UC Irvine

UC Irvine Previously Published Works

Title

The endogenous cannabinoid system and the treatment of marijuana dependence.

Permalink

<https://escholarship.org/uc/item/2j94n02q>

Journal

Neuropharmacology, 47 Suppl 1(SUPPL. 1)

ISSN

0028-3908

Author

Piomelli, Daniele

Publication Date

2004

DOI

10.1016/j.neuropharm.2004.07.018

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

The endogenous cannabinoid system and the treatment of marijuana dependence

Daniele Piomelli^{a,b,*}

^a Department of Pharmacology, University of California, Irvine, Irvine, CA 92697-4625, USA

^b Center for the Neurobiology of Learning and Memory, University of California, Irvine, Irvine, CA 92697-4625, USA

Received 18 May 2004; received in revised form 20 July 2004; accepted 21 July 2004

Abstract

The active principle of marijuana, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), exerts its pharmacological effects by binding to selective receptors present on the membranes of neurons and other cells. These cannabinoid receptors are normally engaged by a family of lipid mediators, called endocannabinoids, which are thought to participate in the regulation of a diversity of brain functions, including pain, mood, appetite and memory. Marijuana use may lead to adaptive changes in endocannabinoid signaling, and these changes might contribute to effects of marijuana as well as to the establishment of marijuana dependence. In the present article, I outline current views on how endocannabinoid substances are produced, released, and deactivated in the brain. In addition, I review recent progress on the development of pharmacological agents that interfere with endocannabinoid deactivation and discuss their potential utility in the treatment of marijuana dependence and other aspects of drug abuse.

© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

The increasing spread of marijuana use, especially among adolescents and young adults (SAMSHA, 2000), has heightened societal awareness of the risks associated with this drug and has highlighted the need to fully understand its mechanism of action. Basic research has shown that Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the main active constituent of marijuana, produces its effects by combining with selective receptors present on the membrane of cells in the brain, the vasculature and the immune system (for review, see Piomelli, 2003). Research has also revealed that a group of lipid-derived substances produced by the body engages these receptors and participates in biological processes as diverse as pain perception, memory formation and blood pressure regulation (Piomelli, 2003). This knowledge has allowed researchers to interpret the pharmacological properties of marijuana, but remains inadequate to the task of developing strategies

for the medicinal management of marijuana dependence. No such strategies exist at present (McRae et al., 2003), despite the fact that pharmacotherapy—alone or in combination with behavioral therapy—is considered a primary treatment option for drug dependence when abuse prevention fails (Goldstein, 2001; Kreek, 2002).

Several basic questions, which are relevant to the pharmacotherapy of marijuana dependence, remain unanswered. For example, while it is clear that Δ^9 -THC acts by hijacking the brain endocannabinoid system, its impact on the various components of this system—synthetic and catabolic enzymes, transporters, and receptors—is still largely undefined. Does Δ^9 -THC produce rapid adaptive changes in neuronal endocannabinoid signaling, as recent evidence indicates (Kelley and Thayer, 2004; Mato et al., 2004)? And, if so, do such changes contribute to the pharmacological actions of the drug? Does prolonged exposure to Δ^9 -THC cause stable alterations in endocannabinoid signaling? And, if so, do such alterations contribute to marijuana dependence and, most importantly, can they be safely reversed to restore normality? Answering these questions may not only help develop effective therapeutic strategies for marijuana dependence, but in light of the

* Corresponding author. Tel.: +1-949-824-6180; fax: +1-949-824-6305.

E-mail address: piomelli@uci.edu (D. Piomelli).

broad roles played by the endocannabinoid system in the control of brain reward processes (Maldonado and Rodriguez de Fonseca, 2002), might also shed new light on fundamental mechanisms of drug addiction. To accomplish this task, it seems important to move forward in two convergent directions: the molecular characterization of endocannabinoid signaling, much of which is still uncharted; and the development of pharmacological agents that interfere with specific components of this system. In the present review, I outline recent progress made in these directions, specifically focusing on endocannabinoid deactivation, and discuss some of the challenges lying ahead.

2. Anandamide

Anandamide was the first endocannabinoid substance to be isolated and structurally characterized (Devane et al., 1992). Its formation in neural cells is thought to require two enzymatic steps, which are illustrated in Fig. 1. The first is the activity-dependent cleavage of the phospholipid precursor N-arachidonoyl-PE (NAPE). This reaction, which is mediated by a unique D-type phospholipase (PLD) (Okamoto et al., 2004), produces anandamide and phosphatidic acid, which is recycled to produce other glycerol-containing phospholipids. The cellular stores of NAPE are small, but can be refilled by an *N*-acyltransferase (NAT) activity, which catalyzes the intermolecular passage of an arachidonic acid group from the *sn*-1 position of phosphatidylcholine (PC) to the head group of phosphatidylethanolamine (PE) (Fig. 1). In cultures of rat cortical neurons, NAT activity is controlled by two intracellular second messengers: Ca^{2+} , which is required to activate the enzyme, and cyclic 3', 5'-adenosine

sine monophosphate (cAMP), which stimulates protein kinase A-dependent protein phosphorylation and, via an unknown mechanism, enhances NAT activity (Cadas et al., 1997). Although separate enzymes catalyze the syntheses of anandamide and NAPE, the two events are likely to occur simultaneously because Ca^{2+} -stimulated anandamide production is often accompanied by *de novo* formation of NAPE (Di Marzo et al., 1994; Cadas et al., 1997).

Anandamide synthesis can be elicited *in vitro* by a variety of agents that elevate intracellular Ca^{2+} levels. For example, the Ca^{2+} ionophore ionomycin stimulates [^3H]anandamide formation in cultures of rat striatal and cortical neurons labeled by incubation with [^3H]ethanolamine (Di Marzo et al., 1994; Cadas et al., 1997). In the same neurons, Ca^{2+} -dependent [^3H]anandamide production may be elicited by the glutamate receptor agonist, kainate, by the K^+ channel blocker 4-aminopyridine, and by membrane-depolarizing concentrations of K^+ ions (Di Marzo et al., 1994; Cadas et al., 1997). Depolarization of neural cells was also shown to evoke Ca^{2+} -dependent anandamide release *in vivo* (Giuffrida et al., 1999).

Along with Ca^{2+} entry, activation of certain G protein-coupled receptors can also initiate anandamide generation. Administration of the dopamine D_2 -receptor agonist quinpirole causes a profound stimulation of anandamide synthesis in the rat basal ganglia, which is prevented by the D_2 antagonist raclopride (Giuffrida et al., 1999; Ferrer et al., 2003). Importantly, cocaine elicits a similar response (Centonze et al., 2004), suggesting a role for anandamide in the actions of these psychostimulant drugs. The ability of the anandamide transport inhibitor AM404 to reduce D_2 agonist-induced hyperactivity, discussed below, further supports this possibility (Beltramo et al., 2000).

2.1. Anandamide deactivation: transport into neurons and glia

The biological elimination of anandamide proceeds through two successive steps of high-affinity transport into cells, followed by intracellular degradation (Fig. 2). Brain neurons and astrocytes in culture internalize anandamide through a process that fulfills all key requirements of a carrier-mediated transport. Plots of the initial rates of [^3H]anandamide internalization in rat brain neurons and astrocytes in culture yield apparent Michaelis constants (K_M) that are consistent with a saturable process and are comparable to the K_M values of brain amine or amino-acid transporters (Beltramo et al., 1997; Hillard and Campbell, 1997; Piomelli et al., 1999). Moreover, neurons and astrocytes in culture internalize [^3H]anandamide, along with a select group of structurally related compounds, in a stereoselective manner (Piomelli et al., 1999). Even further, [^3H]ana-

Fig. 1. Mechanism of anandamide formation in neural cells. This hypothetical diagram illustrates two key steps of anandamide synthesis: first the production of the anandamide precursor N-arachidonoyl-phosphatidylethanolamine (NAPE) from phosphatidylethanolamine (PE) and phosphatidylcholine (PC), catalyzed by the enzyme *N*-acyltransferase; and, second, the hydrolysis of NAPE to yield anandamide, catalyzed by phospholipase D (PLD).

Fig. 2. Mechanisms of anandamide deactivation in neural cells. Anandamide can be internalized by neurons and glia through a high-affinity transport system (AT). After internalization, anandamide may be hydrolyzed by fatty-acid amide hydrolase (FAAH) present on mitochondria and endoplasmic reticulum membranes.

ndamide internalization can be inhibited by drugs that have no effect on the uptake of non-cannabinoid lipid mediators such as fatty acids and eicosanoids (Beltramo et al., 1997; Piomelli et al., 1999). Nevertheless, anandamide is internalized in a Na^+ - and energy-independent manner (Beltramo et al., 1997; Hillard and Campbell, 1997; Piomelli et al., 1999), a feature that differentiates this lipid mediator from most conventional neurotransmitters.

2.2. Anandamide transport inhibitors

The prototype of this class of drugs, the arachidonate derivative AM404 (Fig. 3), has provided impor-

Fig. 3. Chemical structures of the anandamide transport inhibitor AM404 and the FAAH inhibitor URB597.

tant information on the properties of anandamide transport, not only aiding the *in vitro* characterization of this process, but also helping to reveal its possible functions in animals. Importantly, the partial cannabimimetic profile exhibited by this agent *in vivo* suggests that anandamide transport might provide a useful target in disease conditions in which the endocannabinoid system is hypofunctional (Beltramo et al., 2000). Evidence indicates that one such condition could be opiate withdrawal, which is markedly reduced in rodents by administering AM404 (Del Arco et al., 2002).

These theories have been hindered by the fact that the putative transport system responsible for anandamide internalization is still uncharacterized at the molecular level. In fact, the presence of such a system has been recently questioned, based on the observation that [^3H]anandamide uptake in certain cell lines is saturable at longer (>5 min), but not at shorter (<40 s) incubation times (Glaser et al., 2003). This finding has been interpreted to suggest that fatty-acid amide hydrolase (FAAH)—a key enzyme of intracellular anandamide degradation, described in a subsequent section—may be responsible for the saturation of uptake noted at longer incubation times (Glaser et al., 2003). However, the result may also be explained on purely technical grounds, as the high concentration of serum albumin used in the experiments of Glaser and collaborators was previously shown to prevent [^3H]anandamide internalization (Di Marzo et al., 1994; Hillard and Jarrahian 2003). Consistent with this interpretation, recent studies have provided additional evidence for the existence of an anandamide transport system independent of FAAH (Ligresti et al., 2004; Fegley et al., 2004). In particular, one of these studies has shown that cultures of cortical neurons isolated from the brain of FAAH-null mice internalize anandamide as efficiently as do neurons that express normal levels of the enzyme. The same study also demonstrated that the transport inhibitor AM404 is equally effective at reducing anandamide internalization in neurons of FAAH-null and wild-type mice. These results indicate that FAAH does not provide the driving force for anandamide uptake or serve as a target for AM404. *In vivo* experiments further support this conclusion, showing that AM404 not only enhances the actions of exogenous anandamide in FAAH-null mice, but acts more effectively in this mutant strain than it does in control animals. This implies that AM404 is not in fact a FAAH inhibitor, as it has been proposed (Glaser et al., 2003), but a FAAH substrate. In support of this idea, it was found that membranes prepared from the brains of normal mice rapidly hydrolyze AM404, whereas those prepared from mice that lack FAAH are unable to carry out this reaction (Fegley et al., 2004).

The fact that FAAH is not directly involved in anandamide internalization (Ligresti et al., 2004; Fegley et al., 2004) raises the question of what mechanism provides the driving force for this process. One possibility is that an intracellular protein may sequester anandamide at the membrane, driving its internalization and facilitating its movement to the mitochondria and the endoplasmic reticulum, where FAAH is primarily localized (Gulyas et al., 2004). If selective for anandamide, such a protein might participate in the transport process as well as serve as a target for transport inhibitors. This hypothetical model is consistent with fatty-acid transport into cells, which is also thought to require the cooperation of membrane transporters and intracellular fatty-acid binding proteins (Black and DiRusso, 2003).

2.3. Behavioral effects of anandamide transport inhibitors

AM404 increases endogenous anandamide levels in brain tissue and peripheral blood of rats and mice (Fegley et al., 2004; Giuffrida et al., 2000). This effect is accompanied by a series of behavioral responses that, though blocked by the CB₁ antagonist rimonabant (SR141716A), are clearly distinguishable from those of direct cannabinoid agonists. For example, administration of AM404 into the cerebral ventricles of rats decreases exploratory activity without producing catalepsy (rigid immobility) and analgesia, two hallmarks of direct CB₁ receptor activation (Beltramo et al., 2000). In addition, AM404 reduces two characteristic effects caused by activation of D₂ family receptors: the yawning response elicited in mice by low doses of the D₁/D₂-receptor agonist apomorphine; and the stimulation of locomotor activity evoked in rats by the D₂-receptor agonist quinpirole (Beltramo et al., 2000). These effects are observed at doses of AM404 that selectively target anandamide transport and produce only mild hypokinesia when the drug is administered alone (Beltramo et al., 2000). The results of this study, which have been confirmed in several subsequent reports (for review, see Piomelli, 2003), demarcate the pharmacological profile of AM404 from those of direct-acting cannabinoid drugs. This distinction may result from the ability of AM404 to enhance anandamide signaling in an activity-dependent manner, causing anandamide to accumulate in discrete regions of the brain and only when appropriate stimuli initiate its release. Pharmacological activation of D₂ receptors may represent one such stimulus, suggesting that blockade of anandamide transport might offer an innovative strategy to correct abnormalities associated with dysfunction in dopaminergic transmission. Initial tests of this hypothesis have shown that systemic administration of AM404 normalizes movement in sponta-

neously hypertensive rats (SHR), an inbred line in which hyperactivity and attention deficits have been linked to a defective regulation of mesocorticolimbic dopamine pathways (Beltramo et al., 2000).

2.4. Anandamide deactivation: intracellular hydrolysis

FAAH was first identified as an amide hydrolase activity present in rat liver tissue, which catalyzes the hydrolysis of the fatty-acid ethanolamides palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) (Natarajan et al., 1984). That anandamide serves as a substrate for this activity was first suggested on the basis of biochemical evidence (Deutsch and Chin, 1993; Désarnaud et al., 1995; Di Marzo et al., 1994; Ueda et al., 1995) and later demonstrated by molecular cloning, heterologous expression and generation of FAAH-null mice by homologous recombination (Cravatt et al., 1996; Cravatt et al., 2001).

FAAH belongs to a group of enzymes known as 'amidase signature family' (Cravatt et al., 1996; Giang and Cravatt, 1997) and catalyzes the hydrolysis not only of anandamide and other fatty-acid ethanolamides, but also of primary amides such as oleamide (Cravatt et al., 1995) and even of fatty-acid esters such as 2-AG (Goparaju et al., 1998; Lang et al., 1999). Elegant site-directed mutagenesis and X-ray diffraction studies have demonstrated that this unusually broad substrate preference is due to a novel catalytic mechanism involving the amino-acid residue lysine 142. This residue may act as a general acid catalyst, favoring the protonation and consequent detachment of reaction products from the enzyme's active site (Patricelli and Cravatt, 1999; Bracey et al., 2002). Three serine residues that are conserved in all amidase signature enzymes (S241, S217 and S218 in FAAH) also may be essential for enzymatic activity: serine 241 may serve as the enzyme's catalytic nucleophile, while serine 217 and 218 may modulate catalysis through an as-yet-unidentified mechanism (Patricelli et al., 1999). Electron microscopy experiments in the rat and mouse brain have shown that FAAH is predominantly, if not exclusively localized to intracellular membrane compartments, particularly to the endoplasmic reticulum and the mitochondria (Gulyas et al., 2004).

Although FAAH appears to be the predominant route of anandamide hydrolysis in the brain, other enzymes are likely to participate in the breakdown of this endocannabinoid in peripheral tissues. An acid amide hydrolase activity catalytically distinct from FAAH has been characterized in human megakaryoblastic cells and shown to be highly expressed in the rat thymus, lungs and intestine (Ueda et al., 1999, 2001).

2.5. FAAH inhibitors

The search for small-molecule inhibitors of intracellular FAAH activity has led to the emergence of several potent and selective agents, which include substituted sulfonyl fluorides (Gifford et al., 1999), alpha-keto-oxazolopyridines (Boger et al., 2001) and carbamic acid esters (Kathuria et al., 2003; Tarzia et al., 2003). The latter were identified during structure–activity relationship studies aimed at determining whether esters of carbamic acid such as the insecticide carbaryl inhibit FAAH activity. It was found that, although carbaryl is ineffective in this regard, variations in its template result in significant inhibitory potencies. Further structural optimizations yielded a group of highly potent inhibitors, a representative example of which is provided by the compound URB597 (Fig. 3).

Kinetic and dialysis experiments indicate that URB597 interacts non-competitively with FAAH, which is suggestive of an irreversible or slowly reversible association with the enzyme. Importantly, URB597 has no notable effect on CB₁ or CB₂ binding, anandamide transport, or rat brain monoglyceride lipase (MGL), a cytosolic serine hydrolase that catalyzes the hydrolysis of the second endocannabinoid, 2-arachidonoylglycerol (2-AG) (described in a subsequent section) (Kathuria et al., 2003).

Following administration to rats *in vivo*, URB597 produces profound, dose-dependent inhibition of brain FAAH activity. After injection of a maximal dose of compound (0.3 mg kg⁻¹, intraperitoneal), FAAH inhibition is rapid (<15 min), persistent (>16 h) and associated with a 3-fold increase in brain anandamide levels. Furthermore, the inhibitor intensifies and prolongs the effects produced by exogenous anandamide, yet it elicits no overt cannabinoid-like actions when administered alone; for example, it does not cause hypothermia, hot-plate analgesia, or hyperphagia (Kathuria et al., 2003).

2.6. Behavioral effects of FAAH inhibitors

Although URB597 does not display a typical cannabinoid profile in live animals, it exerts several pharmacological effects that might be therapeutically relevant. One such effect, the ability to reduce anxiety-like behaviors in rats, was demonstrated in two distinct experimental models: the elevated ‘zero maze’ test, and the isolation-induced ultrasonic emission test (Kathuria et al., 2003). The ‘zero maze’ consists of an elevated annular platform with two open and two closed quadrants and is based on the conflict between an animal’s instinct to explore its environment and its fear of open spaces where it may be attacked by predators (Shepherd et al., 1994). Benzodiazepines and other clinically used anxiolytic drugs increase the proportion of time spent

in, and the number of entries made into, the open compartments. In a similar fashion, URB597 elicits anxiolytic-like responses at a dose (0.1 mg kg⁻¹, intraperitoneal) that corresponds to those required to inhibit brain FAAH activity. Moreover, these effects are prevented by the CB₁-selective antagonist rimonabant. Analogous results were obtained in the ultrasonic vocalization emission test, which measures the number of stress-induced vocalizations emitted by rat pups removed from their nest (Kathuria et al., 2003). If confirmed in further behavioral models, these findings would suggest that inhibition of intracellular FAAH activity might offer an innovative target for the treatment of anxiety (Gaetani et al., 2003), which is also a feature of marijuana withdrawal (Kouri et al., 1999; Kouri and Pope, 2000; Budney et al., 2003).

3. 2-Arachidonoylglycerol

2-AG was identified as a second endocannabinoid substance in 1995 (Sugiura et al., 1995; Mechoulam et al., 1995). The multiple roles of this lipid compound in cell metabolism and its high levels in brain tissue—about 200-fold higher than those of anandamide—suggest that much of cellular 2-AG may be involved in housekeeping functions. The diversity of roles played by this compound also complicates our efforts to establish biochemical route(s) involved in its physiological formation. Nevertheless, one pathway has emerged as the most likely candidate (Fig. 4). This pathway starts with the phospholipase-mediated generation of 1, 2-diacylglycerol (DAG). This serves as a substrate for two enzymes: DAG kinase, which catalyzes DAG phosphorylation to phosphatidic acid; and DAG lipase (DGL), which hydrolyzes DAG to monoacylglycerol (Bisogno et al., 2003). Pharmacological inhibition of phospholipase C and DGL prevent the Ca²⁺-dependent accumulation of 2-AG in rat cortical neurons, which

Fig. 4. Mechanism of 2-arachidonoylglycerol (2-AG) formation in neural cells. This hypothetical diagram illustrates two steps of 2-AG synthesis: first the production of 1, 2-diacylglycerol (DAG) from phosphoinositides (PIP₂), catalyzed by the enzyme phospholipase C (PLC); and, second, the hydrolysis of 1, 2-DAG to yield 2-AG, catalyzed by diacylglycerol lipase (DGL).

suggests a key role of this pathway in 2-AG generation (Stella and Piomelli, 2001). However, additional routes of 2-AG synthesis also may exist, including phospholipase A₁ (PLA₁), hormone-sensitive lipase or a lipid phosphatase (Piomelli, 2003).

In neurons and glia, 2-AG synthesis may be initiated by a rise in cytosolic Ca²⁺ levels. For example, in cultures of rat cortical neurons, the Ca²⁺ ionophore ionomycin and the glutamate receptor agonist *N*-methyl-D-aspartate (NMDA) stimulate 2-AG production in a Ca²⁺-dependent manner (Stella and Piomelli, 2001). Similarly, in freshly dissected hippocampal slices, electrical stimulation of the Schaffer collaterals, a glutamatergic fiber tract that connects neurons in the CA3 and CA1 fields, causes a Ca²⁺-dependent increase in 2-AG content (Stella et al., 1997). This stimulation has no effect on the levels of non-cannabinoid monoacylglycerols, such as 1(3)-palmitoylglycerol, which indicates that 2-AG formation may not be attributed to a broad, non-specific increase in lipid turnover. Furthermore, electrical stimulation of the Schaffer collaterals does not modify hippocampal anandamide levels, suggesting that the biochemical pathways leading to the production of 2-AG and anandamide may be independently controlled (Stella et al., 1997). In further support of this idea, activation of D₂ receptors, a potent stimulus for anandamide formation in the rat striatum, has no effect on striatal 2-AG levels (Ferrer et al., 2003; Giuffrida et al., 1999).

3.1. 2-AG deactivation: transport into neurons and glia

Neuronal and glial cells internalize 2-AG through a mechanism apparently similar to that implicated in anandamide transport. Thus, human astrocytoma and other tumor cells accumulate [³H]anandamide and [³H]2-AG with similar kinetic properties and this process is blocked by the anandamide transport inhibitor AM404 (Piomelli et al., 1999; Beltramo et al., 2000). In addition, anandamide and 2-AG prevent each other's transport (Beltramo et al., 2000). Nevertheless, there also appear to be differences between anandamide and 2-AG accumulation. For example, [³H]2-AG internalization in astrocytoma cells is reduced by exogenous arachidonic acid, whereas [³H]anandamide internalization is not. This discrepancy may be explained in two ways: arachidonic acid may directly interfere with a 2-AG carrier distinct from anandamide's; or the fatty acid may indirectly prevent the facilitated diffusion of 2-AG by inhibiting its enzymatic conversion to arachidonic acid. If the latter explanation is correct, agents that interfere with the arachidonic acid esterification into phospholipids, such as triacsin C (an inhibitor of fatty acyl-coenzyme A synthetase), should decrease [³H]2-AG uptake. This was found indeed to be the case, at least in astrocytoma cells (Beltramo et al.,

2000). Thus, while anandamide and 2-AG may be internalized through similar transport mechanisms, they appear to differ in how their intracellular breakdown can affect the rate of transport into cells.

3.2. 2-AG deactivation: intracellular hydrolysis

After removal from the external medium, 2-AG is hydrolyzed to arachidonic acid and glycerol. In cell-free preparations, FAAH cleaves anandamide and 2-AG at similar rates, which has led to suggest that this enzyme may contribute to the elimination of both compounds. This appears to be unlikely, however, for three reasons. First, pig brain tissue contains two distinct 2-AG-hydrolase activities, both of which are chromatographically different from FAAH (Goparaju et al., 1999). Second, inhibition of FAAH activity in intact neurons and astrocytoma cells prevents the hydrolysis of anandamide, but has no effect on 2-AG degradation (Kathuria et al., 2003). Finally, 2-AG hydrolysis is entirely preserved in FAAH-null mice (Lichtman et al., 2002). These findings suggest that, although 2-AG can be hydrolyzed by FAAH in vitro, different enzyme(s) may be responsible for its degradation in vivo. A possible candidate for this role is MGL, a cytosolic serine hydrolase that cleaves 2- and 1-monoglycerides into fatty acid and glycerol (Karlsson et al., 1997). The molecular cloning of rat brain MGL has recently allowed the testing of this hypothesis (Dinh et al., 2002). MGL is abundantly expressed in discrete areas of the rat brain—including the hippocampus, cortex, and cerebellum—where CB₁ receptors are also found. Moreover, adenovirus-induced overexpression of MGL enhances the hydrolysis of endogenously produced 2-AG in primary cultures of rat brain neurons (Dinh et al., 2002). Finally, recent experiments indicate that silencing the MGL gene through RNA interference markedly impairs 2-AG degradation in intact HeLa cells (Dinh et al., in press). Although these results strongly support a role of MGL in 2-AG hydrolysis, the development of additional experimental tools (e.g. MGL-null mice, selective MGL inhibitors) will be needed to demonstrate such a role unambiguously.

4. Conclusions

Although the neurobiological bases of drug-seeking behaviors are still poorly understood, it is generally assumed that such behaviors are rooted in intrinsic neural mechanisms that have evolved to serve natural functions such as feeding, drinking and reproduction. For example, current hypotheses on the substrates of opiate abuse invoke an essential role for reward circuits that utilize endogenous opioids as neurotransmitters. It is reasonable to anticipate therefore that information on

the endocannabinoid system will be instrumental in understanding the pharmacological properties of marijuana and will eventually help to identify strategies for the treatment of marijuana dependence and withdrawal.

It is now generally accepted that heavy marijuana use can produce dependence and withdrawal (McRae et al., 2003; Wiesbeck et al., 1996). The latter consists of a constellation of symptoms similar to those seen with nicotine withdrawal, which include irritability, sleep difficulty, decreased appetite, weight loss, and increased anger and irritability (Kouri et al., 1999; Kouri and Pope, 2000; Budney et al., 2003). There have been few pharmacological attempts to alleviate this syndrome (McRae et al., 2003), but one approach using oral Δ^9 -THC has recently shown significant promise (Haney et al., 2004). The broad applicability of a replacement therapy may be limited, however, by undesirable psychotropic and cardiovascular side effects. A possible way to circumvent this limitation may be to develop pharmacological agents that protect anandamide and 2-AG from deactivation and prolong the life span of these endocannabinoid substances in vivo. By analogy with inhibitors of other neurotransmitter deactivation systems, it is reasonable to hypothesize that interfering with the inactivation of these endocannabinoid substances will lead to a more circumscribed and beneficial spectrum of biological responses than those produced by the administration of Δ^9 -THC. As noted above, this prediction has been corroborated by animal studies, which have shown that blockade of anandamide transport or hydrolysis results in a palette of pharmacological effects that are clearly distinguishable from those of Δ^9 -THC. These findings should encourage future investigations aimed at testing endocannabinoid deactivation inhibitors in the treatment of the dependence to marijuana and other drugs of abuse.

Acknowledgements

I wish to thank all the members of my lab for their extraordinary contributions, NIDA for its generous financial support, and M. Kochav for editorial help with this manuscript.

References

- Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A., Piomelli, D., 1997. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* 277, 1094–1097.
- Beltramo, M., Rodríguez de Fonseca, F., Navarro, M., Calignano, A., Gorriti, M.A., Grammatikopoulos, G., Sadile, A.G., Giuffrida, A., Piomelli, D., 2000. Reversal of dopamine D₂ receptor responses by an anandamide transport inhibitor. *J. Neurosci.* 20, 3401–3407.
- Bisogno, T., Howell, F., Williams, G., Minassi, A., Cascio, M., Ligresti, A., Matias, I., Schiano-Moriello, A., Paul, P., Williams, E.J., Gangadharan, U., Hobbs, C., Dimarzo, V., Donerty, P., 2003. Cloning the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *Journal Cell Biol.* 163, 463–468.
- Black, P., DiRusso, C., 2003. Transmembrane movement of exogenous long-chain fatty acids: proteins, enzymes, and vectorial esterification. *Microbiol. Mol. Biol. Rev.* 67, 454–472.
- Boger, D.L., Miyauchi, H., Hedrick, M.P., 2001. Alpha-keto heterocycle inhibitors of fatty acid amide hydrolase: carbonyl group modification and alpha-substitution. *Bioorg. Med. Chem. Lett.* 11, 1517–1520.
- Bracey, M., Hanson, M., Masuda, K., Stevens, R., Cravatt, B., 2002. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science* 298, 1793–1796.
- Budney, A., Moore, B., Vandrey, R., Hughes, J., 2003. The time course and significance of cannabis withdrawal. *J. Abnorm. Psychol.* 112, 393–402.
- Cadas, H., Di Tomaso, E., Piomelli, D., 1997. Occurrence and biosynthesis of endogenous cannabinoid precursor, *N*-arachidonoyl phosphatidylethanolamine, in rat brain. *J. Neurosci.* 17, 1226–1242.
- Centonze, D., Battista, N., Rossi, S., Mercuri, N., Finazzi-Agro, A., Bernardi, G., Calabresi, P., Maccarrone, M., 2004. A critical interaction between dopamine D₂ receptors and endocannabinoids mediates the effects of cocaine on striatal GABAergic transmission. *Neuropsychopharmacology* 29, 1488–1493.
- Cravatt, B.F., Próspero-García, O., Siuzdak, G., Gilula, N.B., Heriksen, S.J., Boger, D.L., Lerner, R.A., 1995. Chemical characterization of a family brain lipids that induce sleep. *Science* 268, 1506–1509.
- Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., Gilula, N.B., 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384, 83–87.
- Cravatt, B.F., Demarest, K., Patricelli, M.P., Bracey, M.H., Giang, D.K., Martin, B.R., Lichtman, A.H., 2001. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc. Natl. Acad. Sci. USA* 98, 9371–9376.
- Del Arco, I., Navarro, M., Bilbao, A., Ferrer, B., Piomelli, D., Fonseca, R., 2002. Attenuation of spontaneous opiate withdrawal in mice by the anandamide transport inhibitor AM404. *Eur. J. Pharmacol.* 454, 103–104.
- Désarnaud, F., Cadas, H., Piomelli, D., 1995. Anandamide amidohydrolase activity in rat brain microsomes. Identification and partial characterization. *J. Biol. Chem.* 270, 6030–6035.
- Deutsch, D.G., Chin, S.A., 1993. Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem. Pharmacol.* 46, 791–796.
- Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258, 1946–1949.
- Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C., Piomelli, D., 1994. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372, 686–691.
- Dinh, T.P., Carpenter, D., Leslie, F.M., Freund, T.F., Katona, I., Sensi, S.L., Kathuria, S., Piomelli, D., 2002. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. USA* 99, 10819–10824.
- Dinh, T., Kathuria, S., Piomelli, D., 2004. RNA interference-mediated silencing reveals a key role for monoglyceride lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. *Mol. Pharmacol.* (in press).

- Fegley, D., Kathuria, S., Mercier, R., Li, C., Makriyannis, A., Piomelli, D., 2004. Anandamide transport is independent of fatty acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. *Proc. Natl. Acad. Sci. USA* 99, 8756–8761.
- Ferrer, B., Asbrock, N., Kathuria, S., Piomelli, D., Giuffrida, A., 2003. Effects of levodopa on endocannabinoid levels in rat basal ganglia: implications for the treatment of levodopa-induced dyskinesias. *Eur. J. Neurosci.* 18, 1607–1614.
- Gaetani, S., Oveisi, F., Piomelli, D., 2003. Modulation of meal pattern in the rat by the anorexic lipid mediator oleoylethanolamide. *Neuropsychopharmacology* 7, 1311–1316.
- Giang, D.K., Cravatt, B.F., 1997. Molecular characterization of human and mouse fatty acid amide hydrolases. *Proc. Natl. Acad. Sci. USA* 94, 2238–2242.
- Gifford, A., Bruneus, M., Lin, S., Goutopoulos, A., Makriyannis, A., Volkow, N., Gatley, S., 1999. Potentiation of the action of anandamide on hippocampal slices by the fatty acid amide hydrolase inhibitor, palmitylsulphonyl fluoride (AM 374). *Eur. J. Pharmacol.* 383, 9–14.
- Giuffrida, A., Parsons, L.H., Kerr, T.M., Rodríguez de Fonseca, F., Navarro, M., Piomelli, D., 1999. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat. Neurosci.* 2, 358–363.
- Giuffrida, A., Rodríguez de Fonseca, F., Nava, F., Loubet-Lescoulié, P., Piomelli, D., 2000. Elevated circulating levels of anandamide after administration of the transport inhibitor, AM404. *Eur. J. Pharmacol.* 408, 161–168.
- Glaser, S., Abumrad, N., Fatade, F., Kaczocha, M., Studholme, K., Deutsh, D., 2003. Evidence against the presence of an anandamide transporter. *Proc. Natl. Acad. Sci. USA* 100, 4269–4274.
- Goldstein, A., 2001. *Addiction: from Biology to Drug Policy*. Oxford University Press, Oxford.
- Goparaju, S.K., Ueda, N., Yamaguchi, H., Yamamoto, S., 1998. Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. *FEBS Lett.* 422, 69–73.
- Goparaju, S.K., Ueda, N., Taniguchi, K., Yamamoto, S., 1999. Enzymes of porcine brain hydrolyzing 2-arachidonoylglycerol, an endogenous ligand of cannabinoid receptors. *Biochem. Pharmacol.* 57, 417–423.
- Gulyas, A.I., Bracey, B.F., Dinh, M.H., Piomelli, D., Boscia, F., Freund, T.F., 2004. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the hippocampus, cerebellum and amygdala. *Eur. J. Neurosci.* 20, 441–458.
- Haney, M., Hart, C., Vosburg, S., Nasser, J., Bennett, A., Zubarán, C., Foltin, R., 2004. Marijuana withdrawal in humans: effects of oral THC or divalproex. *Neuropsychopharmacology* 29, 158–170.
- Hillard, C.J., Campbell, W.B., 1997. Biochemistry and pharmacology of arachidonylethanolamide, a putative endogenous cannabinoid. *J. Lipid. Res.* 38, 2383–2398.
- Hillard, C., Jarrahian, A., 2003. Cellular accumulation of anandamide: consensus and controversy. *Br. J. Pharmacol.* 140, 802–808.
- Karlsson, M., Contreras, J.A., Hellman, U., Tornqvist, H., Holm, C., 1997. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J. Biol. Chem.* 272, 27218–27223.
- Kathuria, S., Gaetani, S., Fegley, D., Valino, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V., Piomelli, D., 2003. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* 9, 76–81.
- Kelley, B., Thayer, S., 2004. Delta-9-tetrahydrocannabinol antagonizes endocannabinoid modulation of synaptic transmission between hippocampal neurons in culture. *Neuropharmacology* 46, 709–715.
- Kouri, E., Pope, H., 2000. Abstinence symptoms during withdrawal from chronic marijuana use. *Exp. Clin. Psychopharmacol.* 8, 483–492.
- Kouri, E., Pope, H., Lukas, S., 1999. Changes in aggressive behavior during withdrawal from long-term marijuana use. *Psychopharmacology* 143, 302–308.
- Kreek, M., 2002. Pharmacotherapy of addictions. *Nat. Rev. Drug Discov.* 1, 710–726.
- Lang, W., Qin, C., Lin, S., Khanolkar, A., Goutopoulos, A., Fan, P., Abouzid, K., Meng, Z., Biegel, D., Makriyannis, A., 1999. Substrate specificity and stereoselectivity of rat brain microsomal anandamide amidohydrolase. *J. Med. Chem.* 42, 896–902.
- Lichtman, A.H., Hawkins, E.G., Griffin, G., Cravatt, B.F., 2002. Pharmacological activity of fatty acid amides is regulated, but not mediated, by fatty acid amide hydrolase in vivo. *J. Pharmacol. Exp. Ther.* 302, 73–79.
- Ligresti, A., Morera, E., Van Der Stelt, M., Monory, K., Lutz, B., Ortar, G., Di Marzo, V., 2004. Further evidence for the existence of a specific process for the membrane transport of anandamide. *Biochem. J.* 380, 265–272.
- Maldonado, R., Rodríguez de Fonseca, F., 2002. Cannabinoid addiction: behavioral models and neural correlates. *J. Neurosci.* 22, 3326–3331.
- Mato, S., Chevalere, V., Robbe, D., Pazos, A., Castillo, P., Manzoni, O., 2004. A single in-vivo exposure to Delta 9-THC blocks endocannabinoid-mediated synaptic plasticity. *Nat. Neurosci.* 7, 585–586.
- McRae, A., Budney, A., Brady, K., 2003. Treatment of marijuana dependence: a review of the literature. *J. Subst. Abuse. Treat.* 24, 369–376.
- Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N., Schatz, A., Gopher, A., Almog, S., Martin, B., Compton, D., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* 50, 83–90.
- Natarajan, V., Schmid, P.C., Reddy, P.V., Schmid, H.H., 1984. Catabolism of *N*-acylethanolamine phospholipids by dog brain preparations. *J. Neurochem.* 42, 1613–1619.
- Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T., Ueda, N., 2004. Molecular characterization of a phospholipase D generating anandamide and its congeners. *J. Biol. Chem.* 279, 5298–5305.
- Patricelli, M.P., Cravatt, B.F., 1999. Fatty acid amide hydrolase competitively degrades bioactive amides and esters through a nonconventional catalytic mechanism. *Biochemistry* 38, 14125–14130.
- Patricelli, M.P., Lovato, M.A., Cravatt, B.F., 1999. Chemical and mutagenic investigations of fatty acid amide hydrolase: evidence for a family of serine hydrolases with distinct catalytic properties. *Biochemistry* 38, 9804–9812.
- Piomelli, D., 2003. The molecular logic of endocannabinoid signaling. *Nat. Rev. Neurosci.* 4, 873–882.
- Piomelli, D., Beltramo, M., Glasnapp, S., Lin, Y., Goutopoulos, A., Xie, X.Q., Makriyannis, A., 1999. Structural determinants for recognition and translocation by the anandamide transporter. *Proc. Natl. Acad. Sci. USA* 96, 5802–5807.
- SAMSHA, 2000. *National Household Survey on Drug Abuse*. Author, Rockville, MD.
- Shepherd, J.K., Grewal, S.S., Fletcher, A., Bill, D.J., Dourish, C.T., 1994. Behavioural and pharmacological characterisation of the elevated “zero-maze” as an animal model of anxiety. *Psychopharmacology* 116, 56–64.
- Stella, N., Piomelli, D., 2001. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur. J. Pharmacol.* 425, 189–196.
- Stella, N., Schweitzer, P., Piomelli, D., 1997. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388, 773–778.

- Sugiura, T., Kondo, S., Sukagawa, A., Shinoda, A., Itoh, K., Yamashita, A., Waku, K., 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* 215, 89–97.
- Tarzia, G., Duranti, A., Tontini, A., Piersanti, G., Mor, M., Rivara, S., Plazzi, P.V., Park, C., Kathuria, K., Piomelli, D., 2003. Synthesis and structure–activity relationship of a series of pyrrole cannabinoid receptor ligands. *Bioorg. Med. Chem.* 11, 3965–3973.
- Ueda, N., Kurahashi, Y., Yamamoto, S., Tokunaga, T., 1995. Partial purification and characterization of the porcine brain enzyme hydrolyzing and synthesizing anandamide. *J. Biol. Chem.* 270, 23823–23827.
- Ueda, N., Yamanaka, K., Terasawa, Y., Yamamoto, S., 1999. An acid amidase hydrolyzing anandamide as an endogenous ligand for cannabinoid receptors. *FEBS Lett.* 454, 267–270.
- Ueda, N., Liu, Q., Yamanaka, K., 2001. Marked activation of the *N*-acylphosphatidylethanolamine-hydrolyzing phosphodiesterase by divalent cations. *Biochim. Biophys. Acta* 1532, 121–127.
- Wiesbeck, G., Schuckit, M., Kalmijn, J., Tipp, J., Bucholz, K., Smith, T., 1996. An evaluation of the history of a marijuana withdrawal syndrome in a large population. *Addiction* 91, 1469–1478.