocannabinol 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. CB_1 and CB_2 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; Sugiura 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; Gerdeman 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.

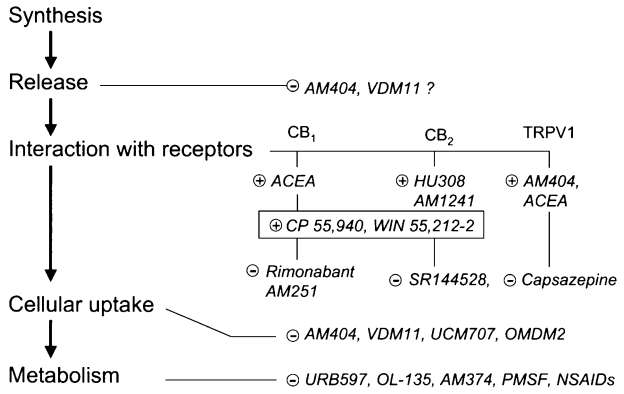

Fig. 1. Pharmacological manipulation of the release, removal and interaction of AEA with its receptors. "⊕" indicates an agonist at the receptors involved, "⊖" indicates either a receptor antagonist or an inhibitor of the process shown, as appropriate. The compounds are by no means an exhaustive list [JWH133 (6a *R*,10a *R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6 *H*-dibenzo[b,d]pyran), for example, is a CB_2 receptor-selective agonist (Huffman et al., 1999) that has been used among others to characterise the role of CB_2 receptors in the proliferation of glioma cells (see Sánchez et al., 2001) and the reponses of wide dynamic range dorsal horn neurons in different models of inflammatory and neuropathic pain (Elmes et al., 2004)] but have been presented here simply because they are discussed in the present review. Abbreviations of compounds (when structures are not shown or given elsewhere): ACEA, *N*-(2-chloroethyl)-5*Z*,8 *Z*,11 *Z*,14 *Z*-eicosatetraenamide; AM374, palmitylsulfonyl fluoride; AM404, *N*-(4-hydroxyphenyl)-5 *Z*,8 *Z*,11 *Z*,14 *Z*-eicosatetraenamide; CP 55,940, (-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; NSAIDs, nonsteroidal anti-inflammatory agents, in this case indomethacin and flurbiprofen (see Fowler et al., 2003); OL-135, 1-oxo-1[5-(2-pyridyl)-2-yl]-7-phenylheptane); OMDM-2, (9*Z*)-*N*-[1-((*R*)-4-hydroxbenzyl)-2-hydroxyethyl]-9-octadecenamide; PMSF, phenylmethylsulfonyl fluoride; SR144528, *N*-[(1S)-*endo*-1,3,3-trimethyl *bicyclo* [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylben-zyl)-pyrazole-3-carboxamide; UCM707, *N*-(fur-3-ylmethyl) 5*Z*,8 *Z*,11 *Z*,14 *Z*-eicosatetraenamide; URB597, 3'-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate; VDM11, (5*Z*,8*Z*,11*Z*,14*Z*)-*N*-(4-hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide; WIN 55,212-2, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate.

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 tetrahydrolipstatin 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 [^{3}H]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 endocannabinoid)? 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

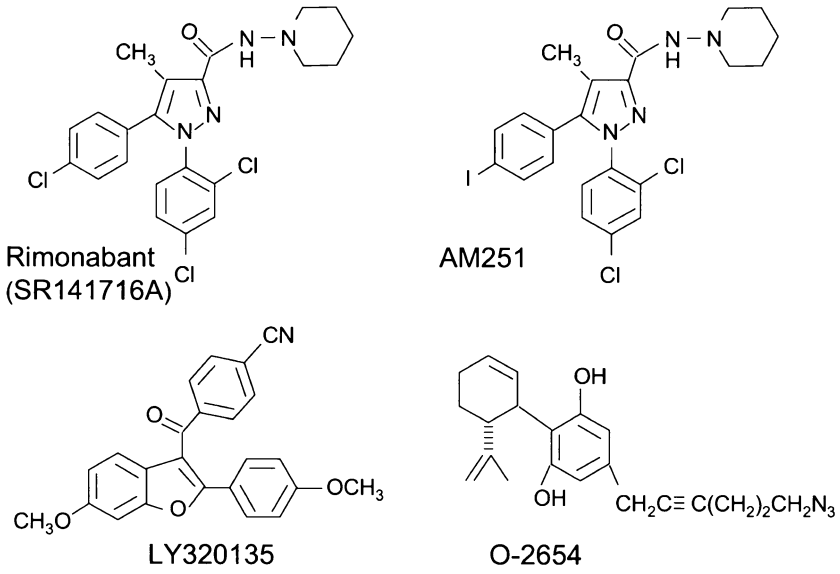

Fig. 2. Chemical structures of four CB_1 receptor antagonists/inverse agonists.

cellular AEA accumulation (see the section below relating to AEA uptake inhibitors).

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 CB_1 and CB_2 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). CB_1 and CB_2 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 CB_1 and CB_2 receptors to endocannabinoid effects, and rimonabant is now in phase III clinical trials for weight reduction and an aid to smoking cessation[1]. 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 CB_1 receptors are constitutively active. In physiological systems, a situation whereby, for example, a CB_1 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 CB_1 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
In vivo effects of putative anandamide transporter inhibitors

Compound and dose	Finding	Reference
AM404		
10 mg/kg i.v. ♂Swiss mice	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	Beltramo et al. (1997)
10 mg/kg i.v. ♂ guinea pigs	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	Calignano et al. (1997)
10 mg/kg i.p ♂ Wistar rats	↓ plasma prolactin but not luteinizing hormone levels; ↑ inactivity and ↓ ambulation but not exploration or frequency of stereotypy in open field test	González et al. (1999)
10 mg/kg i.p. ♂ Wistar rats	↑ AEA but not PEA levels in plasma; ↑ immobility and ↓ locomotion; not seen in rats pretreated with 0.5 mg/kg i.p. rimonabant	Giuffrida et al. (2000)
10 μg i.c.v. ♂ Wistar rats	↑ 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	Beltramo et al. (2000)
2 μg i.c.v. ♂ Wistar rats	↓ apomorphine induced yawning, not seen in rats pretreated with 0.2 mg/kg i.v. rimonabant.	Beltramo et al. (2000)
10 mg/kg i.v. Biozzi ABH mice (gender not given)	↓ spasticity in mice induced to display chronic relapsing experimental allergic encephalomyelitis	Baker et al. (2001)
62.5 μg topically (eye) normotensive ♂ and ♀ Dutch Belted rabbits	↓ intraocular pressure when administered in 2-hydroxy-β-cyclodextrin; initial ↑ when administered in propylene glycol	Laine et al. (2001)
3 nmol i.t. C57/B6 mice (gender not given)	↓ 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.)	Gühring et al. (2002)
10 mg/kg i.p. C57/B6 mice (gender not given)	↓ 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	Del Arco et al. (2002)
10 mg/kg i.p.♂ Sprague–Dawley rats	↓ 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.)	Lastres-Becker et al. (2002, 2003b)
1 mg/kg s.c. repeated injections (prenatal days E11–E20), ♂ and ♀ Naples High Excitability (NHE) rats	↓ activity in novelty situations on postnatal day 60, as assessed by the Làt-maze.	Viggiano et al. (2003)
0.1–1 mg/kg i.p. ♂ Sprague–Dawley rats	No effect on motor activity produced by L-DOPA (150 mg/kg i.p.)+ benserazide (50 mg/kg i.p.) in reserpinised animals	Segovia et al. (2003)
1 and 5 mg/kg i.p.; 1 and 5 μg intrastriatally, ♂ Wistar rats	↓ 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-HT_{1B} receptors	Fernandez-Espejo et al. (2004)
5 mg/kg i.p. ♂ Swiss mice	↓ prepulse inhibition (PPI) after both acute or chronic administration; effect blocked by rimonabant (1 mg/kg)	Fernandez-Espejo and Galan-Rodriguez (2004)
10 mg/kg i.p. $FAAH^{+/+}$mice	No effect on body temperature alone. ↑ AEAs (5 mg/kg i.p.) hypothermic effects.	Fegley et al. (2004)
10 mg/kg i.p. $FAAH^{-/-}$mice	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.)	Fegley et al. (2004)
VDM11		
10 mg/kg i.v. Biozzi ABH mice (gender not given)	↓ spasticity in mice induced to display chronic relapsing experimental allergic encephalomyelitis	Baker et al. (2001)
10 mg/kg i.p. ♂ ICR mice	Potentiates the reduction of intestinal transit produced by i.p. administration of acetic acid. Effect blocked by 1 mg/kg i.p. rimonabant	Mascolo et al. (2002)
5 mg/kg i.p. ♂ Sprague–Dawley rats	No effect on ambulatory activity in 3-nitropropionic acid-lesioned rats or in control rats	Lastres-Becker et al. (2003b)
10 mg/kg i.p. ♂ ICR mice	Blocks intestinal fluid accumulation produced by cholera toxin; effect antagonised by 0.3 mg/kg rimonabant	Izzo et al. (2003)
5 mg/kg i.p. ♂ Wistar rats	↑ 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	de Lago et al. (2004)

(*continued on next page*)

Table 1 (*continued*)

Compound and dose	Finding	Reference
VDM11		
5 mg/kg into tumour, twice weekly to athymic ♂ mice (Charles-River) inocculated with *Kras*-transformed thyroid cells	↓ tumour growth after 5 weeks to 37% of that seen for controls. ↑2-AG but not AEA levels in the tumours.	Bifulco et al., 2004
UCM707		
0.1–10 mg/kg ♂ Wistar rats	↑ time spent in inactivity (10 mg/kg); no effects per se on ambulatory, exploratory or stereotypic activity. No effects on hot plate test. In follow up, 0.5 mg/kg potentiates effect of subeffective dose (0.3 mg/kg i.p.) of AEA (on ↓ ambulatory activity and ↑ time spent in inactivity); a similar result was seen for hot plate latency for 1 mg/kg UCM707 and 2 mg/kg i.p AEA	de Lago et al. (2002)
3 mg/kg s.c., ♂ C57BL/6N mice	Small (~15%) but significant reduction in seizure scores following i.p. kainic acid administration (35 mg/kg). The opposite effect was seen with rimonabant (3 mg/kg s.c.)	Marsicano et al. (2003)
OMDM-2		
5 mg/kg i.p. ♂ Wistar rats	↑ 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 upon 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	de Lago et al. (2004)
5 mg/kg i.v. Biozotti ABH mice (gender not given)	↓ spasticity in mice induced to display chronic relapsing experimental allergic encephalomyelitis. Also seen with OMDM-1 at this dose	de Lago et al. (2004)

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 CB_1 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 CB_1 receptors.

The interaction with A1 receptors is by no means a unique effect of these compounds at μM concentrations, since non-CB_1 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), Ca^{2+}-induced relaxation of mesenteric branch arteries from CB_1 receptor knockout ($CB_1^{-/-}$) 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 $CB_1^{-/-}$ 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 CB_1 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" CB_1 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

uptake to a passive diffusion process driven to a varying extent by the AEA metabolising enzyme fatty acid amide 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 enantiomeric 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 (Rakhshan 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 (Zygmunt 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 CB_1 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 CB_1 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, propofol 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; Arizzi 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 (prostamide $F_{2\alpha}$, 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 $F_{2\alpha}$ 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_{50} values <1 μM) MAGL-selective inhibitors or genetically modified animals have yet been reported in the literature. "Standard" FAAH inhibitors like PMSF and acyltrifluoromethyl 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; Ghafouri et al., 2004). However, compounds that have equal or greater selectivity for MAGL than FAAH in vitro (albeit with IC_{50} values >10 μM) have been reported (Ghafouri et al., 2004; Cascio 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 cannnabis 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™ 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_1 receptor agonists or the use of CB_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 antinociceptive effects of cannabinoids involve supraspinal, spinal and peripheral CB_1 receptors, as well as peripheral CB_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 palmitoylethanolamide have antinociceptive actions (Jaggar et al., 1998; Calignano et al., 1998; Huang et al., 2001). The case of palmitoylethanolamide is particularly interesting, since the antinociceptive effects of this compound are blocked by SR144528 (Jaggar et al., 1998; Calignano et al., 1998), although palmitoylethanolamide itself has no affinity for CB_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 (endo)cannabinoids in $CB_1^{-/-}$ 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_1 receptors

The peripheral component of CB_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 antinociceptive 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 CB_1 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. CB_2 receptor agonists

The lack of central CB_2 receptors (other than on activated microglia) make them an attractive target for drug development. Initially, it was demonstrated that the CB_2 receptor agonist HU308 (structure shown in Fig. 3) was efficaceous in the formalin model in a manner blocked by SR144528 (Hanus et al., 1999). Subsequent studies have demonstrated that the CB_2 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 CB_1 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 spinally administered, in the formalin test involve CB_1 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.

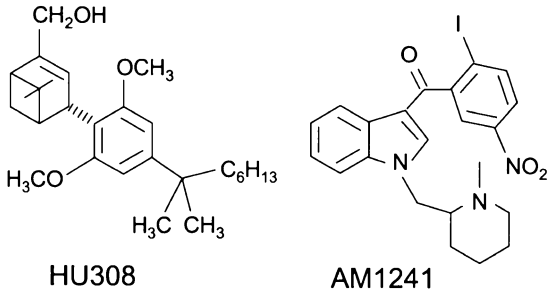

Fig. 3. Chemical structures of two CB_2 receptor agonists. Note that the structure of AM1241 is that shown in the study of Ibrahim et al. (2003).

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 CB_1 receptors to a degree resulting in unwanted effects, whereas in the affected region, CB_1 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 CB_1 receptors in the affected region would be more preferable to a generalised effect on all CB_1 receptors. Whether or not a local potentiation of AEA is therapeutically desirable, however, is a matter of some contention (see below).

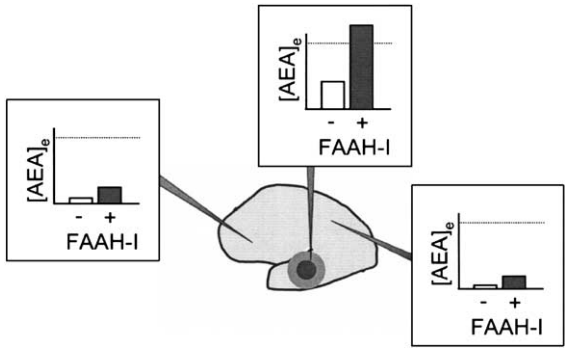

Fig. 4. Schematic representation of the hypothetical changes in the concentration of AEA in different areas of the brain following an ischemic insult. Unfilled columns ("−") and filled columns ("+") are the expected levels of anandamide in the absence and presence, respectively, of an FAAH inhibitor. In non-affected regions, the AEA level remains low (Berger et al., 2004), and in consequence the effect of an FAAH inhibitor is presumed to be insufficient to raise AEA levels sufficiently to produce significant activation of CB_1 receptors (the threshold level is illustrated as a dotted line). In contrast, in the affected region, a dramatic increase in AEA levels is found (Schäbitz et al., 2002; Berger et al., 2004) and the concomitant inhibition of FAAH is presumed to increase extracellular levels to those required for the activation of local CB_1 receptors.

- 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; Martínez-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 Dirnagl 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 excacerbate 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 tonus. 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 for obesity (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 CB_1 receptor in the CNS, make it difficult to predict the net impact of CB_1 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.

Acknowledgements

The authors would like to take this opportunity to thank the Swedish Research Council (Grant no. 12158, medicine), Konung Gustav V's and Drottning Victorias Foundation, Gun and Bertil Stohne's Foundation, Stiftelsen för Gamla Tjänarinnor, and the Research Funds of the Medical Faculty, Umeå University for their generous financial support of our research into the pharmacology of the endocannabinoid system.

References

Adams IB, Martin BR. Cannabis: pharmacology and toxicology in animals and humans. Addiction 1996;91:1585–614.

Adams R, Baker BR, Wearn RB. Structure of cannabinol: III. Synthesis of cannabinol, 1-hydroxyl-3-*n*-amyl-6,6,9-trimethyl-6-dibenzopyran. J Am Chem Soc 1940a;62:2204–7.

Adams R, Hunt M, Clark JH. Structure of cannabidiol, a product isolated from the marihuana extract of Minnesota wild hemp I. J Am Chem Soc 1940b;62:196–200.

Andersson DA, Adner M, Högestätt ED, Zygmunt PM. Mechanisms underlying tissue selectivity of anandamide and other vanilloid receptor agonists. Mol Pharmacol 2002;62:705–13.

Arizzi MN, Cervone KM, Aberman JE, Betz A, Liu Q, Lin S, et al. Behavioral effects of inhibition of cannabinoid metabolism: the amidase inhibitor AM374 enhances the suppression of lever pressing produced by exogenously administered anandamide. Life Sci 2004;74:1001–11.

Ates M, Hamza M, Seidel K, Kotalla CE, Ledent C, Gühring H. Intrathecally applied flurbiprofen produces an endocannabinoid-dependent antinociception in the rat formalin test. Eur J Neurosci 2003;17:597–604.

Azad SC, Monory K, Marsicano G, Cravatt BF, Lutz B, Zieglgänsberger W, et al. Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. J Neurosci 2004;24:9953–61.

Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Huffman JW, et al. Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature 2000;404:84–7.

Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Makriyannis A, et al. Endocannabinoids control spasticity in a multiple sclerosis model. FASEB J 2001;15:300–2.

Baker D, Pryce G, Giovannoni G, Thompson AJ. The therapeutic potential of cannabis. Lancet Neurol 2003;2:291–8.

Baskfield CY, Martin BR, Wiley JL. Differential effects of Δ^9-tetrahydrocannabinol and methanandamide in CB1 knockout and wild-type mice. J Pharmacol Exp Ther 2004;309:86–91.

Bátkai S, Pacher P, Jarai Z, Wagner JA, Kunos G. Cannabinoid antagonist SR141716 inhibits endotoxic hypotension by a cardiac mechanism not involving CB1 or CB2 receptors. Am J Physiol 2004;287:H595–600.

Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 1997;277:1094–7.

Beltramo M, Rodruíguez de Fonseca F, Navarro M, Calignano A, Gorriti MA, Grammatikopoulos G, et al. Reversal of dopamine D_2 receptor responses by an anandamide transport inhibitor. J Neurosci 2000;20:3401–7.

Berdyshev EV, Schmid PC, Krebsbach RJ, Hillard CJ, Huang C, Chen N, et al. Cannabinoid-receptor-independent cell signalling by *N*-acylethanolamines. Biochem J 2001;360:67–75.

Berger C, Schmid PC, Schabitz W-R, Wolf M, Schwab S, Schmid HHO. Massive accumulation of *N*-acylethanolamines after stroke. Cell signalling in acute cerebral ischemia? J Neurochem 2004;88:1159–67.

Berman JS, Symonds C, Birch R. Efficacy of two cannabis based medicinal extracts for relief of central neuropathic pain from brachial plexus avulsion: results of a randomised controlled trial. Pain 2004;112:299–306.

Bifulco M, Laezza C, Valenti M, Ligresti A, Portella G, Di Marzo V. A new strategy to block tumor growth by inhibiting endocannabinoid inactivation. FASEB J 2004;18:1606–8.

Bisogno T, Sepe N, Melck D, Maurelli S, De Petrocellis L, Di Marzo V. Biosynthesis, release and degradation of the novel endogenous cannabimimetic metabolite 2-arachidonoylglycerol in mouse neuroblastoma cells. Biochem J 1997;322:671–7.

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, et al. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 2003;163:463–8.

Boger DL, Sato H, Lerner AE, Hedrick MP, Fecik RA, Miyauchi H, et al. Exceptionally potent inhibitors of fatty acid amide hydrolase: the enzyme responsible for degradation of endogenous oleamide and anandamide. Proc Natl Acad Sci U S A 2000;97:5044–9.

Bukoski RD, Bátkai S, Járai Z, Wang Y, Offertaler L, Jackson WF, et al. CB_1 receptor antagonist SR141716A inhibits Ca^{2+}-induced relaxation in CB_1 receptor-deficient mice. Hypertension 2002;39:251–7.

Burstein SH, Karst M, Schneider U, Zurier RB. Ajulemic acid: a novel cannabinoid produces analgesia without a "high". Life Sci 2004;75: 1513–22.

Calignano A, La Rana G, Beltramo M, Makriyannis A, Piomelli D. Potentiation of anandamide hypotension by the transport inhibitor, AM404. Eur J Pharmacol 1997;337:R1–2.

Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature 1998;394:277–81.

Cascio MG, Bisogno T, Matias I, De Petrocellis L, Orlando P, Di Marzo V. Enzymes for 2-AG biosynthesis and metabolism in cell lines, and their pharmacological inhibition. 2004 Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society; 2004. p. 110.

Cernak I, Vink R, Natale J, Stoica B, Lea PM, Movsesyan V, et al. The "dark side" of endocannabinoids: a neurotoxic role for anandamide. J Cereb Blood Flow Metab 2004;24:564–78.

Clayton N, Marshall FH, Bountra C, O'Shaughnessy CT. CB1 and CB2 cannabinoid receptors are implicated in inflammatory pain. Pain 2002;96:253–60.

Clement AB, Hawkins EG, Lichtman AH, Cravatt BF. Increased seizure susceptibility and proconvulsant activity of anandamide in mice lacking fatty acid amide hydrolase. J Neurosci 2003;23:3916–23.

Compton DR, Martin BR. The effect of the enzyme inhibitor phenylmethylsulfonyl fluoride on the pharmacological effect of anandamide in the mouse model of cannabimimetic activity. J Pharmacol Exp Ther 1997;283:1138–43.

Connolly ES, Winfree CJ, Springer TA, Naka Y, Liao H, Yan SD, et al. Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. J Clin Invest 1996;97:209–16.

Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci U S A 2001;98:9371–6.

Cravatt BF, Saghatelian A, Hawkins EG, Clement AB, Bracey MH, Lichtman AH. Functional disassociation of the central and peripheral fatty acid amide signaling systems. Proc Natl Acad Sci U S A 2004; 101:10821–6.

Croxford JL, Miller SD. Immunoregulation of a viral model of multiple sclerosis using the synthetic cannabinoid R(+)WIN55,212. J Clin Invest 2003;111:1231–40.

de Lago E, Fernández-Ruiz J, Ortega-Gutiérrez S, Viso A, López-Rodríguez ML, Ramos JA. UCM707, a potent and selective inhibitor of endocannabinoid uptake, potentiates hypokinetic and antinociceptive effects of anandamide. Eur J Pharmacol 2002;449:99–103.

de Lago E, Ligresti A, Ortar G, Morera E, Cabranes A, Pryce G, et al. In vivo pharmacological actions of two novel inhibitors of anandamide cellular uptake. Eur J Pharmacol 2004;484:249–57.

De Petrocellis L, Bisogno T, Davis JB, Pertwee RG, Di Marzo V. Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity. FEBS Letts 2000;483:52–6.

De Petrocellis L, Bisogno T, Maccarrone M, Davis JB, Finazzi-Agró A, Di Marzo V. The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. J Biol Chem 2001;276:12856–63.

De Petrocellis L, Cascio MG, Di Marzo V. The endocannabinoid system: a general view and latest additions. Br J Pharmacol 2004; 141: 765–74.

Del Arco I, Navarro M, Bilbao A, Ferrer B, Piomelli D, Rodríguez de Fonseca F. Attention of spontaneous opiate withdrawal in mice by the anandamide transport inhibitor AM404. Eur J Pharmacol 2002;454:103–4.

Deutsch DG, Chin SA. Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. Biochem Pharmacol 1993;46: 791–6.

Deutsch DG, Lin S, Hill WAG, Morse KL, Salehani D, Arreaza G, et al. Fatty acid sulfonyl fluorides inhibit anandamide metabolism and bind to the cannabinoid receptor. Biochem Biophys Res Commun 1997;231: 217–21.

Devane WA, Dysarz FA, Johnson RM, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 1988;34:605–13.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LG, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992;258:1946–9.

Di Marzo V, Bisogno T, De Petrocellis L, Melck D, Orlando P, Wagner JA, et al. Biosynthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in circulating and tumoral macrophages. Eur J Biochem 1999;264:258–67.

Di Marzo V, Breivogel CS, Tao Q, Bridgen DT, Razdan RK, Zimmer AM, et al. Levels, metabolism, and pharmacological activity of anandamide in CB_1 cannabinoid receptor knockout mice: evidence for non-CB_1, non-CB_2 receptor-mediated actions of anandamide in mouse brain. J Neurochem 2000;75:2434–44.

Di Marzo V, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 2001;410:822–5.

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci U S A 2002;99:10819–24.

Dinh TP, Kathuria S, Piomelli D. RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. Mol Pharmacol 2004;66:1260–4.

Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999;22:391–7.

Dogrul A, Gul H, Akar A, Yildiz O, Bilgin F, Guzeldemir E. Topical cannabinoid antinociception: synergy with spinal sites. Pain 2003;105: 11–6.

D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu Y-t, et al. The psychotomometic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology 2004;29:1558–72.

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

Fedorova I, Hashimoto A, Fecik RA, Hedrick MP, Hanus L, Boger DL, et al. Behavioral evidence for the interaction of oleamide with multiple neurotransmitter systems. J Pharmacol Exp Ther 2001;299:332–42.

Fegley D, Kathuria S, Mercier R, Li C, Goutopoulos A, Makriyannis A, et al. Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. Proc Natl Acad Sci 2004;101:8756–61.

Felder CC, Joyce KE, Briley EM, Glass M, Mackie KP, Fahey KJ, et al. LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. J Pharmacol Exp Ther 1998;284:291–7.

Fernandez-Espejo E, Galan-Rodriguez B. Sensorimotor gating in mice is disrupted after AM404, an anandamide reuptake and degradation inhibitor. Psychopharmacology 2004;175:220–4.

Fernandez-Espejo E, Carabello I, Rodríguez de Fonseca F, Ferrer B, El Banoua F, Flores JA, et al. Experimental parkinsonism alters anandamide precursor synthesis, and functional deficits are improved by AM404: a modulator of endocannabinoid function. Neuropsychopharmacology 2004;29:1134–42.

Finsterer J, Christian P, Wolfgang K. Occipital stroke shortly after cannabis consumption. Clin Neurol Neurosurg 2004;106:305–8.

Fowler CJ. Plant-derived, synthetic and endogenous cannabinoids as neuroprotective agents Non-psychoactive cannabinoids, "entourage" compounds and inhibitors of *N*-acyl ethanolamine breakdown as therapeutic strategies to avoid pyschotropic effects. Brain Res Rev 2003;41:26–43.

Fowler CJ. Metabolism of the endocannabinoids anandamide and 2-arachidonoyl glycerol, a review, with emphasis on the pharmacology of fatty acid amide hydrolase, a possible target for the treatment of neurodegenerative diseases and pain. Curr Med Chem Cent Nerv Sys Agents 2004a;4:161–74.

Fowler CJ. Possible involvement of the endocannabinoid system in the actions of three clinically used drugs. Trends Pharmacol Sci 2004b;25:59–61.

Fowler CJ, Holt S, Tiger G. Acidic non-steroidal anti-inflammatory drugs inhibit rat brain fatty acid amide hydrolase in a pH-dependent manner. J Enz Inhib Med Chem 2003;18:55–8.

Fowler CJ, Tiger G, Ligresti A, López-Rodríguez ML, Di Marzo V. Selective inhibition of anandamide cellular uptake versus enzymatic hydrolysis—a difficult issue to handle. Eur J Pharmacol 2004;492: 1–11.

Fox A, Kesingland A, Gentry C, McNair K, Patel S, Urban L, et al. The role of central and peripheral cannabinoid$_1$ receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. Pain 2001;92:91–100.

Franklin A, Parmentier-Batteur S, Walter L, Greenberg DA, Stella N. Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. J Neurosci 2003;23:7767–75.

Fride E, Foox A, Rosenberg E, Faigenboim M, Cohen V, Barda L, et al. Milk intake and survival in newborn cannabinoid CB_1 receptor knockout mice: evidence for a "CB_3" receptor. Eur J Pharmacol 2003; 461:27–34.

Gaoni Y, Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 1964;86:1646–7.

Gatley SJ, Gifford AN, Volkow ND, Lan R, Makriyannis A. ^{123}I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB_1 receptors. Eur J Pharmacol 1996;307:331–8.

Gerdeman GL, Lovinger DM. Emerging roles for endocannabinoids in long-term synaptic plasticity. Br J Pharmacol 2003;140:781–9.

Ghafouri N, Tiger G, Razdan RK, Mahadevan A, Pertwee RG, Martin BR, et al. Inhibition of monoacylglycerol lipase and fatty acid amide hydrolase by analogues of 2-arachidonoylglycerol. Br J Pharmacol 2004;143:774–84.

Giuffrida A, Rodruíguez de Fonseca F, Nava F, Loubet-Lescoulié P, Piomelli D. Elevated circulating levels of anandamide after administration of the transport inhibitor, AM404. Eur J Pharmacol 2000;408: 161–8.

Glaser S, Abumrad N, Fatade F, Kaczocha M, Studholme K, Deutsch D. Evidence against the presence of an anandamide transporter. Proc Natl Acad Sci U S A 2003;100:4269–74.

González S, Romero J, de Miguel R, Lastres-Becker I, Villanua MA, Makriyannis A, et al. Extrapyramidal and neuroendocrine effects of AM404, an inhibitor of the carrier-mediated transport of anandamide. Life Sci 1999;65:327–36.

Goparaju SK, Ueda N, Taniguchi K, Yamamoto S. Enzymes of porcine brain hydrolyzing 2-arachidonoylglycerol, an endogenous ligand of cannabinoid receptors. Biochem Pharmacol 1999;57:417–23.

Gubellini P, Picconi B, Bari M, Battista N, Calabresi P, Centonze D, et al. Experimental parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission. J Neurosci 2002;22: 6900–7.

Gühring H, Hamza M, Sergejeva M, Ates M, Kotalla CE, Ledent C, et al. A role for endocannabinoids in indomethacin-induced spinal antinociception. Eur J Pharmacol 2002;454:153–63.

Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, et al. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus; cerebellum and amygdala. Eur J Neurosci 2004;20:441–58.

Guzmán M. Cannabinoids: potential anticancer agents. Nature Rev, Cancer 2003;3:745–55.

Haller J, Varga B, Ledent C, Barna I, Freund TF. Context-dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behaviour in mice. Eur J Neurosci 2004;19:1906–12.

Hansen HH, Schmid PC, Bittigau P, Lastres-Becker I, Berrendero F, Manzaneres J, et al. Anandamide, but not 2-arachidonoylglycerol, accumulates during in vivo neurodegeneration. J Neurochem 2001;78: 1415–27.

Hansen HH, Azcoitia I, Pons S, Romero J, García-Segura LM, Ramos JA, et al. Blockade of cannabinoid CB1 receptor function protects against in vivo disseminating brain damage following NMDA-induced excitotoxicity. J Neurochem 2002;82:154–8.

Hanus L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M, et al. HU-308: a specific agonist for CB_2, a peripheral cannabinoid receptor. Proc Natl Acad Sci U S A 1999;96:14228–33.

Harrold JA, Williams G. The cannabinoid system: a role in both the homeostatic and hedonic control of eating? Br J Nutr 2003;90:729–34.

Hillard CJ, Jarrahian A. Cellular accumulation of anandamide: consensus and controversy. Br J Pharmacol 2003;140:802–8.

Hillard CJ, Edgemond WS, Jarrahian A, Campbell WB. Accumulation of *N*-arachidonoylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. J Neurochem 1997;69:631–8.

Hively RL, Mosher WA, Hoffmann FW. Isolation of *trans*-Δ^6-tetrahydrocannabinol from marijuana. J Am Chem Soc 1966;88:1832–3.

Hohmann AG, Farthing JN, Zvonok AM, Makriyannis A. Selective activation of cannabinoid CB_2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. J Pharmacol Exp Ther 2004;308: 446–53.

Holt S, Costa B, Fowler CJ. FAAH inhibitors and indomethacin reduce carrageenan induced hind paw inflammation in the mouse—role of cannabinoid receptors. 2004 Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society; 2004. p. 51.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International union of pharmacology XXVII Classification of cannabinoid receptors. Pharmacol Rev 2002;54:161–202.

Huang SM, Bisogno T, Petros TJ, Chang SY, Zavitsanos PA, Zipkin RE, et al. Identification of a new class of molecules, the arachidonoyl amino acids, and characterization of one member that inhibits pain. J Biol Chem 2001;276:42639–44.

Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, et al. Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry 2001;58:322–8.

Huffman JW, Liddle J, Yu S, Aung MM, Abood ME, Wiley JL, et al. 3-(1′,1′-dimethylbutyl)-1-deoxy-Δ^8-THC and related compounds: synthesis of selective ligands for the CB_2 receptor. Bioorg Med Chem 1999;7:2905–14.

Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, et al. Activation of CB_2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci U S A 2003;100:10529–33.

Izzo AA, Capasso F, Costagliola A, Bisogno T, Marsicano G, Ligresti A, et al. An endogenous cannabinoid tone attenuates cholera toxin-induced fluid accumulation in mice. Gastroenterology 2003;125:765–74.

Jackson SJ, Baker D, Cuzner ML. Cannabinoid-mediated neuroprotection following interferon-gamma treatment in a three-dimensional mouse brain aggregate cell culture. Eur J Neurosci 2004;20:2267–75.

Jaggar SI, Hasnie FS, Sellaturay S, Rice ASC. The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. Pain 1998;76:189–99.

Járai Z, Wagner JA, Goparaju SK, Wang L, Razdan RK, Sugiura T, et al. Cardiovascular effects of 2-arachidonoyl glycerol in anesthetized mice. Hypertension 2000;35:679–84.

Jarrahian A, Manna S, Edgemond WS, Campbell WB, Hillard CJ. Structure–activity relationships among *N*-arachidonoylethanolamine (anandamide) head group analogues for the anandamide transporter. J Neurochem 2000;74:2597–606.

Jin KL, Mao XO, Goldsmith PC, Greenberg DA. CB1 cannabinoid receptor induction in experimental stroke. Ann Neurol 2000;48:257–61.

Jonsson K-O, Andersson A, Jacobsson SOP, Vandevoorde S, Lambert DM, Fowler CJ. AM404 and VDM 11 non-specifically inhibit C6 glioma cell proliferation at concentrations used to block the cellular accumulation of the endocannabinoid anandamide. Arch Toxicol 2003;77:201–7.

Kagaya M, Lamb J, Robbins J, Page CP, Spina D. Characterization of the anandamide induced depolarisation of guinea-pig isolated vagus nerve. Br J Pharmacol 2002;137:39–48.

Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. J Biol Chem 1997;272:27218–23.

Karlsson M, Påhlsson C, Fowler CJ. Reversible, temperature-dependent, and AM404-inhibitable adsorption of anandamide to cell culture wells as a confounding factor in release experiments. Eur J Pharm Sci 2004;22:181–9.

Karst M, Salim K, Burstein S, Conrad I, Hoy L, Schneider U. Analgesic effect of the synthetic cannabinoid CT-3 on chronic neuropathic pain. A randomized controlled trial. JAMA 2003;290:1757–62.

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

Kelley BG, Thayer SA. Anandamide transport inhibitor AM404 and structurally related compounds inhibit synaptic transmission between rat hippocampal neurons in culture independent of cannabinoid CB_1 receptors. Eur J Pharmacol 2004;496:33–9.

Kelly S, Jhaveri MD, Sagar DR, Kendall DA, Chapman V. Activation of peripheral cannabinoid CB_1 receptors inhibits mechanically evoked responses of spinal neurons in noninflamed rats and rats with hindpaw inflammation. Eur J Neurosci 2003;18:2239–43.

Kim J, Alger BE. Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. Nat Neurosci 2004;7:697–8.

Kozak KR, Marnett LJ. Oxidative metabolism of endocannabinoids. Prostaglandins Leukot Essent Fat Acids 2002;66:211–20.

Laine K, Järvinen T, Savinainen J, Laitinen JT, Pate DW, Järvinen K. Effects of topical anandamide-transport inhibitors, AM404 and olvanil, on intraocular pressure in normotensive rabbits. Pharm Res 2001;18:494–9.

Lambert DM, Dipaolo FG, Sonveaux P, Kanonyo M, Govaerts SJ, Hermans E, et al. Analogues and homologues of *N*-palmitoylethanolamide, a putative endogenous CB_2 cannabinoid, as potential ligands for the cannabinoid receptors. Biochim Biophys Acta 1999;1440:266–74.

Lastres-Becker I, Hansen HH, Berrendero F, De Miguel R, Perez-Rosado A, Manzanares J, et al. Alleviation of motor hyperactivity and neurochemical deficits by endocannabinoid uptake inhibition in a rat model of Huntington's disease. Synapse 2002;44:23–35.

Lastres-Becker I, De Miguel R, Fernandez-Ruiz JJ. The endocannabinoid system and Huntington's disease. Curr Drug Target CNS Neurol Disord 2003a;2:335–47.

Lastres-Becker I, de Miguel R, De Petrocellis L, Makriyannis A, Di Marzo V, Fernández-Ruiz J. Compounds acting at the endocannabinoid and/or endovanilloid systems reduce hyperkinesia in a rat model of Huntington's disease. J Neurochem 2003b;84:1097–109.

Liao C, Zheng J, David LS, Nicholson RA. Inhibition of voltage-sensitive sodium channels by the cannabinoid 1 receptor antagonist AM251 in mammalian brain. Basic Clin Pharmacol Toxicol 2004;94:73–8.

Lichtman AH, Shelton CC, Advani T, Cravatt BF. Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. Pain 2004a;109:319–27.

Lichtman AH, Leung D, Shelton CC, Saghatelian A, Hardouin C, Boger DL, et al. Reversible inhibitors of fatty acid amide hydrolase that promote analgesia: evidence for an unprecedented combination of potency and selectivity. J Pharmacol Exp Ther 2004b;311:441–8.

Ligresti A, Morera E, Van Der Stelt MM, Monory K, Lutz B, Ortar G, et al. Further evidence for the existence of a specific process for the membrane transport of anandamide. Biochem J 2004;380:265–72.

López-Rodríguez ML, Viso A, Ortega-Gutiérrez S, Lastres-Becker I, González S, Fernández-Ruiz J, et al. Design, synthesis and biological evaluation of novel arachidonic acid derivatives as highly potent and selective endocannabinoid transporter inhibitors. J Med Chem 2001;44:4505–8.

López-Rodríguez ML, Viso A, Ortega-Gutiérrez S, Fowler CJ, Tiger G, de Lago E, et al. Design, synthesis and biological evaluation of new endocannabinoid transporter inhibitors: comparison with effects upon fatty acid amidohydrolase. J Med Chem 2003;46:1512–22.

Luk T, Jin W, Zvonok A, Lu D, Lin X-Z, Chavkin C, et al. Identification of a potent and highly efficacious, yet slowly desensitizing CB1 cannabinoid receptor agonist. Br J Pharmacol 2004;142:495–500.

Maccarrone M. Inhibition of anandamide hydrolysis: cells also know how to do it. Trends Mol Med 2004;10:13–4.

Maccarrone M, Bari M, Battista N, Finazzi-Agrò A. Estrogen stimulates arachidonoylethanolamine release from human endothelial cells and platelet activation. Blood 2002;100:4040–8.

Maccarrone M, Piccirilli S, Battista N, Del Duca C, Nappi G, Corasanti MT, et al. Enhanced anandamide degradation is associated with neuronal apoptosis induced by the HIV-1 coat glycoprotein gp120 in the rat neocortex. J Neurochem 2004;89:1293–300.

Makara JK, Fegley D, Kathuria S, Duranti A, Tontini A, Mor M, Tarzia G, Freund TF, Piomelli D, 2004. Selective inhibition of monoglyceride lipase suggests a key role for 2-arachidonoylglycerol in hippocampal depolarization-induced suppression of inhibition Program No. 623.13.2004 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience.

Malan TP, Ibrahim MM, Deng H, Liu Q, Mata HP, Vanderah T, et al. CB_2 cannabinoid receptor-mediated peripheral antinociception. Pain 2001;93:239–45.

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 2003;302:84–8.

Martínez-Orgado J, Fernández-Frutos B, González R, Romero E, Urigüen L, Romero J, et al. Neuroprotection by the cannabinoid agonist WIN-55,212 in an in vivo newborn rat model of acute severe asphyxia. Mol Brain Res 2003;114:132–9.

Mascolo N, Izzo AA, Ligresti A, Costagliola A, Pinto L, Cascio MG, et al. The endocannabinoid system and the molecular basis of paralytic ileus in mice. FASEB J 2002;16:1973–5.

Mato S, Pazos A, Valdizán EM. Cannabinoid receptor antagonism and inverse agonism in response to SR141716A on cAMP production in human and rat brain. Eur J Pharmacol 2002;443:43–6.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561–4.

Mauler F, Mittendorf J, Horváth E, De Vry J. Characterization of the diarylether sulfonylester (-)-(R)-3-(2-hydroxymethylindanyl-4-oxy)-phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271) as a potent cannabinoid receptor agonist with neuroprotective properties. J Pharmacol Exp Ther 2002;302:359–68.

McFarland MJ, Barker EL. Anandamide transport. Pharmacol Ther 2004;104:117–35.

McFarland MJ, Porter AC, Rakhshan FR, Rawat DS, Gibbs RA, Barker EL. A role for caveolae/lipid rafts in the uptake and recycling of the endogenous cannabinoid anandamide. J Biol Chem 2004;279:41991–7.

McHugh D, Ross RA. Effects of anandamide on human neutrophil migration. 2004 Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society; 2004. p. 79.

Mechoulam R, Hanus L. A historical overview of chemical research on cannabinoids. Chem Phys Lipids 2000;108:1–13.

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

Melis M, Perra S, Muntoni AL, Pillolla G, Lutz B, Marsicano G, et al. Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. J Neurosci 2004;24: 10707–15.

Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–5.

Murillo-Rodríguez E, Blanco-Centurion C, Sanchez C, Piomelli D, Shiromani PJ. Anandamide enhances extracellular levels of adenosine and induces sleep: an in vivo microdialysis study. Sleep 2003;26: 943–7.

Muthian S, Rademacher DJ, Roelke CT, Gross GJ, Hillard CJ. Anandamide content is increased and CB_1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia. Neuroscience 2004;129:743–50.

Nackley AG, Makriyannis A, Hohmann AG. Selective activation of cannabinoid CB2 receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. Neuroscience 2003a;119:747–57.

Nackley AG, Suplita RL, Hohmann AG. A peripheral cannabinoid mechanism suppresses spinal Fos protein expression and pain behavior in a rat model of inflammation. Neuroscience 2003b;117:659–70.

Nagayama T, Sinor AD, Simon RP, Chen J, Graham SH, Jin K, et al. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. J Neurosci 1999;19:2987–95.

Neef M, Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Zbinden A, Brenneisen R. The analgesic effect of oral delta-9-tetrahydrocannabinol (THC), morphine, and a THC-morphine combination in healthy subjects under experimental pain conditions. Pain 2003;105:79–88.

Ni X, Geller EB, Eppihimer MJ, Eisenstein TK, Adler MW, Tuma RF. Win 55212-2, a cannabinoid receptor agonist, attenuates leukocyte/endothelial interactions in an experimental autoimmune encephalomyelitis model. Mult Scler 2004;10:158–64.

Nie J, Lewis DL. Structural domains of the CB1 cannabinoid receptor that contribute to constitutive activity and G-protein sequestration. J Neurosci 2001;21:8758–64.

Ortar G, Ligresti A, De Petrocellis L, Morera E, Di Marzo V. Novel selective and metabolically stable inhibitors of anandamide cellular uptake. Biochem Pharmacol 2003;65:1473–81.

Ortega-Gutiérrez S, Hawkins EG, Viso A, López-Rodríguez ML, Cravatt BF. Comparison of anandamide transport in FAAH wild-type and knockout neurons: evidence for contributions by both FAAH and the CB1 receptor to anandamide uptake. Biochemistry 2004;43:8184–90.

Pacher P, Bátkai S, Kunos G. Haemodynamic profile and responsiveness to anandamide of $TRPV_1$ receptor knock-out mice. J Physiol 2004;558: 647–57.

Panikashvili D, Simeonidou C, Ben-Shabat S, Hanus L, Breuer A, Mechoulam R, et al. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature 2001;413:527–31.

Paria BC, Deutsch DD, Dey SK. The uterus is a potential site for anandamide synthesis and hydrolysis: differential profiles of anandamide synthase and hydrolase activities in the mouse uterus during the periimplantation period. Mol Reprod Dev 1996;45:183–92.

Parmentier-Batteur S, Jin K, Mao XO, Xie L, Greenberg DA. Increased severity of stroke in CB1 cannabinoid receptor knock-out mice. J Neurosci 2002;22:9771–5.

Patel S, Wohlfeil ER, Rademacher DJ, Carrier EJ, Perry LJ, Kundu A, et al. The general anesthetic propofol increases brain *N*-arachidonylethanolamine (anandamide) content and inhibits fatty acid amide hydrolase. Br J Pharmacol 2003;139:1005–13.

Patricelli MP, Cravatt BF. Proteins regulating the biosynthesis and inactivation of neuromodulatory fatty acid amides. Vit Hormon 2001; 62:95–131.

Pertwee RG. Cannabinoid receptors and pain. Prog Neurobiol 2001;63: 569–611.

Piomelli D. The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 2003;4:873–84.

Price TJ, Patwardhan A, Akopian AN, Hargreaves KM, Flores CM. Modulation of trigeminal sensory neuron activity by the dual cannabinoid–vanilloid agonists anandamide, *N*-arachidonoyl-dopamine and arachidonyl-2-chloroethylamide. Br J Pharmacol 2004;141: 1118–30.

Pryce G, Ahmed Z, Hankey DJ, Jackson SJ, Croxford JL, Pocock JM, et al. Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. Brain 2003;126:2191–202.

Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A, et al. Inhibition of inflammatory hyperalgesia by activation of peripheral CB_2 cannabinoid receptors. Anesthesiology 2003;99:955–60.

Quistad GB, Sparks SE, Segall Y, Nomura DK, Casida JE. Selective inhibitors of fatty acid amide hydrolase relative to neuropathy target esterase and acetylcholinesterase: toxicological implications. Toxicol Appl Pharmacol 2002;179:57–63.

Rakhshan F, Day TA, Blakely RD, Barker EL. Carrier-mediated uptake of the endogenous cannabinoid anandamide in RBL-2H3 cells. J Pharmacol Exp Ther 2000;292:960–7.

Razdan RK. Structure–activity relationships in cannabinoids. Pharmacol Rev 1986;38:75–149.

Reynolds JR. On the therapeutical uses and toxic effects of *Cannabis indica*. Lancet 1890;135:637–8.

Rice ASC. Cannabinoids and pain. Curr Opin Inv Drugs 2001;2:399–414.

Richardson JD, Kilo S, Hargreaves KM. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB_1 receptors. Pain 1998;75:111–9.

Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Letts 1994;350:240–4.

Rinaldi-Carmona M, Barth F, Millan J, Derocq J-M, Casellas P, Congy C, et al. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J Pharmacol Exp Ther 1998;284:644–50.

Roberts LA, Christie MJ, Connor M. Anandamide is a partial agonist at native vanilloid receptors in acutely isolated mouse trigeminal sensory neurons. Br J Pharmacol 2002;137:421–8.

Ronesi J, Gerdeman GL, Lovinger DM. Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. J Neurosci 2004;24:1673–9.

Ross RA. Anandamide and vanilloid TRPV1 receptors. Br J Pharmacol 2003;140:790–801.

Saario SM, Savinainen JR, Laitinen JT, Järvinen T, Niemi R. Monoglyceride lipase-like enzymatic activity is responsible for hydrolysis of 2-arachidonoylglycerol in rat cerebellar membranes. Biochem Pharmacol 2004;67:1381–7.

Sánchez C, de Ceballos ML, del Pulgar TG, Rueda D, Corbacho C, Velasco G, et al. Inhibition of glioma growth in vivo by selective activation of the CB_2 cannabinoid receptor. Cancer Res 2001;61:5784–9.

Santucci V, Storme J-J, Soubrié P, Le fur G. Arousal-enhancing properties of the CB1 cannabinoid receptor antagonist SR141716A in rats as assessed by electroencephalographic spectral and sleep-waking cycle analysis. Life Sci 1996;58:PL103–10.

Savinainen JR, Saario SM, Niemi R, Järvinen T, Laitinen JT. An optimized approach to study endocannabinoid signaling: evidence against constitutive activity of rat brain adenosine A_1 and cannabinoid CB_1 receptors. Br J Pharmacol 2003;140:1451–9.

Schäbitz W-R, Giuffrida A, Berger C, Aschoff A, Schwaninger M, Schwab S, et al. Release of fatty acid amides in a patient with hemispheric stroke: a microdialysis study. Stroke 2002;33:2112–4.

Scott DA, Wright CE, Angus JA. Evidence that CB-1 and CB-2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat. Pain 2004;109:124–31.

Segovia G, Mora F, Crossman AR, Brotchie JM. Effects of CB1 cannabinoid receptor modulating compounds on the hyperkinesia induced by high-dose levodopa in the reserpine-treated rat model of Parkinson's disease. Mov Disord 2003;18:138–49.

Sim-Selley L, Brunk LK, Selley DE. Inhibitory effects of SR141716A on G-protein activation in rat brain. Eur J Pharmacol 2001;414:135–43.

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

Sugiura T, Kodaka T, Nakane S, Miyashita T, Kondo S, Suhara Y, et al. Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor Structure–activity relationship of 2-arachidonoylglycerol, ether-linked analogues and related compounds. J Biol Chem 1999; 274:2794–801.

Svendsen KB, Jensen TS, Bach FW. Does the cannabinoid dronabinol reduce central pain in multiple sclerosis? Randomised double blind placebo controlled crossover trial. BMJ 2004;329:253.

Thomas A, Ross RA, Saha B, Mahadevan A, Razdan RK, Pertwee RG. 6"-Azidohex-2"-yne-cannabidiol: a potential neutral, competitive cannabinoid CB_1 receptor antagonist. Eur J Pharmacol 2004;487:213–21.

Trettel J, Levine ES. Endocannabinoids mediate rapid retrograde signaling at interneuron → pyramidal neuron synapses of the neocortex. J Neurophysiol 2003;89:2334–8.

Urigüen L, Pérez-Rial S, Ledent C, Palomo T, Manzanares J. Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB_1 receptors. Neuropharmacology 2004;46:966–73.

van der Stelt M, Veldhuis WB, Bär PR, Veldink GA, Vliegenthart JFG, Nicolay K. Neuroprotection by Δ^9-tetrahydrocannabinol, the main active compound in marijuana, against ouabain-induced in vivo excitotoxicity. J Neurosci 2001a;21:6475–9.

van der Stelt M, Veldhuis WB, van Haaften GW, Fezza F, Bisogno T, Bär PR, et al. Exogenous anandamide protects rat brain against acute neuronal injury in vivo. J Neurosci 2001b;21:8765–71.

van Oosten BW, Killestein J, Mathus-Vliegen EMH, Polman CH. Multiple sclerosis following treatment with a cannabinoid receptor-1 antagonist. Mult Scler 2004;10:330–1.

Veldhuis WB, van der Stelt M, Wadman MW, van Zadelhoff G, Maccarrone M, Fezza F, et al. Neuroprotection by the endogenous cannabinoid anandamide and arvanil against in vivo excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases. J Neurosci 2003;23:4127–33.

Verty ANA, McGregor IS, Mallet PE. Consumption of high carbohydrate, high fat, amd normal chow is equally suppressed by a cannabinoid receptor antagonist in non-deprived rats. Neurosci Letts 2004;354: 217–20.

Viggiano D, Ruocco LA, Pignatelli M, Grammatikipoulos G, Sadile AG. Prenatal elevation of endocannabinoids corrects the unbalance between dopamine systems and reduces activity in the Naples High Excitability rats. Neurosci Biobehav Rev 2003;27:129–39.

Wade DT, Robson P, House H, Makela P, Aram J. A preliminary controlled study to determine whether whole-plant cannabis extracts can improve intractable neurogenic symptoms. Clin Rehab 2003;17:21–9.

Wade MR, Tzavara ET, Nomikos GG. Cannabinoids reduce cAMP levels in the striatum of freely moving rats: an in vivo microdialysis study. Brain Res 2004;1005:117–23.

Walker JM, Huang SM. Endocannabinoids in pain modulation. Prostaglandins Leukot Essenst Fat Acids 2002;66:235–42.

Walker JM, Huang SM, Strangman NM, Tsou K, Sañudo-Peña MC. Pain modulation by release of the endogenous cannabinoid anandamide. Proc Natl Acad Sci U S A 1999;96:12198–203.

Walter L, Stella N. Cannabinoids and neuroinflammation. Br J Pharmacol 2004;141:775–85.

Walter L, Franklin A, Witting A, Wade C, Xie Y, Kunos G, et al. Nonpsychotrophic annabinoid receptors regulate microglial cell migration. J Neurosci 2003;23:1398–405.

Weber A, Ni J, Ling K-HJ, Acheampong A, Tang-Liu DD-S, Burk R, et al. Formation of prostamides from anandamide in FAAH knockout mice analyzed by HPLC with tandem mass spectrometry. J Lipid Res 2004; 45:757–63.

Wiley JL, Dewey MA, Jefferson RG, Winckler RL, Bridgen DT, Willoughby KA, et al. Influence of phenylmethylsulfonyl fluoride on anandamide brain levels and pharmacological effects. Life Sci 2000; 67:1573–83.

Wingerchuk D. Cannabis for medical purposes: cultivating science, weeding out the fiction. Lancet 2004;364:315–6.

Woodward DF, Chen J, Lu T, Dinh T, Cravatt BF, Matias I, et al. Tissue distribution of 2-AG, PEA, anandamide and prostamides E_2 and $F_{2\alpha}$ in FAAH −/− and FAAH +/+ mice. 2004 Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society; 2004. p. 78.

Yesilyurt O, Dogrul A, Gul H, Seyrek M, Kusmez O, Ozkan Y, et al. Topical cannabinoid enhances topical morphine antinociception. Pain 2003;105:303–8.

Zajicek J, Fox P, Sanders H, Wright D, Vickery J, Nunn A, et al. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. Lancet 2003;362:1517–26.

Zygmunt PM, Chuang H-h, Movahed P, Julius D, Högestätt ED. The anandamide transport inhibitor AM404 activates vanilloid receptors. Eur J Pharmacol 2000;396:39–42.

Zygmunt PM, Petersson J, Andersson DA, Chuang H-h, Sørgård M, Di Marzo V, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 1999;400:452–7.