



Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system

Benjamin F Cravatt* and Aron H Lichtman

The medicinal properties of exogenous cannabinoids have been recognized for centuries and can largely be attributed to the activation in the nervous system of a single G-protein-coupled receptor, CB1. However, the beneficial properties of cannabinoids, which include relief of pain and spasticity, are counterbalanced by adverse effects such as cognitive and motor dysfunction. The recent discoveries of anandamide, a natural lipid ligand for CB1, and an enzyme, fatty acid amide hydrolase (FAAH), that terminates anandamide signaling have inspired pharmacological strategies to augment endogenous cannabinoid ('endocannabinoid') activity with FAAH inhibitors, which might exhibit superior selectivity in their elicited behavioral effects compared with direct CB1 agonists.

Addresses

The Skaggs Institute for Chemical Biology and Departments of Cell Biology and Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

*e-mail: cravatt@scripps.edu

Current Opinion in Chemical Biology 2003, 7:469–475

This review comes from a themed issue on
Next-generation therapeutics
Edited by John Kozarich and Hugh Rosen

1367-5931/\$ – see front matter
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DOI 10.1016/S1367-5931(03)00079-6

Abbreviations

2-AG	2-arachidonoyl glycerol
CB	cannabinoid
FAA	fatty acid amide
FAAH	fatty acid amide hydrolase
GPCR	G-protein-coupled receptor
KO	knockout
NAE	<i>N</i> -acyl ethanolamine
THC	Δ^9 -tetrahydrocannabinol

Introduction

Over the past decade, great strides have been made in our understanding of the endogenous cannabinoid (endocannabinoid) system and its molecular constituents. In the early 1990s, two G-protein-coupled receptors (GPCRs), CB1 [1] and CB2 [2], were characterized that recognize Δ^9 -tetrahydrocannabinol (THC), the active component of marijuana [3]. Nearly concurrently with these discoveries, a lipid constituent of porcine brain, *N*-arachidonoyl ethanolamine (anandamide; **1**, Figure 1), was isolated and shown to act as an endogenous ligand for the CB1 receptor [4]. The subsequent identification of 2-arachidonoyl

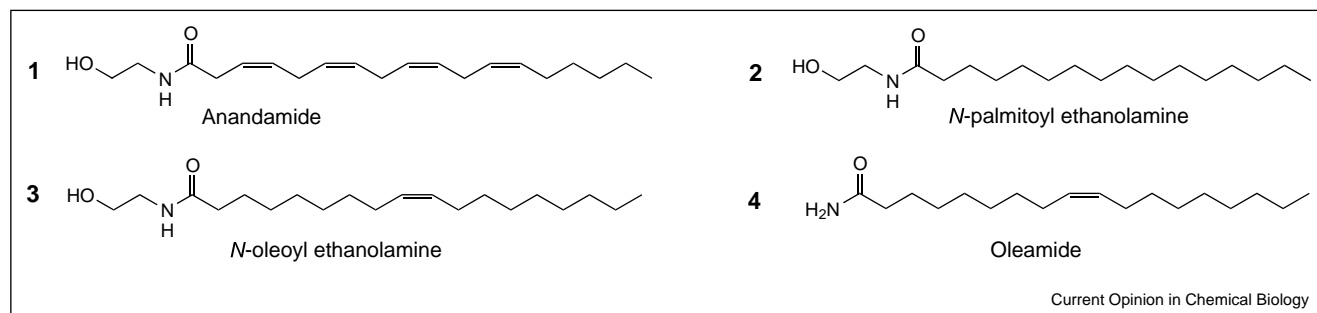
glycerol (2-AG) as a second endocannabinoid [5,6] has fortified the hypothesis that cannabinoid (CB) receptors are part of the sub-class of GPCRs that recognize lipids as their natural ligands. Consistent with this notion, based on primary structural alignment of the GPCR superfamily, CB receptors are most homologous to the *edg* receptors, which also bind endogenous lipids such as lysophosphatidic acid and sphingosine 1-phosphate [7].

The identification of anandamide and 2-AG as endocannabinoids has stimulated efforts to understand the mechanisms for their biosynthesis and inactivation. Both anandamide and 2-AG belong to large classes of natural lipids, the fatty acid amides (FAAs) [8] and monoacylglycerols [9], respectively. Notably, several FAAs in addition to anandamide appear to serve as endogenous signaling lipids, including the *N*-acyl ethanolamines (NAEs) *N*-palmitoyl ethanolamine (**2**, Figure 1) and *N*-oleoyl ethanolamine (**3**, Figure 1), which modulate pain sensation [10,11] and feeding [12], respectively, and the primary FAA oleamide (**4**, Figure 1), which has been shown to promote sleep [13]. For the purposes of this article, we focus on the enzymatic mechanisms for FAA biosynthesis and degradation; for more detailed discussion of monoacylglycerol metabolism, the reader may consult recent reviews [14,15].

Enzymatic routes for the biosynthesis and catabolism of FAAs: implications for therapeutic strategies that target the endocannabinoid system

Candidate routes for the biosynthesis and catabolism of FAAs were originally put forth by Schmid and colleagues during the 1980s. In these elegant studies, the authors described a two-step enzymatic pathway for the biosynthesis of NAEs, involving first a calcium-dependent transacylase that catalyzes the formation of *N*-acyl phosphatidylethanolamines [16] followed by the hydrolysis of these constituents by a phospholipase D to release NAEs [17] (Figure 2). An enzymatic activity was also described that hydrolyzes NAEs to their corresponding acids [18], which was suggested to represent a general mechanism for terminating FAA signaling *in vivo* (e.g. arachidonic acid does not bind to CB receptors) [13,19]. Nonetheless, the actual enzymes involved in FAA metabolism remained unknown until the late 1990s, when a rat oleamide hydrolyase activity was affinity purified and its cDNA cloned [20]. This enzyme was recombinantly expressed, shown to hydrolyze oleamide, anandamide and several other endogenous FAAs, and named fatty acid amide hydrolyase (FAAH).

Figure 1

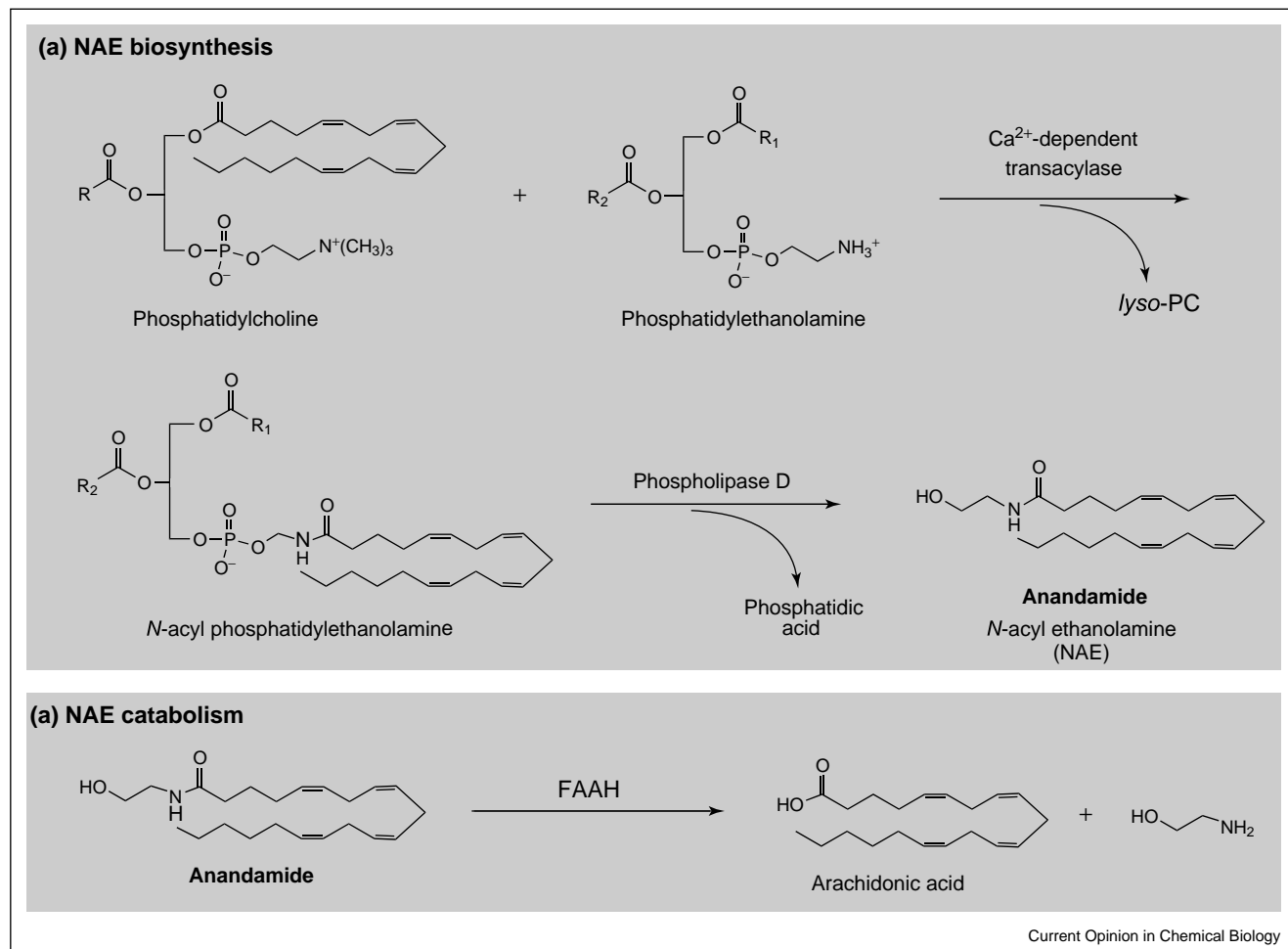


Representative endogenous fatty acid amides (FAAs).

The enzymes involved in FAA biosynthesis remain poorly characterized. Nevertheless, the determination that anandamide and related NAEs are produced by neurons in a calcium-dependent (i.e. activity-dependent)

manner [21] does have significant therapeutic implications. Indeed, this finding suggests that augmentation of endocannabinoid signaling through, for example, the inhibition of FAAH may produce behavioral effects that

Figure 2



Postulated routes for (a) biosynthesis and (b) catabolism of the NAE class of endogenous FAAs, using anandamide as the example. Lyso-PC, lysophosphatidyl choline.

Table 1

A comparison of the behavioral effects produced by CB1 agonists versus the genetic (KO) or chemical (inhibitor) inactivation of FAAH.

	Potential therapeutic effects (application)					Side-effects		
	Analgesia (pain)	Anxiolysis (anxiety)	Anti-spasticity (multiple sclerosis)	Anti-emesis (nausea)	Decrease IOP [‡] (glaucoma)	Hypomotility	Hypothermia	Catalepsy
CB1 agonist*	Yes	Yes/No [†]	Yes	Yes	Yes	Yes	Yes	Yes
FAAH KO mice	Yes	Unknown	Unknown	Unknown	Unknown	No	No	No
FAAH inhibitor	Yes	Yes	Unknown	Unknown	Unknown	No	No	No

For a more comprehensive review of the potential therapeutic uses and adverse effects of CB1 agonists, see [22]. †CB1 agonists have complex effects on emotional state and have been reported to both alleviate and exacerbate anxiety [33**]. ‡IOP, intraocular pressure.

differ significantly from those elicited by CB1 agonists, which, despite numerous suggested clinical uses, cause several adverse effects that limit their therapeutic utility [22*] (Table 1). The myriad pharmacological properties displayed by CB1 agonists may reflect the broad expression pattern of this receptor in the nervous system [23], and medicinal chemistry efforts to create CB1 agonists that possess a subset of these activities have, to date, met with little success. Thus, a need remains for pharmacological agents that can interface with the endocannabinoid system and promote a restricted set of the behavioral effects produced by direct CB1 agonists. It is suggested here that inhibitors of FAAH might fulfill this criterion, a hypothesis that is supported by recent studies describing the neurochemical and behavioral consequences that accompany the genetic/chemical inactivation of this enzyme.

The genetic inactivation of FAAH

Despite biochemical and cell biological studies supporting a role for anandamide as an endogenous CB1 ligand, the behavioral effects elicited by this FAA are very weak and transient compared with those produced by THC [24]. Preliminary evidence suggested that the limited pharmacological activity of anandamide may be due to its rapid catabolism *in vivo*, as this lipid is hydrolyzed to arachidonic acid within minutes of exogenous administration [25]. Nonetheless, the relative contribution made by FAAH to the hydrolysis of anandamide *in vivo* remained unclear until a mouse model was generated in which this enzyme was genetically disrupted (FAAH-knockout (KO) mice) [26**]. FAAH-KO mice were born at the expected Mendelian frequency and were viable, fertile and normal in their general cage behaviour. Tissues from these animals were found to display a 50–100-fold reduction in hydrolysis rates for anandamide and related FAAs. In contrast to wild-type mice, in which administered anandamide failed to produce significant behavioral effects, FAAH-KO mice exhibited robust responses to this FAA, becoming hypomotile, analgesic, cataleptic and hypothermic. Notably, all of the behavioural effects of anandamide in FAAH-KO mice were blocked by pre-treatment with the CB1 receptor antagonist SR141716A, indicating that anandamide acted as a

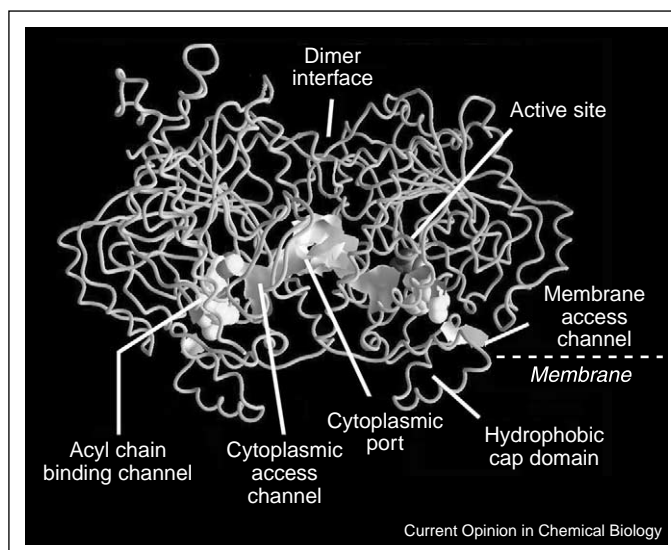
potent and selective CB1 ligand in these animals. Consistent with this notion, the apparent binding affinity of anandamide for the CB1 receptor increased approximately 15-fold in brain homogenates from FAAH-KO mice [27].

Neurochemical studies revealed that endogenous levels of anandamide and other NAEs were elevated 10–15 fold in several brain regions of FAAH-KO mice, including hippocampus, cortex and cerebellum [26**,28]. Interestingly, these augmented central nervous system levels of FAAs correlated with a CB1-dependent reduction in pain sensation in FAAH-KO mice [26**]. Collectively, these findings indicate that FAAH is a key enzyme involved in FAA catabolism *in vivo* and suggest that pain pathways are under the influence of a FAAH-regulated endocannabinoid tone. Notably, however, FAAH-KO mice exhibited normal motility, body weight and body temperature, indicating that several other neurobehaviors affected by exogenously applied CB1 agonists were not under tonic control of anandamide in these animals.

The chemical inactivation of FAAH

FAAH-KO mice represent a powerful model system in which to examine the neurochemical and behavioral consequences of constitutive inactivation of FAA catabolism. However, to understand the pharmacological effects of acute disruption of FAAH activity, potent and selective inhibitors of this enzyme are required. Several FAAH inhibitors have been described, including trifluoromethyl ketones [29], α -keto heterocycles [30*], sulfonyl fluorides [31], and fluorophosphonates [32]. Recently, a series of carbamate inhibitors of FAAH were generated by Piomelli and colleagues and shown to be efficacious *in vivo* [33**]. Within two hours after administration to rodents, these FAAH inhibitors URB532 and URB597 (Figure 3) augmented endogenous brain levels of anandamide and other NAEs approximately 3–5 fold and produced CB1-dependent anxiolytic and analgesic effects. Importantly, FAAH inhibitors did not induce catalepsy, hypothermia or changes in appetite, common side effects of pure CB1 agonists. Nearly concurrently with this study, scientists at Bristol-Myers Squibb reported a distinct set of carbamate inhibitors of FAAH (e.g. BMS-1, Figure 3) that promoted

Figure 4



X-ray crystal structure of FAAH [37**] highlighting several key regions, including the membrane access, cytoplasmic access and acyl chain (substrate) binding channels. The hydrophobic cap predicted to form a monotopic membrane-binding domain is also shown.

hydrolase and a novel enzyme, KIAA1363. Interestingly, despite their overlapping inhibitor sensitivity profiles, neither of these enzymes shares any sequence homology with FAAH. These findings highlight the value of proteome-wide screens that can detect unanticipated sites of action for inhibitors of enzymes, such as FAAH, that are members of large superfamilies.

Conclusions and future directions

Over the past two years, a remarkable number of advances have been made in our understanding of the molecular and physiological functions of the FAAH-endocannabinoid system. These studies have provided strong evidence that FAAH may serve as an attractive therapeutic target for the treatment of pain and neuropsychiatric disorders. Nevertheless, many important challenges remain as FAAH advances toward the status of a validated target. First, can reversible inhibitors of this enzyme be generated that display strong efficacy and target selectivity *in vivo*? If so, will these inhibitors elicit beneficial behavioral effects, such as relief of pain and anxiety, without displaying adverse properties? So far, pharmacological and genetic evidence in rodents indicates that the inactivation of FAAH produces a select number of behavioral effects without promoting gross changes in nervous system function as is observed with direct CB1 agonists. Still, the recent discovery of a polymorphism in human FAAH that is over-represented in patients with problem drug use may suggest that changes in FAAH function could influence addictive behaviour [41]. Additionally, FAAH-KO mice have been found to show enhanced sensitivity to chemically induced limbic seizures [28], consistent with a disinhibitory role for endo-

cannabinoids in the hippocampus [42]. These findings indicate that a deeper understanding of the relationship between the FAAH-endocannabinoid system and neural disorders such as addiction and epilepsy is warranted. Another important issue relates to the impact of prolonged administration of FAAH inhibitors. Will such treatments result in desensitization of the endocannabinoid system, thereby limiting their clinical utility for diseases such as chronic pain? Preliminary evidence from FAAH-KO mice would argue against this notion, given that these animals possess constitutively elevated anandamide levels and appear to maintain a functional CB1 receptor system throughout life [26**]. Finally, FAAH regulates the levels of several endogenous FAAs, most of which do not interact with CB receptors, but appear to possess distinct sites of action and functions in the nervous system and periphery. What are the functions of these 'orphan' FAAs and how might these activities be affected by FAAH inhibition?

In conclusion, it is noteworthy that the provocative neurochemical and behavioural effects that accompany the genetic or chemical inactivation of FAAH resemble, in a general way, the outcome of disruption of monoamine reuptake [43]. In both cases, the magnitude and duration of signaling by endogenous chemical transmitters are selectively augmented in a subset of the neural circuits activated by the administration of direct receptor agonists, resulting in a very selective profile of affected behaviors. One can only hope that this analogy can be extended further, perhaps all the way to the clinic, where FAAH inhibitors may also prove of value for the treatment of nervous system disorders.

Acknowledgements

The authors thank Dr Michael Bracey for assistance with the design of Figure 4 and gratefully acknowledge support from the Skaggs Institute for Chemical Biology, the NIH/NIDA (DA13173 and DA15197), and The Helen L Dorris Institute for the Study of Neurological and Psychiatric Disorders of Children and Adolescents.

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