Enhancement of endocannabinoid signaling by fatty acid amide hydrolase inhibition: A neuroprotective therapeutic modality

Jeannie Hwang\textsuperscript{a,b}, Crista Adamson\textsuperscript{a}, David Butler\textsuperscript{a}, David R. Janero\textsuperscript{b}, Alexandros Makriyannis\textsuperscript{b}, and Ben A. Bahr\textsuperscript{a,b}

\textsuperscript{a}Department of Pharmaceutical Sciences and the Neurosciences Program, University of Connecticut, Storrs, Connecticut U.S.A

\textsuperscript{b}Center for Drug Discovery, Northeastern University, Boston, Massachusetts U.S.A

Abstract

\textbf{Aim}—This review posits that fatty acid amide hydrolase (FAAH) inhibition has therapeutic potential against neuropathological states including traumatic brain injury, Alzheimer's, Huntington's, and Parkinson's diseases, and stroke.

\textbf{Main Methods}—This proposition is supported by data from numerous in vitro and in vivo experiments establishing metabolic and pharmacological contexts for the neuroprotective role of the endogenous cannabinoid ("endocannabinoid") system and selective FAAH inhibitors.

\textbf{Key Findings}—The systems biology of endocannabinoid signaling involves two main cannabinoid receptors, the principal endocannabinoid lipid mediators \(\text{N}-\text{arachidonoylethanolamine} \) ("anandamide") (AEA) and \(2\)-arachidonoyl glycerol (2-AG), related metabolites, and the proteins involved in endocannabinoid biosynthesis, biotransformation, and transit. The endocannabinoid system is capable of activating distinct signaling pathways on-demand in response to pathogenic events or stimuli, thereby enhancing cell survival and promoting tissue repair. Accumulating data suggest that endocannabinoid system modulation at discrete targets is a promising pharmacotherapeutic strategy for treating various medical conditions. In particular, neuronal injury activates cannabinoid signaling in the central nervous system as an intrinsic neuroprotective response. Indirect potentiation of this salutary response through pharmacological inhibition of FAAH, an endocannabinoid-deactivating enzyme, and consequent activation of signaling pathways downstream from cannabinoid receptors, have been shown to promote neuronal maintenance and function.

\textbf{Significance}—This therapeutic modality has the potential to offer site- and event-specific therapeutic relief in those tissues where endocannabinoids are being produced as part of a physiological protective mechanism. In contrast, direct application of cannabinoid receptor agonists to the central nervous system may activate CB receptors indiscriminately and invite unwanted psychotrophic effects.
Keywords

Alzheimer's disease; anandamide; brain trauma; cannabinoid; CB1 receptor; endocannabinoid; FAAH; Huntington's disease; neurodegeneration; neuroinflammation; Parkinson's disease; stroke

Introduction

Demonstration that cannabinoid (CB) constituents of the Cannabis plant, including Δ⁹-tetrahydrocannabinol (THC), exert their effects by engaging and activating discrete cellular CB receptors prompted the search in animals for naturally produced CB-receptor agonist ligands. This quest led to the discovery of a ubiquitous mammalian signaling system in which endogenous cannabinoids (endocannabinoids) synthesized by the body act as signaling lipids play varied homeostatic and regulatory roles (Mackie 2006; Pacher et al. 2006; Pertwee 2008). Experimental and clinical data have unequivocally demonstrated that one of the most important functions of the endocannabinoid signaling system is tissue protection against pathological insult or injury-- a function that has opened several attractive therapeutic modalities for pharmacological endocannabinoid-system modulation (Chang et al. 2006; Mackie 2008; Pacher and Haskó 2008; Vemuri et al. 2008; Janero and Makriyannis, 2009a,b). In particular, great advances have been made toward targeted pharmacotherapeutic manipulation of endocannabinoid signaling for neuroprotection. One well-studied endocannabinoid in particular, N-arachidonoylethanolamine (or “anandamide”) (AEA), has been linked to the neuroprotective property of CB-receptor transmission (Table 1) (see reviews: Bahr et al. 2006; Pavlopoulos et al. 2006; Janero and Makriyannis 2007; Micale et al. 2007). Distributed throughout the brain as an integral membrane protein, the enzyme fatty acid amide hydrolase (FAAH) is primarily responsible for inactivating AEA and quenching AEA-induced biological responses (Basavarajappa 2007; Vandevoorde and Lambert 2007; Di Marzo 2008; Ahn et al. 2008; Fezza et al. 2008). Whereas THC and other direct CB-receptor agonists can negatively influence psychomotor, cognitive, and appetitive behaviors (Freund 2003; Iversen 2003; Di Marzo and Matias 2005), selective FAAH inhibitors appear to offer site- and event-specific therapeutic relief in those tissues where endocannabinoids are being produced as part of a physiological protective mechanism (Vemuri et al. 2008; Janero and Makriyannis, 2009a). By virtue of this pharmacological mode of action, FAAH inhibitors are advantageously poised to exploit the neuroprotective nature of endocannabinoid signaling without risk of eliciting adverse psychotropic (or other) effects associated with chronic application of cannabinoid receptor agonists.

The foregoing implies that endocannabinoid signaling may be enhanced indirectly to therapeutic levels through FAAH inhibition, making FAAH an attractive pharmacotherapeutic target and selective FAAH inhibitors attractive drug candidates for various neurological and neurodegenerative/neuroinflammatory disorders (Table 2). This proposition will be examined herein from the perspectives of endocannabinoid metabolism and endocannabinoid-system neuropharmacology.

I. Endocannabinoid Metabolic Pathways

A growing number of endocannabinoid amide and ester long-chain fatty-acid derivatives are either known agonists or candidate ligands for one or both principal CB receptor subtypes, CB1 and CB2 (Mackie 2008; Pei et al. 2008; Pertwee 2008; Wood et al. 2008). It should also be noted that there have been other studies identifying potential cannabinoid receptors including a species named GPR55 (Begg et al. 2005; Pertwee 2007; Lauckner et al. 2008). The two signaling lipids first identified as endocannabinoids, AEA and 2-AG, remain nonetheless the most intensively studied and best characterized CB-receptor agonists (Bisogno 2008). AEA
is a partial CB1-receptor activator with modest affinity \([K_i = 61 \text{ nM (rat) and 240 \text{ nM (human)}]}\) and a relatively weak CB2-receptor ligand \((K_i = 440-1930 \text{ nM for rodent and human CB2 receptors})\) with low overall efficacy. 2-AG is a full agonist at the CB1 and CB2 receptors, albeit with lower affinity \((K_i = 472 \text{ and 1400 nM, respectively})\) and greater efficacy relative to AEA (Vemuri et al. 2008; Janero and Makriyannis, 2009a). Their distinctive biochemical and pharmacological properties notwithstanding, homeostatic regulation of AEA and 2-AG signaling depends critically upon controlled endocannabinoid generation and biotransformation/inactivation. The balanced actions of endocannabinoid synthesizing and metabolizing enzymes help localize and direct the intrinsically broad influence these lipid mediators may exert (Alexander and Kendall 2007). It is not surprising, therefore, that spatiotemporal changes in the expression/activity of endocannabinoid biosynthetic and inactivating enzymes are associated with both normal physiological processes as well as pathological states in humans (Wang et al. 2007; Ludányi et al. 2008). As distinct from most water-soluble neurotransmitters that are mobilized from membrane-delimited storage vesicles in bioactive form, AEA and 2-AG are synthesized on-demand in response to (patho) physiological stimuli (Figure 1), and their signaling functions are efficiently terminated by cellular uptake through a presently ill-defined transporter system and the rapid, enzyme-catalyzed hydrolytic inactivation (Figure 2) (Di Marzo 2008; Ahn et al. 2008). Reflecting the importance of AEA and 2-AG to intercellular communication within the central nervous system (CNS), investigations into the metabolism of these endocannabinoids have made extensive use of brain tissue and neuronal cells (Ahn et al. 2008; Lovinger 2008). For this reason, a synopsis of key metabolic pathways implicated in the regulation of endocannabinoid biosignaling primarily in the CNS is presented here. Recent reviews may be consulted for additional primary literature as well as details on the molecular and structural properties of various endocannabinoid-metabolizing enzymes, their physiological chemistry, and their potential as therapeutic targets (Bahr et al. 2006; Basavarajappa 2007; Vandevoorde and Lambert 2007; Saario and Laitinen 2007; Schneider et al. 2007; Ahn et al. 2008; Fezza et al. 2008; Vemuri et al. 2008; Janero and Makriyannis 2009a,b; Kano et al. 2009).

### A. Endocannabinoid Biosynthesis

Enzymatic synthesis of both AEA and 2-AG draws upon pools of membrane phospholipids such as phosphatidylethanolamine (PE), phosphatidylcholine (PC), and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)\(P_2\)) (Figure 1) (Ahn et al. 2008;Lovinger 2008) (Figure 1). The pathways by which \(N\)-acyl ethanolamines (NAEs) such as AEA are synthesized had been studied well before AEA was identified as an endocannabinoid. Early data suggested a two-step pathway involving the sequential action of a calcium-dependent transacylase (Ca-TA) that transfers a fatty-acyl chain from a membrane phospholipid molecule onto the primary amine of membrane PE to generate \(N\)-acyl phosphatidylethanolamine (NAPE) and a NAPE-selective phospholipase D (NAPE-PLD) that hydrolyzes NAPE to NAEs such as AEA (Natarajan et al. 1983,1984;Cadas et al. 1997). Two NAPE-PLD-independent pathways have also been implicated in NAE/AEA formation. One involves the hydrolysis of NAPE (and, perhaps, lyso-NAPE) to a glycerophospho(GP)-NAE intermediate by phospholipase A1/A2 (PLA1/2) or--perhaps more importantly in the CNS--by the serine hydrolase \(\alpha/\beta\)-hydrolase-4 (ABHD4). GP-NAE ester cleavage by glycerophosphodiesterase-1 (GDE1) then generates NAE/AEA (Simon and Cravatt 2006,2008). Another candidate NAPE-PLD-independent pathway for AEA biosynthesis involves the conversion of NAPE to phospho-AEA (pAEA) by phospholipase C (PLC) followed by the phosphatase-mediated hydrolysis of pAEA (Liu et al. 2006,2008). Formation of polyunsaturated NAEs such as AEA appears to rely more upon NAPE-PLD-independent routes, whereas synthesis of long-chain saturated or monounsaturated NAEs proceeds mainly through NAPE-PLD (Ahn et al. 2008). All of these candidate pathways for NAE/AEA formation, however, likely have NAPEs as key lipid precursors.
As is the case for AEA, multiple, incompletely characterized pathways for 2-AG biosynthesis by neurons have been proposed, each of which involves sequential enzyme-catalyzed transformations (Figure 1). The first and best-studied pathway is implicated in 2-AG formation elicited by intracellular calcium elevation or by \( G_{\alpha q/11} \)-coupled receptor activation. Both of these signals stimulate membrane-bound phospholipase C\( \beta \) (PLC-\( \beta \)) to hydrolyze the minor membrane phospholipid PtdIns(4,5)\( P_2 \), generating inositol 1,4,5-triphosphate (Ins\( P_3 \)) and diacylglycerol (DAG). Two developmentally-regulated serine hydrolases, the \( sn-1 \)-selective DAG lipases (DAGLs) DAGL-\( \alpha \) and -\( \beta \), then catalyze the final step of 2-AG formation from DAG (Figure 1) (Jung et al. 2005, 2007; Vandervoorde and Lambert 2007; Ahn et al. 2008). Although both DAGL-\( \alpha \) and -\( \beta \) are highly enriched in brain, the latter is expressed primarily in early development, whereas DAGL-\( \alpha \) expression is sustained throughout adulthood and shifts from a predominantly pre- to post-synaptic locus during neuronal maturation (Bisogno et al. 2003). Another presumptive pathway for 2-AG formation involves the synergistic actions of calcium elevation and receptor activation leading to DAG hydrolysis by DAGL-\( \alpha/\beta \) as its final step (Hashimotodani et al. 2007, 2008).

Most enzymes implicated in AEA or 2-AG biosynthesis presently lack sufficiently selective genetic or pharmacological tools (e.g., potent small-molecule inhibitors) for experimental provocation to quantify the relative contribution of each endocannabinoid biosynthetic pathway discussed. Among the recognized endocannabinoid biosynthesizing enzymes, DAGL is the focus of much current research. The strategic positioning of DAGL in multiple 2-AG biosynthetic pathways suggests that selective DAGL inhibition might offer an attractive therapeutic modality for medical conditions to which heightened 2-AG signaling through the cannabinoid 1 (CB1) receptor may contribute, such as obesity, metabolic syndrome, and substance abuse disorders (Vemuri et al. 2008; Ortar et al. 2008; Hoover et al. 2008; Janero and Makriyannis 2009a,b).

### B. Endocannabinoid Inactivation and Biotransformation

In marked contrast to the multiplicity and partial redundancy of putative pathways for AEA and 2-AG biosynthesis, each of these two principal endocannabinoids is inactivated in the nervous system primarily by a specific, relatively well-characterized hydrolase that generates the biologically important polyunsaturated fatty acid, arachidonic acid (AA) (Figure 2). Fatty acid amide hydrolase (FAAH) is the principal enzyme responsible for terminating AEA signaling and is widely expressed throughout the CNS as an integral membrane protein (Basavarajappa 2007; Vandervoorde and Lambert 2007; Di Marzo 2008; Ahn et al. 2008; Fezza et al. 2008). Another amidase-signature serine hydrolase with FAAH activity shares limited sequence identity with the original, so-called FAAH-1 enzyme and has been designated as FAAH-2 (Wei et al. 2006). FAAH-1 and -2 display several distinct biochemical, phylogenetic, and tissue-distribution characteristics. In the CNS, FAAH-1 is the more abundant FAAH and it hydrolyzes NAEs (including AEA) at greater rates than FAAH-2, which is enriched in select peripheral tissues but is absent in lower mammals, including rodents (Wei et al. 2006). FAAH-1 and -2 also display quantitatively distinct inhibitor sensitivities (Wei et al. 2006). An NAE-hydrolyzing acid amidase (NAAA) identified in immune system cells and select peripheral tissues has been cloned (Tsuboi et al. 2007a,b). NAAA is not a serine hydrolase and is also quite different from FAAH in its intracellular (lysosomal) localization, acidic pH optimum, and lack of preference for AEA as substrate. The contribution of NAAA to AEA inactivation in vivo is unknown.

Genetic polymorphisms in FAAH that disturb endocannabinoid biosignaling are associated with increased risk for substance abuse (Flanagan et al. 2006), gastrointestinal dysfunction (Storr et al. 2008a), and overweight/obesity issues (Aberle et al. 2008) in certain human subpopulations. Because of the important roles of AEA in neuronal plasticity, memory, and...
protection against neurological insult, selective FAAH inhibitors are a prime translational focus as potential drugs for important medical problems including pain management, psychobehavioral states (anxiety, depression), inflammation, excitotoxic events (i.e., stroke, seizures), and age-related neurodegenerative disorders including Huntington’s, Parkinson’s, and Alzheimer’s diseases (Bahr et al. 2006; Karanian et al. 2005b, 2007; Centonze et al. 2007; Scherma et al. 2008; Storr et al. 2008b; Naidu et al. 2009) (vide infra). Targeted FAAH inhibition (i.e., blocking AEA inactivation) is considered an attractive therapeutic approach to enhance indirectly cannabinergic signaling, for it would take place preferentially in regions where endocannabinoid synthesis and release are ongoing as a salutary, tissue-protective response. The site- and event-specific character of the pharmacological inhibition of endocannabinoid deactivating enzymes such as FAAH may offer increased selectivity with less risk of the undesirable side effects that have been observed with CB-receptor agonists capable of activating all accessible receptors indiscriminately (Vemuri et al. 2008; Janero and Makriyannis 2009).

Although FAAH hydrolyzes both AEA and 2-AG at similar rates in vitro (Goparaju et al. 1998), multiple lines of genetic, biochemical, and pharmacological evidence suggest that enzyme(s) other than FAAH regulate 2-AG inactivation in vivo. In particular, a soluble serine hydrolase, monoacylglycerol lipase (MGL), is considered the principal enzyme responsible for terminating 2-AG signaling in the CNS by catalyzing 2-AG’s conversion to AA (Figure 2) (Vandevoorde and Lambert 2007; Saario and Laitinen 2007; Ahn et al. 2008; Zvonok et al. 2008a and 2008b). Four other hydrolases are capable of generating AA from 2-AG: α/β-hydrolase-6 (ABHD6), α/β-hydrolase-12 (ABHD4), neuropathy-target esterase (NTE), and hormone-sensitive lipase (H-S lipase) (Figure 2) (Belfrage et al. 1977; van Tienhoven et al. 2002; Blankman et al. 2007). Some 85% of total brain 2-AG hydrolysis has been assigned to MGL, the remaining ~ 15% catalyzed mostly by ABHD6 and ABHD12 (Blankman et al. 2007). The distinct subcellular distributions of MGL, ABHD6, and ABHD12 suggest that they may modulate specific CNS 2-AG pools (Ahn et al. 2008).

Enzymatic conversion of AEA and 2-AG to AA constitutes the hydrolytic deactivation mode of endocannabinoid metabolism. AEA and 2-AG can also serve as indirect and direct sources of substrates for oxidative transformation to various lipid derivatives (Figure 2). The AA product of both FAAH and MGL catalysis is a second-messenger molecule whose positional oxygenation, epoxidation, or hydroxylation by cyclooxygenase (COX), lipoxygenase (LOX), epoxygenase, or hydroxylase enzymes can generate an array of fatty acid derivatives including prostaglandin, prostacyclin, thromboxane, and leukotriene eicosanoids and hydroxyeicosatetraenoic and epoxyeicosatrienoic acids (Khanapure et al. 2007). Many of these AA derivatives have important physiological functions: e.g., prostaglandin E2 (PGE2) is a potent modulator of vascular tone and mediates diverse immune and inflammatory responses (Khanapure et al. 2007). Both AEA and 2-AG can be oxygenated directly by COX to generate prostaglandin ethanolamides (“prostamides”) [e.g., prostaglandin E2 ethanolamine (PGE2- EA)] and glyceryl prostaglandins [e.g., prostaglandin E2 glycerol ester (PGE2-G)], respectively (Figure 2). COX-1 is much less effective than COX-2 in catalyzing oxidative endocannabinoid metabolism (Woodward et al. 2008). Likewise, AEA and 2-AG can serve as LOX and cytochrome P450 substrates, leading to an array of oxygenated compounds including hydroxyeicosatetraenoic acid ethanolamides [e.g., 12-S-OH arachidonoyl ethanolamine (12-S-HEA), hydroxyeicosatetraenoic acid glycerol esters [e.g., 12-S-OH arachidonoylglycerol (12-S-HG)] (Figure 2), epoxygenoicosatetraenoic acid ethanolamides, and epoxyeicosatetraenoic acid glycerol esters (Prusakiewicz et al. 2007;Chen et al. 2008). The production and physiological activity of most products of direct AEA and 2-AG oxidative metabolism in vivo largely remain to be established (Guindon and Hohmann 2008). In this regard, it has recently been observed that COX-2 converts 2-AG into PGE2-G, which induces hyperalgesia in the rat (Shu-Jung et al. 2008), and that fat ingestion elicits NAPE biosynthesis to suppress food intake.
II. Neuroprotection Through Endocannabinoid Modulation

Responses to CB receptor activation include opening of potassium channels, inhibition of calcium currents, and stimulation of various protein kinases (Deadwyler et al. 1995; Gomez Del Pulgar et al. 2000; Galve-Roperh et al. 2002; Molina-Holgado et al. 2005; Karanian et al. 2005b, 2007). Some of the many such signaling elements activated by endocannabinoids play important roles in neuronal maintenance (Bahr et al. 2006; Galve-Roperh et al. 2008). CB receptor transmission elicits modulatory effects on calcium channels, resulting in reduced neurotransmitter (e.g., GABA, glutamate) release (Hajos et al. 2000; Kreitzer and Regehr 2001; Ohno-Shosaku et al. 2001; Wilson et al. 2001). One particular mitogen-activated protein kinase, extracellular signal-regulated kinase (ERK), is involved in cannabinergic signaling, as are focal adhesion kinase (FAK) and phosphatidylinositol 3'-kinase (PI3K). These signaling elements appear to play key roles in the neuroprotective nature of the endocannabinoid system, and the associated signaling pathways are disrupted by blocking CB receptor activation (Wallace et al. 2003; Khaspekov et al. 2004; Karanian et al. 2005a,b). Compromise of basal cross-talk among pathways involving ERK, FAK, and growth-factor receptors during endocannabinergic blockade elicits a corresponding increase in neuronal pathogenic susceptibility and a decrease in synaptic maintenance. Several reports further indicate that genetic ablation of CB receptors and their associated downstream signaling increases susceptibility to seizure induction, traumatic and ischemic brain injury, and neuronal inflammatory damage (Parmentier-Batteur et al. 2002; Marsicano et al. 2003; Jackson et al. 2005; Panikashvili et al. 2005). Conversely, promoting CB receptor responses through the action of exogenous or endogenous ligands can generate enhanced levels of signaling via ERK, FAK, and other pathways. This makes FAAH and other endocannabinoid deactivation mechanisms ideal targets for neuroprotective modulation of the cannabinergic system. Selective FAAH inhibitors and FAAH knockout studies have described the outcome of elevated endogenous signaling through CB1 receptors (Cravatt et al. 2001; Kathuria et al. 2003; Karanian et al. 2005b, 2007). Dual modulation of endocannabinoid action was achieved by combining a FAAH inhibitor with another blocker of endocannabinoid deactivation (anandamide transport inhibitor), resulting in pronounced increases in FAK and ERK responses, to levels similar to those triggered by CB1-receptor agonists. The effects of exogenously applied AEA are also potentiated by FAAH inhibition (Gifford et al. 1999; Arizzi et al. 2004). Together, these studies indicate that positive modulation of endocannabinoid responses can harness endogenous signaling networks that support neuroprotection.

A. Neuroprotective Effects of FAAH Inhibitors

In the CNS, endocannabinoids are produced by neurons on demand (Figure 1), and their efficient catalytic inactivation (Figure 2) helps ensure that they act locally near their site of synthesis. Endocannabinoids are synthesized and released in response to neuronal injury, and the cannabinergic action of the released CB-receptor ligands can be enhanced by attenuating FAAH activity. Since AEA is effectively inactivated by the endocannabinoid-hydrolyzing enzyme FAAH (vide supra), inhibiting FAAH would increase the availability of released AEA to activate local CB receptors to elevate the initial neuroprotective response to a therapeutic level. The significance of FAAH inhibition as a means of enhancing cannabinergic signaling
has been demonstrated through both knockout strategies as well as pharmacological FAAH blockade by synthetic inhibitors (Cravatt et al. 2001; Karanian et al. 2005a; Moreira et al. 2008). Not surprisingly, potential therapeutic indications to which this strategy could be applied for therapeutic purposes are expanding and include management of multiple sclerosis (Benito et al. 2007), spinal cord injury (García-Ovejero et al. 2009), ischemic events (Nucci et al. 2007), and other disorders, particularly those involving the CNS (Table 2).

Endocannabinoid production and signaling through CB receptors elicit neuronal-cell survival responses that could play a salutary role against diverse neuropathologies. Table 3 lists the growing number of neuroprotective effects associated with FAAH inhibition. For example, the FAAH inhibitor URB597 attenuated hippocampal neuronal hyperactivity (Coomb et al. 2008), and systemic administration of URB597 minimized the retinal damage observed in ischemic-reperfusion studies (Nucci et al. 2007). Another FAAH inhibitor, palmitoyl sulfonate fluoride (AM374), enhanced the effects of AEA (Gifford et al. 1999; Arizzi et al. 2004) and promoted endogenous CB-related signaling in brain tissue (Karanian et al. 2005b). Pharmacological modulation of endocannabinoid deactivation to promote signaling resulted in excitotoxic protection in both hippocampal slice and animal models. The neuroprotection was demonstrated cellularly and functionally, i.e., by a reduction in neuronal loss in the hippocampus, enhanced synaptic integrity, reduced behavioral abnormalities, and improved memory performance (Karanian et al. 2005b). These neuroprotective effects were completely abrogated by a selective CB1-receptor antagonist, thus indicating that CB1-receptor transmission was responsible for the neuroprotection.

Another example of neuroprotective FAAH modulation is provided by data from the kainate rat model. As an excitotoxin, kainic acid (KA) induces seizures and damage to the hippocampus, a brain region involved in higher-order brain functions. Uncontrolled seizures produce excitotoxic brain damage reminiscent of that in many disease states. Recurring seizures can result from epilepsy, brain injury, and genetic conditions. Among the medical complications associated with substance abuse/drug addiction, excitotoxic brain injury can be caused by alcohol (Wilkins et al., 2006), diverse amphetamine derivatives (Itzhak and Ali, 2006), and cocaine (Kosten et al., 1994; Mets et al., 2003; Matsumoto et al., 2004; Witkins et al., 2008). The selective FAAH inhibitor AM374 was shown to enhance AEA levels and decrease seizure severity in KA-treated rats (Karanian et al. 2007). This study also documented a dose-dependent and accelerated rate of seizure recovery with AM374 treatment, which protected against excitotoxic cytoskeletal damage and synaptic decay. The controlled modulation of cannabinergic signals also protected against the cellular and functional deficits in the kainate rats. These salutary responses were prevented by the CB1-receptor antagonist AM251, indicating that the neuroprotective action of FAAH inhibition was mediated through endocannabinoid transmission. This FAAH-inhibitor study further implicated the activated MAPK/ERK pathway in the reduction of neuronal damage. Another selective FAAH inhibitor, URB597, has been found to attenuate the damaging effects of KA-induced neuronal activity (Coomb et al. 2008). Together, these studies show dose-dependent congruence in vivo among: 1) reduced seizure severity, 2) enhanced synaptic integrity, and 3) improved behavioral performance. The endocannabinoid enhancement attenuated seizure severity, perhaps by reducing intracellular calcium via cannabinergic actions on voltage-gated calcium channels through inhibition of adenylyl cyclase (Deadwyler et al., 1993; Shen and Thayer, 1996, 1998; Mu et al., 1999) or by eliciting nonspecific and synapse-specific depression of excitatory circuits (Shen et al., 1996; Kim and Thayer, 2000; Gerdeman and Lovinger, 2001; Singla et al., 2007). Clinical studies indicate that epileptic seizures in humans can result from perturbation of the endocannabinoid system, including the down-regulation of CB receptors located in hippocampal glutamatergic terminals (Ludanyi et al., 2008). In sum, manipulation of the endocannabinoid system, a key regulator of synaptic transmission in the brain, through FAAH inhibition is a potentially attractive approach for treating seizures of diverse etiology.
The neuroprotective action of FAAH inhibitors is reminiscent of the compensatory protective response in which AEA levels are elevated 2- to 13-fold after KA-induced seizures (Marsicano et al., 2003), ischemia (Amantea et al., 2007), concussive head trauma (Hansen et al., 2001), and neurotoxin exposure (Hansen et al., 2001; Maccarrone et al., 2003). In humans experiencing stroke-related excitotoxic insult, AEA release during the injury process has been observed as a compensatory protective response to potentiate intrinsic survival signaling (Schäbitz et al., 2002). Cannabinergic modulation of network excitability may also have the potential to offset the neuronal over-activation produced by drugs of abuse that can involve various transmitters including dopamine, serotonin, norepinephrine, GABA, and glutamate. Note that inhibitors of the strategy of developing FAAH inhibitors as neuroprotective drugs gains attractiveness from findings that FAAH inhibitors are devoid of cataleptic effects (Beltramo et al., 2000; Arizzi et al., 2004; Karanian et al., 2007) and do not carry an abuse potential themselves (Justinova et al., 2008). These properties suggest that FAAH inhibitors could represent novel, safe drugs for treating brain damage from epileptic seizures, traumatic injury, and drugs of abuse.

Other medical indications where FAAH inhibition may be an effective pharmacotherapeutic strategy include multiple sclerosis and neurodegenerative/neuroinflammatory diseases such as Alzheimer’s, Huntington’s, and Parkinson’s diseases (Benito et al. 2003; Maccarrone et al. 2003; Ramirez et al. 2005; Micale et al. 2007; Bisogno and Di Marzo 2008). Recent data presented in abstract indicate that the content of AEA and a prime AEA precursor, N-arachidonoyl phosphatidylethanolamine (Figure 1), were markedly reduced in the temporal and frontal cortices of Alzheimer’s patients, whereas 2-AG and 2-AG-related related lipids were largely unchanged (Jung et al. 2008). Interestingly, AEA levels in different brain regions of the Alzheimer’s patients correlated with region-specific cognitive test scores, implicating the importance of endocannabinoid signaling for cognition. In another clinical Alzheimer’s study, AEA was shown to prevent toxicity of the human amyloid-β peptide (Milton 2002), which is believed responsible for the neurodegenerative changes in the Alzheimer’s brain. In an animal model of multiple sclerosis, AM374 significantly reduced a hallmark symptom, spasticity, a therapeutic response similar to that elicited by treatment with potent cannabinergic agonists (Baker et al. 2001).

Further support for beneficial effects of FAAH inhibition in multiple sclerosis was recently demonstrated in a chronic encephalitis model with the observation that that FAAH knockout mice exhibited a more substantial remission compared to wild-type mice (Webb et al. 2008). The reduced endocannabinoid signaling associated with Huntington’s disease could be reversed through the blockade of FAAH activity (see review: Micale et al. 2007). FAAH inhibition by methylarachidonoyl fluorophosphonate (MAFP) also restored normal glutamatergic activity in an animal model of Parkinson’s disease (Maccarrone et al. 2003). These experimental and clinical data suggest that FAAH inhibition will gain importance as a potential therapeutic modality for age-related neurodegenerative diseases.

III. Conclusion

The growing understanding of the biosynthetic and inactivation pathways that help regulate endocannabinoid signaling and the successful biochemical description and functional annotation of several molecular constituents of the endocannabinoid metabolome have suggested new treatment approaches for several important disease states ill-satisfied by currently available drugs. Therapeutic exploitation of “on-demand,” tissue-protective endocannabinoid responses is particularly attractive with respect to many neuropathological conditions. Blocking the inactivation of the endogenous, tissue-protective CB-receptor ligands promotes beneficial neuromodulatory and neuroprotective events (e.g., reduced transmitter release) as well as various downstream signaling pathways. Enhancing AEA levels by
inhibiting a critical deactivation step-- i.e., the hydrolytic enzyme FAAH-- is a particularly noteworthy example of exploiting the protective nature of the endocannabinoid system. Selective inhibitors of FAAH boost the endogenous tissue-protective responses in a site- and event-specific manner, thereby avoiding the risk of unwanted psychotropic effects that may result from the prolonged systemic application of CB-receptor agonists. FAAH inhibition is especially effective at attenuating excitotoxic progression in the brain and protecting against synaptic compromise, neuronal death, and the behavioral symptoms of excitotoxic brain damage. Future advances in identifying critical targets of endocannabinoid metabolism and metabolic intermediates whose pharmacological modulation can enhance endocannabinoid signaling to therapeutic levels will undoubtedly open new drug-discovery avenues. Accumulated data already encourage continued preclinical profiling of newer-generation FAAH inhibitors with the aim of promoting lead development candidates into the clinic for first-in-man proof-of-principal studies (Janero and Makriyannis, 2009a).

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Life Sci. Author manuscript; available in PMC 2011 April 10.


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Figure 1.
Diagrammatic representation of the major metabolic pathways implicated in the biosynthesis of the principal endocannabinoids, anandamide and 2-arachidonoyl glycerol.
Figure 2.
Diagrammatic representation of the major hydrolytic inactivation and oxidative biotransformation pathways implicated in anandamide and 2-arachidonoyl glycerol catabolism.
### Table 1

Pathogenic events that increase anandamide levels.

<table>
<thead>
<tr>
<th>Pathogenic insult</th>
<th>Alteration to the endocannabinoid system</th>
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<tr>
<td>traumatic brain injury</td>
<td>increases in anandamide</td>
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<td>NMDA-induced excitotoxicity</td>
<td>increases in anandamide</td>
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<td>kainic acid-induced seizures</td>
<td>increases in anandamide</td>
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<td>6-Hydroxydopamine toxicity</td>
<td>increases in anandamide</td>
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<td>model of multiple sclerosis</td>
<td>increases in anandamide</td>
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**Table 2**

Medicinal applications for controlled FAAH inhibitors.

<table>
<thead>
<tr>
<th>Potential indications</th>
<th>Example FAAH inhibitors</th>
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<tr>
<td>exposure to excitotoxic neurotoxins</td>
<td>AM374, URB597</td>
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<tr>
<td>stroke/ischemia</td>
<td>URB597</td>
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<tr>
<td>epileptic seizures</td>
<td>AM374</td>
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<tr>
<td>multiple sclerosis</td>
<td>AM374</td>
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<tr>
<td>spinal cord injury</td>
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<tr>
<td>traumatic brain injury</td>
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<td>addiction</td>
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### Table 3

**Effects of FAAH inhibitors**

- elevation of anandamide levels
- activation of ERK, FAK, and other pathways
- reduced intra-neuronal calcium
- decreased glutamate release probability
- reduction in seizure severity and period of duration
- attenuation of excitotoxic progression
- protection of synaptic integrity
- reduced excitotoxic neuronal death
- protection against excitotoxic behavioral changes
- reduced spasticity in multiple sclerosis models