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Fatty Acid Amide Hydrolase

FAAH is an anandamide-metabolizing enzyme and is an important part of the endocannabinoid catabolism pathway.

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Related terms:

Anandamide, Cannabinoid Receptor, Endocannabinoid System, Monoacylglycerol Lipase, 2-Arachidonoylglycerol, Amidase, N-Acylethanolamine, Enzymatic Hydrolysis, Degradation

Fatty Acid Amide Hydrolase (FAAH)

Steve P.H. Alexander, in xPharm: The Comprehensive Pharmacology Reference, 2009

Introduction

Fatty acid amide hydrolase (FAAH) is a serine hydrolase with a prominent role in the hydrolysis of endocannabinoids. As such, it is a target for the development of inhibitors, with potential therapeutic roles in the treatment of chronic pain, inflammation, depression and eating disorders.

The finding that some non-steroidal anti-inflammatory drugs, conventionally thought to be clinically effective through inhibition of cyclooxygenase activity, are also inhibitors of FAAH Fowler (2007), raises the possibility that FAAH has already been a target of clinically-relevant agents.

In man, two forms, FAAH1 and FAAH2, have been identified Wei et al (2006), while rodents and other lower mammals express only one isoform. Intriguingly, while FAAH1 is found on intracellular membranes, suggested to be Golgi or endoplasmic reticulum, with a cytosolic orientation of the active site, FAAH2 has been suggested to be membrane-bound with a luminal orientation (potentially extracellular) of the active site Wei et al (2006).

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Cannabinoids and Their Receptors

Heather B. Bradshaw, Emma Leishman, in Methods in Enzymology, 2017

6.1 The Many Faces of FAAH

FAAH as the name implies is hypothesized to be responsible for the hydrolysis of

the so-called fatty acid amides, among which the most famous of this lipid species is the endogenous cannabinoid, Anandamide (Cravatt et al., 1996). As mentioned earlier, the nomenclature for this lipoamine family of lipids is a somewhat moving target; however, our data show that even those lipids that clearly fall under the fatty acid amide subspecies are not ubiquitously hydrolyzed by FAAH (Leishman, Cornett, et al., 2016). To understand this statement more fully, it is important to state that it was also previously hypothesized that the hydrolysis of AEA (*aka* *N*-arachidonoyl ethanolamine) results in the production of ethanolamine and "free" AA. In large part, this hypothesis was supported by the measurement of what was considered to be radio-labeled AA on TLC plates. It is important to note that even those who did the pioneering work in elucidating what has come to be known as FAAH (e.g., Dale Deutsch) stated that the ethanolamine measurement method was preferred over the use of a TLC step when measuring FAAH activity (Deutsch, 2016). TLC, while an effective tool, lacks the specificity of mass spectrometric analysis. It is possible that the polarity of other AA derivatives, such as NAGly, might have a similar TLC migration pattern and be mistaken for AA. Our work showed that in an intact cell system AEA is a better substrate for the production of NAGly than AA (Bradshaw, Lee, et al., 2009; Bradshaw, Rimmerman, Hu, Benton, et al., 2009; Bradshaw, Rimmerman, Hu, Burstein, et al., 2009), suggesting that there is a more direct conversion between these two molecules than the conjugation of free AA with glycine. Perhaps the additional bands on the TLC plate were not just AA.

Data published from our laboratory certainly continue to support the hypothesis that FAAH is a primary metabolic enzyme for AEA; in that AEA is significantly increased in the FAAH KO mouse; however, our data suggest that this hydrolysis is not accompanied by a release of free AA. One key point of evidence for this is that unlike in MAGL KO mice that showed significant decreases in AA across the brain, there was no change in levels of AA in any region of FAAH KO brains examined compared to WT (Leishman, Cornett, et al., 2016). On the contrary, our data strongly suggest that FAAH is acting as a hydrolytic enzyme for specific lipids and a biosynthetic enzyme for other lipids (Bradshaw, Rimmerman, Hu, Benton, et al., 2009; Bradshaw, Rimmerman, Hu, Burstein, et al., 2009; Hu et al., 2009; Leishman, Cornett, et al., 2016). As mentioned earlier, data from our lab demonstrated that AEA was acting as a precursor molecule for the biosynthesis of NAGly (Bradshaw, Rimmerman, Hu, Benton, et al., 2009; Bradshaw, Rimmerman, Hu, Burstein, et al., 2009) and *N*-arachidonyl dopamine (Hu et al., 2009). These more recent data suggest that at least eight additional AA-derived lipoamines are also likely products of AEA FAAH-dependent metabolism (Leishman, Cornett, et al., 2016). This is evidenced by the fact that these AA-derived lipoamines were significantly decreased in the FAAH KO mouse. Therefore, the most parsimonious explanation for the lack of a change in AA levels and significant decreases in AA-derived lipoamines is that AA generated from AEA hydrolysis by FAAH is being used directly as a substrate for AA-derived lipoamines. It is extremely important to recognize that the interdependence this class of lipids appears to have when we contemplate inhibiting this enzyme pharmacologically when viewed in the light that there are so many other signaling molecules that can be effected. This may also explain the lack of efficacy for FAAH inhibitors in clinical trials in which the goal was to increase AEA in order to decrease pain (Schlosburg, Kinsey, & Lichtman, 2009).

When it comes to potentially targeting FAAH for therapeutics, it is also important to remember that FAAH is produced throughout the brain and not just in those

areas that are related to pain. It is highly expressed with particular density in the cerebellum, hippocampus and cortex and is also significantly correlated with CB1 expression (Egertova, Giang, Cravatt, & Elphick, 1998). Clearly, the drive to use FAAH inhibitors for pain comes from the data that tail flick latencies were increased and that pain behavior was attenuated in the formalin test in FAAH KO mice (Cravatt et al., 2001). There is a long-standing argument; however, in the pain field that while these discreet measures for the nocifensive response in an animal are reproducible and real, they are not particularly translatable to pain that is being treated in the clinic, which is typically chronic and often visceral. This led to a question that was recently addressed in a collaboration with Andrea Hohmann's lab in which FAAH KO mice showed an *increased* pain response (Carey et al., 2016). In this study, we showed that context matters. If animals are pretreated with the TRPV1 agonist, capsaicin, which does normally cause a general nocifensive response in the WT, the FAAH KO responses are dramatically increased. Lipidomics of the paw skin and the spinal cord revealed that there was the expected increase in baseline NAEs in the FAAH KO mouse; however, capsaicin treatment actually further increased some of the NAEs as well as PGs (Carey et al., 2016). That PGs were not changed in the WT after capsaicin treatment and only in the FAAH KO suggests further dysregulation of lipid metabolism as a by-product of FAAH inactivation.

Our data demonstrating that AA-derived lipoamines had the most species that were significantly decreased in the FAAH KO mouse is potentially enough to give pause when considering modifying the effects of this enzyme systemically; however, they were not the only subclass of lipids effected. Additional longer chain unsaturated fatty acid-derived lipoamines such as the docosahexaenoic acid (DA)- and linoleic acid (LA)-derived lipoamines were also significantly decreased. In our screen we showed a downregulation of LA-glycine, and DA-glycine and DA-leucine. Perhaps more intriguing was the pattern of downregulation of shorter chain saturated *N*-palmitoyl GABA and *N*-oleoyl GABA paralleled the decreases in *N*-arachidonyl-GABA; whereas the other three *N*-acyl GABA species were significantly increased paralleling the increases in the NAEs. Likewise, PA-derived lipoamines of leucine, methionine, proline, tryptophan, and valine were all downregulated as well as the stearic acid (SA) derivatives of valine and tryptophan in at least one brain area in the KO mice. This represents a departure from the long chain, unsaturated AA-derived lipids and suggests both a functional relevance of both the FAAH enzyme and the lipid signaling of brain regions and perhaps a "shuffling" of substrates and products. This may represent a drive for homeostatic balance in how these molecules are produced and distributed is in continuous flux. Deletion of one of the enzymes within this regulatory system completely changes this balance.

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Annual Reports in Medicinal Chemistry

Lei Zhang, Anabella Villalobos, in Annual Reports in Medicinal Chemistry, 2012

5.3 FAAH PET tracers

The fatty acid amide hydrolase (FAAH) enzyme is a serine hydrolase responsible for degrading the fatty acid amide family of signaling lipids, including the

endocannabinoid anandamide. The involvement of FAAH in pain and nervous system disorders has made it an attractive target for molecular imaging. Three [^{11}C]-labeled FAAH inhibitors have been recently reported as potential PET tracers for FAAH brain imaging (Fig. 8.3). Two were based on the irreversible covalent carbamate inhibitor URB597 (**12**) and one was based on a reversible FAAH inhibitor (MK-3168, **15**). **12** has been shown to inhibit FAAH irreversibly via carbamylation of FAAH's catalytic Ser^{241}, which acts as a nucleophile. A moderately active URB597 analog **13** (IC_{50} = 436 nM) was targeted as a PET lead, and a [^{11}C]-methoxy group was introduced into the aniline moiety which was expected to remain attached to the enzyme upon carbamylation. Subsequent biodistribution studies showed no retention of radioactivity in brain, substantial peripheral metabolism, and minimal differences in the biodistribution patterns of wild-type and FAAH knock-out mice.[25] As a follow-up to this effort, the same group reported PET imaging results of an improved URB597 analog, [^{11}C]CURB (**14**), which was labeled at the carbonyl position using a novel [^{11}C]CO_2 fixation methodology. In contrast to the previous tracer, **14** showed heterogeneous brain binding with little washout over time which was consistent with irreversible binding. The specific binding of **14** to FAAH was demonstrated by blocking experiments with a high dose of URB597, with the highest specific binding ratio observed in cortex and the lowest in hypothalamus.[26] More recently, another novel FAAH PET tracer, [^{11}C]MK-3168 (**15**), was disclosed, which unlike previously described tracers binds to FAAH in a reversible noncovalent manner with a K_d of 0.8 nM (human cortex tissue binding). PET imaging in rhesus monkeys demonstrated heterogeneous, specific brain uptake, consistent with known regional FAAH distribution.[27] Clinical imaging studies have been carried out with **15** to provide receptor occupancy information supporting the development of a clinical candidate. Good brain uptake and test/retest variability have been reported.

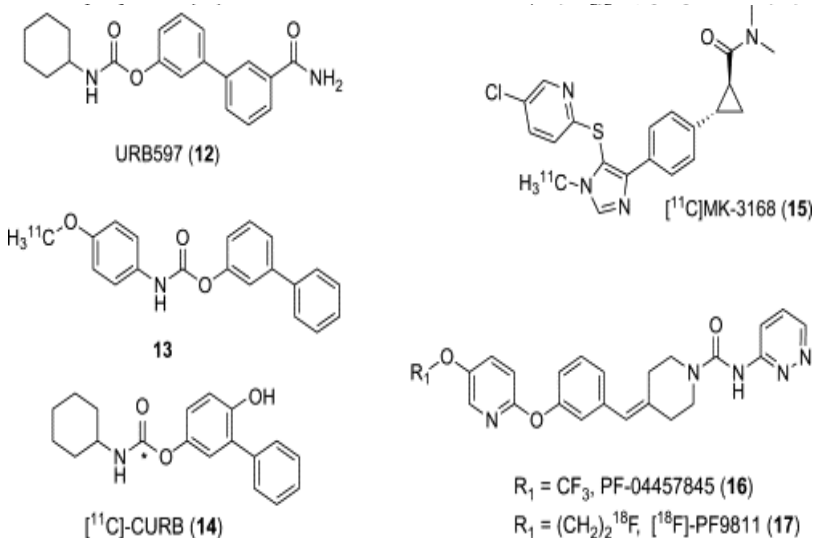

Figure 8.3. Structure of FAAH PET tracers.

Recently, our group disclosed SAR efforts in a novel urea series leading to the identification of PF-04457845 (**16**) as a clinical candidate.[28] The excellent potency, selectivity, and pharmacokinetic properties of **16** make it an attractive scaffold for PET tracer development. Toward this end, we developed [^{18}F]PF-9811 (**17**), based on a close-in analog of **16**, wherein the trifluoromethyl moiety was replaced with a fluoroethoxy group, without impact in *in vitro* FAAH potency (IC_{50} = 16 nM) and *in*

vivo FAAH inhibition activity (complete inhibition of FAAH *in vivo* at 10 mg/kg p.o. in C57B1/6 mice). Biodistribution experiments of **17** in rats showed good uptake in all regions of the brain, with preferential binding in the cortex, hippocampus, and cerebellum, and a statistically significant radiosignal increase from the 10 to 90-min time points, consistent with the characteristics of an irreversible inhibitor. Specificity of **17** for FAAH was demonstrated by pretreatment with **16**, which reduced uptake across all brain regions (37–73% at 90 min). In addition, **17** was evaluated in rat microPET studies and the results largely mirrored those of the biodistribution study, with high brain uptake and specific FAAH binding. The favorable outcome suggests that **17** represents a promising PET tracer for FAAH imaging with the potential advantage of a [^{18}F] radionuclide for higher resolution and flexibility in scan times.[29]

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Animal Models for Medications Screening to Treat Addiction

T.J. Phillips, ... C. Reed, in International Review of Neurobiology, 2016

3.4 Fatty Acid Amide Hydrolase

Fatty acid amide hydrolase is a membrane-associated serine hydrolase located in brain and liver that inactivates endocannabinoids (Cravatt et al., 2001). The Pro129Thr variant results in normal catalytic activity, but enhanced sensitivity to proteolytic degradation and was associated with drug dependence in earlier research (Sipe, Chiang, Gerber, Beutler, & Cravatt, 2002). Further investigation in MA-dependent populations noted no association in one case (Morita et al., 2005), and a significant association in another (Sim, Hatim, Reynolds, & Mohamed, 2013). Differences in outcome could be population based, as the former study was in a Japanese population and the latter in a Malaysian population.

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Computational chemistry methods in structural biology

Alessio Lodola, ... Marco Mor, in Advances in Protein Chemistry and Structural Biology, 2011

I Introduction

Fatty acid amide hydrolase (FAAH) is a mammalian membrane protein responsible for the hydrolysis and inactivation of biologically active amides (Piomelli, 2003), including the endocannabinoid anandamide and agonists of the peroxisome proliferator-activated receptors, such as oleoylethanolamide and palmitoylethanolamide (Muccioli, 2010).

The catalytic mechanism of FAAH is unique among mammalian enzymes in that it involves a catalytic triad consisting of two serine residues (Ser217 and Ser241) and one lysine residue (Lys142), rather than the more common serine–histidine–aspartate triad found in classical serine hydrolases (McKinney and Cravatt, 2005). It has been proposed that Lys142 might serve as a key acid and base in distinct steps

of the catalytic cycle (Fig. 1). As a base, it would activate the Ser241 nucleophile for attack on the substrate carbonyl. As an acid, Lys142 would protonate the substrate leaving group, leading to its expulsion. The effect of Lys142 on Ser241 nucleophile strength and on leaving group protonation occurs indirectly, via the bridging Ser217 of the triad which acts as a "proton shuttle" (Lodola et al., 2005; McKinney and Cravatt, 2005).

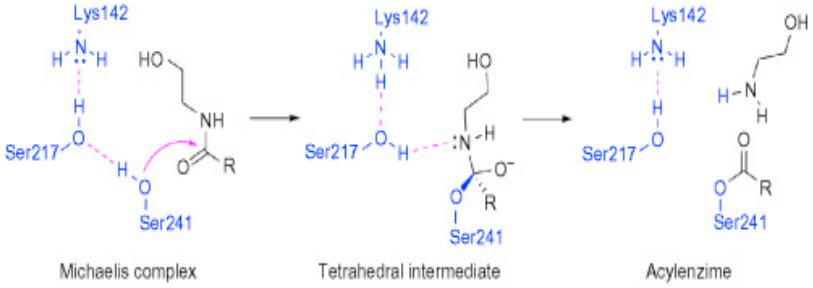

Fig. 1. Proposed catalytic mechanism of FAAH in presence of fatty acid ethanolamides. R represents the lipophilic chain of the substrate. Hydrogen bonds are displayed with pink dotted lines.

Genetic or pharmacological inactivation of FAAH enzyme leads to analgesic, anti-inflammatory, anxiolytic, and antidepressant effects in animal models (Bambico et al., 2009), without producing the undesirable side effects observed with cannabinoid receptor agonists (Piomelli, 2005). FAAH represents therefore an attractive therapeutic target for the treatment of several central nervous system disorders (Petrosino and Di Marzo, 2010).

FAAH enzyme activity is blocked by a variety of classical serine hydrolase inhibitors such as sulfonyl fluorides, fluorophosphonates, α-ketoesters, α-ketoamides, trifluoromethylketones, and acyl-heterocycles (Seierstad and Breitenbucher, 2008). Other classes of inhibitors, characterized by an improved drug-like profile, have also been reported (Minkkilä et al., 2010). These include piperazinyl-(pyridinyl)urea- and carbamate-based compounds (Mor and Lodola, 2009) which have been shown to inhibit FAAH by covalently modifying the enzyme's active site, that is, through carbamoylation of the nucleophile Ser241 (Alexander and Cravatt, 2005; Ahn et al., 2007).

Among these carbamoylating agents, *N*-alkylcarbamic acid aryl esters emerged as the first promising class of compounds capable to inhibit FAAH *in vivo*, gaining considerable interest for the treatment of anxiety, inflammation, and pain (Kathuria et al., 2003; Piomelli et al., 2006; Sit et al., 2007). More recently, other classes of carbamate derivatives and related compounds (Gattinoni et al., 2010) have been developed by academic and industrial groups. For more detailed information, the reader is referred to reviews dedicated to FAAH inhibitors (Seierstad and Breitenbucher, 2008; Minkkilä et al., 2010).

The design of *N*-alkylcarbamic acid aryl esters as FAAH inhibitors has been widely supported by the application of computer-aided drug design (CADD) techniques (Marshall and Beusen, 2003). By definition, CADD uses computational methods to discover and improve biologically active compounds. This was also the case for FAAH, as both ligand-based drug design (LBDD) and structure-based drug design (SBDD) have been applied to rationalize structure–activity relationships (SARs), helping the design of novel FAAH inhibitors.

The LBDD approach is usually applied when structural information on the target

macromolecule is missing (Marshall and Beusen, 2003). LBDD relies on the hypothesis that compounds with comparable physicochemical properties behave similarly in biological systems. Pharmacophore models as well as quantitative SARs (QSARs) can therefore be developed based on the analysis of known ligands. The QSAR approach is based on the search for a mathematical relationship between the biological activity of a series of compounds and their structural descriptors, usually encoding a chemical or physicochemical information (e.g., lipophilicity, electronic properties, steric hindrance, etc.) (Hansch and Leo, 1995). Classical QSAR variables usually account for the magnitude of a structural property, but they do not provide information about their spatial distribution in the molecular surroundings (Selassie, 2003). Thanks to computer graphics, vector descriptors have been developed, allowing the rationalization of structure–activity data within a three-dimensional (3D) setting. The possibility to represent molecular properties in a 3D space is evocative of the supposed ligand–receptor interaction process and makes intuitive the meaning of the QSAR models (Favia, 2011). The most popular 3D-QSAR methodologies are comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) (Tropsha, 2003). These methods, correlating differences in biological activity with changes in shape and in the intensity of noncovalent interaction fields “around” (CoMFA) or “on” (CoMSIA) the molecules, have been successfully applied in numerous drug-discovery projects, both in retrospective analysis and in supporting the design of new compounds (Tropsha, 2003; Mor et al., 2005).

The SBDD approach is based on availability of the 3D structure of the biological target, usually obtained by X-ray crystallography or NMR studies (Hardy et al., 2003). If an experimental structure of the target is not available, homology models can be developed based on the experimental structure of a related protein (Fiser et al., 2002). Given the 3D-structure of the target, ligands can be (i) designed directly into the target binding site using interactive graphic tools (Marshall and Beusen, 2003) or (ii) built and placed within the binding site using a molecular docking approach (Kitchen et al., 2004). Molecular docking attempts to predict the preferred conformation and orientation of a compound into a specific cavity (i.e., the binding site) of the target molecule, assigning a “score” to all the identified binding modes (Kroemer, 2007). The reliability of a docking strategy mainly relies on the quality of the scoring function (Leach et al., 2006). In the past decades, several approaches have been developed to estimate the free energy of binding, with different levels of accuracy. The most rapid and less computationally demanding methods are the empirical or knowledge-based scoring approaches, which are based either on simple energy functions or on the frequency of occurrence of different atom–atom contact pairs in complexes of known structure (Klebe, 2006). The minimalism of the energy function together with the lack of conformational sampling make these approaches extremely fast, but rather inaccurate (Michel and Essex, 2010). However, the most rigorous and accurate methods, which involve slow gradual transformations between the states of interest, by using molecular dynamics (MD) simulations, are extremely time-consuming (Deng and Roux, 2009). In this respect, computational approaches based on enhanced sampling methods (Branduardi et al., 2007; Colizzi et al., 2010; Woods et al., 2011) seem quite promising, as they have the potential to make accurate predictions at reasonable computational costs.

One of the most important aspects when trying to predict the binding mode of an active compound along with the potencies of a set of similar ligands is the time required for calculating their affinity. While screening of virtual libraries demands a

high throughput of ligands, and thus the time spent on evaluating a single compound needs to be short, when the binding mode of a "lead" compound is relatively certain it may be desirable to perform time-consuming calculations, to improve the accuracy of the prediction (Jorgensen, 2009). In spite of the theoretical aspects behind the "scoring problem," various lead identification (Villoutreix et al., 2009) and optimization (Andricopulo et al., 2009; Carmi et al., 2010; Solorzano et al., 2010) projects have been successfully carried out by applying SBDD techniques, indicating that theoretical approaches can give a practical and valuable contribution to the design of bioactive compounds.

This review focuses on the application of computational methods to the design and development of FAAH inhibitors belonging to the class of *N*-alkylcarbamic acid aryl esters. Early investigations, when the 3D structure of FAAH was still unknown, were based on LBDD techniques, including QSAR and 3D-QSAR methods, while more recent advancements were obtained applying SBDD approaches. These included (i) molecular docking, (ii) combined quantum mechanics/molecular mechanics (QM/MM) simulations, and (iii) linear interaction energy (LIE) calculations.

Obesity

Guoxun Chen, Zhen Pang, in Vitamins & Hormones, 2013

4.4 Effects of FAAH and MAGL KO in mice

FAAH was originally purified from rat liver membrane preparations with the activity to hydrolyze oleamide into oleic acid and ammonia (Cravatt et al., 1996). This enzyme has activity against fatty acid amides including high activity to anandamide and is highly expressed in brain and liver (Cravatt et al., 1996). The FAAH KO mice have elevated brain anandamide levels, heightened sensitivity to anandamide, but normal BW (Cravatt et al., 2001). It will be very interesting to elucidate the role of FAAH in liver which does not seem to express enzymes for anandamide synthesis (Cohen & Grahame Hardie, 1991; Okamoto et al., 2004).

Since plasma 2-AG level is higher in the obese patients than in the normal subjects (Blüher et al., 2006), its production has been manipulated in animals to determine its effects on obesity. Recombinant adenovirus-mediated overexpression of MAGL in rat brain causes reduction of 2-AG, but not anandamide content (Dinh et al., 2002). Conversely, administration of a specific MAGL inhibitor (JZL184) dramatically increases the brain 2-AG, but not anandamide level, and causes CB1-dependent behavior changes (Long et al., 2009). In agreement with these results, the MAGL KO mice have elevated monoacylglycerol, including 2-AG, levels in their adipose tissue, brain, and liver. However, the MAGL KO mice have normal fat mass, locomotor activity, energy expenditure, and food consumption, probably due to the development of tolerance to the high 2-AG concentrations, suggesting desensitization of the cannabinoid receptors. Interestingly, the KO mice on HFD have improved glucose tolerance and insulin sensitivity comparing to the WT DIO mice (Taschler et al., 2011).

In addition to having agonist activities on CB1 and CB2, 2-AG is also a substrate for the generation of AA for the cyclo-oxygenase (COX)-mediated prostaglandin production (Fig. 14.1) in the brain, liver, and lung, but not gut and spleen (Nomura

et al., 2011). The disruption of MAGL activity either by genetic inactivation or by specific inhibitors results in the accumulation of 2-AG in the brain and liver (Nomura et al., 2011; Taschler et al., 2011) and reduction of several prostaglandins and other eicosanoids, such as prostaglandin E2 (Nomura et al., 2011). It also blocks liposaccharide (LPS)-induced inflammatory responses in the brain and provides protection against chemically induced Parkinsonism in mice (Nomura et al., 2011). Traditionally, the supply of AA for eicosanoid production has been considered the role of phospholipase A2 (Buczynski, Dumlao, & Dennis, 2009). However, the reduction of AA contents in the brain and liver was not observed in phospholipase A2 KO mice, but rather in MAGL KO (Nomura et al., 2011). The link between AA generation for prostaglandin production and the rise of cAMP levels in adipocytes has been indicated (Wolf, 2009). The cannabinoid system can also regulate the production of cAMP in adipocytes (Di Marzo et al., 2001; Roche et al., 2006). Further investigations are needed to understand relationships of these two pathways.

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Endocannabinoids

Martin A. Sticht, ... Linda A. Parker, in International Review of Neurobiology, 2015

2.5.3 Dual FAAH and MAGL Inhibition

Dual inhibition of FAAH and MAGL also effectively reduced contextually elicited gaping in rats. The dual FAAH–MAGL inhibitor JZL195 (10 mg/kg, ip) suppressed contextually elicited gaping and elevated AEA, PEA, and OEA (Limebeer et al., 2014). This effect was blocked by SR141716 (but not by AM630) indicating a CB_1 receptor-mediated effect. The suppressive effect of JZL195 on gaping, along with the corresponding elevation of AEA and 2-AG, was augmented by pretreatment with either AEA or 2-AG. Furthermore, AEA alone (but not 2-AG) also suppressed gaping; an effect that was blocked by CB_1 receptor antagonism. These results indicate that JZL195 reduced AN primarily by inhibiting FAAH, but inhibition of MAGL is also indicated. Further work should clarify the interaction between AEA and 2-AG, through the use of dual FAAH and MAGL inhibition, in the suppression of contextually elicited gaping in rats.

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Epigenetics and cognitive disorders

Andrea Stoccoro, Fabio Coppedè, in Epigenetics in Psychiatry (Second Edition), 2021

Markers of DNA methylation in blood DNA: candidate genes

An increase in fatty acid amide hydrolase (*FAAH*) and 5-lipoxygenase (*5-LOX*) gene expression and a reduction of their promoter methylation were observed in PBMCs of AD subjects with respect to age-matched controls [56,57]. The peptidyl-prolyl *cis*/*trans*-isomerase Pin1 acts on both tau and APP to regulate their functions by influencing tau phosphorylation and APP processing; the analysis of PBMCs of AD

subjects and matched controls revealed a statistically significant reduction in promoter methylation, paralleled by a significantly increased Pin1 expression in AD [58]. The analysis of promoter polymorphisms of the *DNMT3B* gene revealed no association with AD risk when the gene was evaluated independently from other factors [83]. However, when a panel of polymorphic genes involved in one-carbon unit transport and metabolism, SAM production, and DNA methylation reactions was evaluated simultaneously with circulating biochemical markers of B-vitamin status (serum folate, vitamin B_{12}, and plasma homocysteine), a combination of those biomarkers allowed discrimination with significant accuracy between AD patients and matched controls [84]. Several other studies investigated gene specific methylation levels in peripheral blood of AD patients. The genes that showed the most consistently significant findings were *BDNF* and *PIN1*. *BDNF* methylation levels have been found to be higher in individuals with AD compared to controls in two studies of Asian populations [85,86], but this difference was not shown in a study of AD patients of Caucasian origin [87]. Interestingly, in a later longitudinal study conducted in 458 MCI individuals, 128 of whom converted to AD within 5 years, it was observed that *BDNF* promoter methylation was able to predict the conversion from MCI to AD [59]. Regarding *PIN1*, two studies found a decrease in methylation levels in association with AD [58,88], although one study did not observe differences between AD and control subjects [89].

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Neuropharmacology of Nicotine

Bernard Le Foll, in Biological Research on Addiction, 2013

Peroxisome Proliferator-Activated Receptors (PPARs)

The inhibition of FAAH increases the levels of several endogenous substances in the brain, including the endocannabinoid anandamide and the noncannabinoid fatty acid ethanolamides oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), which are ligands for alpha type peroxisome proliferator-activated nuclear receptors (PPAR-α). Originally discovered as orphan nuclear receptors in the early 1990s, the PPARs were first discovered to be targets of a group of compounds known as the peroxisome proliferators which are named due to their ability to induce an proliferation in the cellular organelle peroxisomes in rodents. Though eventually discovered not to be involved in the induction of peroxisome proliferation in humans, the name has remained unchanged. There are three PPAR isoforms identified (alpha, delta, and gamma) with all three being transcribed from different genes. PPAR-α, once activated by endogenous or synthetic ligands, heterodimerizes with the retinoid X receptor-α (RXR-α) and undergoes conformational changes. This subsequently allows for the binding to a DNA sequence known as the peroxisome proliferator response element (PPRE), which is located in the promoter region of the target gene. PPAR-α also has nongenomic effects. The primary function of PPAR-α is as a fatty acid sensor, and through the activation of several target genes, it is a major regulator of lipid and lipoprotein metabolism and energy homeostasis. OEA/PEA, which are endogenous PPAR-α agonists, are able to block nicotine addictive properties. The effects of OEA/PEA in this regard were inhibited by MK 886, a PPAR-α antagonist, thereby demonstrating specific PPAR-α involvement. The rapid onset of OEA/PEA effects suggested a

nongenomic mechanism of PPAR-α stimulation of tyrosine kinases. Further work showed PPAR-α agonists through tyrosine kinase-induced phosphorylation of nAChRs containing β2 subunits (β2 nAChR) caused β2 nAChR negative modulation. These effects prevent nicotine from causing excitation of dopamine neurons in the VTA, a process key in its addictive potential. Finally, the experimental PPAR-α agonists, WY 14643 and methyl OEA, have been demonstrated to attenuate both nicotine-taking and nicotine-seeking behavior in both rats and nonhuman primates, but have no effects on food or cocaine-taking. PPAR-α agonists were also able to replicate the ability of OEA/PEA to induce excitation of dopamine neurons in the VTA and increase extracellular dopamine levels in the NAc. All of these results provide a solid body of evidence suggesting that PPAR-α in the brain, may be a potential new pharmacotherapy for the treatment of nicotine addiction. However, to date no study has validated the PPAR-hypothesis of nicotine addiction in humans.

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Effects of Δ9-Tetrahydrocannabinol, Synthetic Cannabinoids, and Fatty Acid Amide Hydrolase Inhibitors on Mood and Serotonin Neurotransmission

Gabriella Gobbi, ... Francis Bambico, in Neuropathology of Drug Addictions and Substance Misuse, 2016

Fatty Acid Amide Hydrolase Inhibition, 5-HT Firing, and Antidepressant-Like Activity

The selective inhibitor of the enzyme FAAH, which catalyzes the intracellular hydrolysis of the endocannabinoid anandamide, has been proposed to be a useful alternative to direct CB1R agonists because of their capacity to increase endogenous CB1R signaling without inducing the typical cannabis-related side effects such as dependence and sedation (see Bambico & Gobbi, 2008 for review).

One of the first experiments carried out in our laboratory was to test whether the FAAH inhibitor URB 597 influences DR 5-HT transmission. We first measured spontaneous activity of 5-HT neurons in the DR of anesthetized rats. Single injections of URB 597 (0.03–0.3 mg/kg, iv) evoked a slow increase in 5-HT neuron firing activity, which was half-maximal at a dose of ≈0.06 mg/kg and was blocked by pretreatment with rimonabant (1 mg/kg, iv; Figure 3). Interestingly, the increase in firing was not as immediate as observed with the CB1R direct agonist, occurring only after 15–20 min; this delay is compatible with the pharmacodynamics of URB 597, which after passing the blood–brain barrier, inhibits FAAH, leading to a gradual accumulation in anandamide content, which subsequently activates CB1Rs (Gobbi et al., 2005).

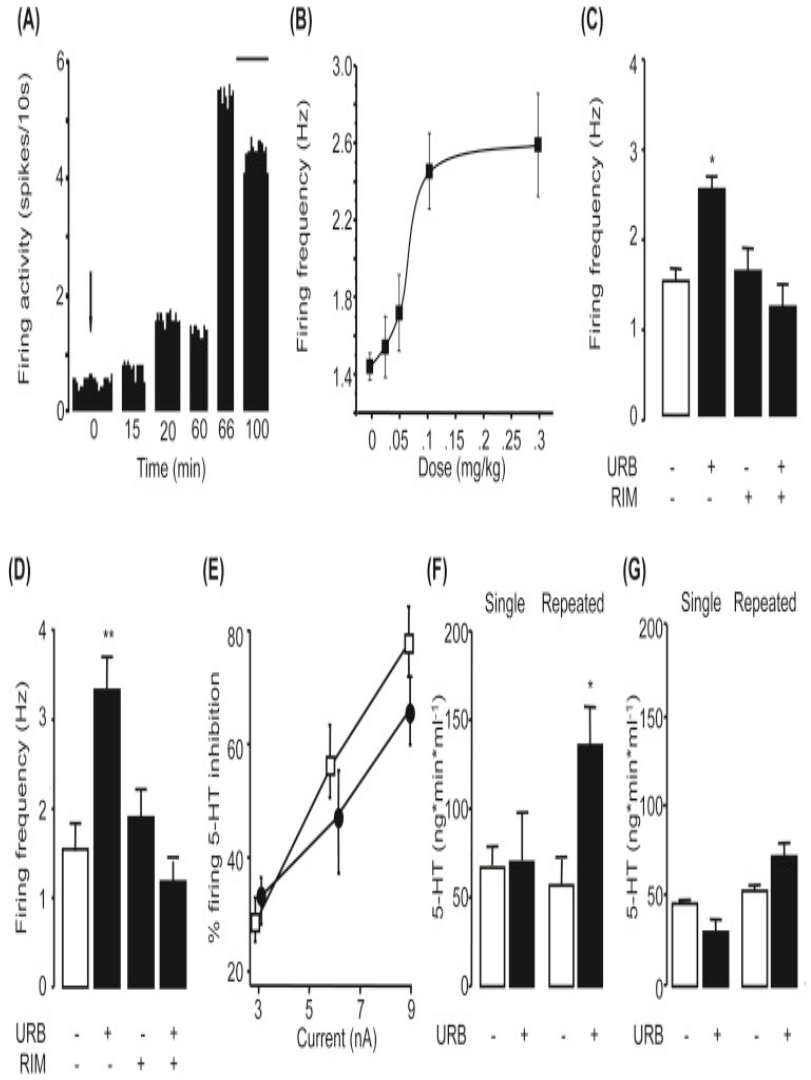

Figure 3. Effects of URB 597 on 5-HT neuron firing in the rat DR. (A) Integrated firing rate histogram of DR neurons, illustrating the time-dependent effects of URB 597; arrow indicates time of URB 597 injection (0.1 mg/kg, iv; calibration bar, 1 min). (B) Dose-dependent effects of URB 597 on spontaneous firing rate. (C and D) Single administration of rimonabant (RIM) (1 mg/kg, iv) prevents the effects of single (0.1 mg/kg) (C) and repeated (D) URB 597 injections (0.1 mg/kg, ip, once daily for 4 days) on 5-HT neuron firing. (E) Repeated URB 597 administration does not affect the response of 5-HT neurons to 8-hydroxy-2-(di-*n*-propylamino)tetralin, expressed as percentage inhibition of 5-HT-neuron firing rate. Open symbols represent vehicle. (F and G) Effects of single or repeated URB 597 injections on 5-HT outflow over 3 h in hippocampus (F) and PFC (G) of awake rats. ∗*p* < 0.05 vs vehicle; ∗∗*p* < 0.01 vs vehicle.

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This response was recapitulated following subchronic URB 597 treatment. Indeed, 4-day treatment with URB 597 (0.1 mg/kg, ip, once daily for 4 days) evoked an even stronger response, which was also reversed by rimonabant (1 mg/kg, ip) (Gobbi et al., 2005). This sustained 5-HT increase after a subchronic treatment regimen was also associated with an increase in 5-HT bursting activity and sustained 5-HT release in the hippocampus, but not in the PFC, as assessed by in vivo microdialysis in awake rats (Gobbi et al., 2005). A single injection of URB 597 had no such effect.

Finally, 4-day treatment with URB 597 did not affect the responsiveness of 5-HT neurons to local iontophoretic administration of the 5-HT1A receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), suggesting that URB 597, unlike classical antidepressants (Artigas, Romero, de Montigny, & Blier, 1996; Gobbi & Blier, 2005), did not produce desensitization of 5-HT1A autoreceptors. Importantly, at the same doses (0.1 and 0.3 mg/kg) and duration (once a day for 4 days), URB 597 induced antidepressant-like effects in the mouse TST and the rat FST. This effect was more robust after repeated injections (4 days) and was reversed by the preadministration of the CB1R antagonist rimonabant (Figure 3; Gobbi et al., 2005).

Since we have previously shown that intra-mPFCv administration of WIN 55,212-2 elicits antidepressant-like responses as well as increases in DR 5-HT firing, we next examined whether *endogenous* medial prefrontocortical cannabinoid signaling modulates coping behaviors in the FST using a combination of behavioral, pharmacological, biochemical, and electrophysiological approaches. We first examined how FST exposure affects endocannabinoid ligand content in the mPFC using mass spectrometry and revealed that anandamide content experiences a rapid and robust decline immediately following the first FST exposure session. Anandamide content was partially (but not fully) restored when examined 24 h later, but was subject to an even greater decline following a second FST exposure session, and this was accompanied by behavioral despair (i.e., increased immobility) (McLaughlin et al., 2012). Thus, fluctuations in anandamide signaling in the mPFC were suspected to mediate the transition between active and passive coping strategies. To support this claim, we next demonstrated that local inhibition of FAAH in the mPFCv with URB 597 reduced the expression of passive, despair-like coping responses in the FST and consequently augmented the expression of a subset of active coping responses (i.e., swimming) that are known to be 5-HT mediated (McLaughlin et al., 2012). The enhancement of swimming cannot be attributed to a general increase in locomotion, since previous reports have demonstrated that URB 597 does not significantly affect basal locomotor activity (Adamczyk, Mccreary, & Filip, 2008). This effect in the FST was blocked by coadministration of the CB1R inverse agonist AM251, as well as by global pharmacological depletion of 5-HT precursors, suggesting that the ability of FAAH inhibition within the mPFCv to promote active coping strategies in the FST is both CB1R dependent and 5-HT mediated. Finally, using in vivo single-unit extracellular recordings, we demonstrated that local inhibition of FAAH within the mPFCv enhanced the firing rate of DR 5-HT neurons on a time course that mirrors the behavioral effects in the FST (McLaughlin et al., 2012). Together, these studies argue that anandamide/CB1R activity in the mPFCv mediates the expression of active coping responses in the FST via an enhancement of DR 5-HT neuronal firing, which is in line with previous findings from our group following intra-mPFCv administration of exogenous CB1R agonists (Bambico et al., 2007).

We were also able to recapitulate these electrophysiological and behavioral findings in FAAH-knockout mice ($FAAH^{-/-}$), in which we observed a marked increase (+34.68%) in DR 5-HT neural firing compared to their littermates, which was reversed by rimonabant (Bambico et al., 2010). This effect was particularly significant in a subset of neurons exhibiting high firing rates (33.15% mean decrease). $FAAH^{-/-}$ mice also showed a resilience profile characterized by reduced immobility in the FST and TST, predictive of antidepressant activity, which again was attenuated by rimonabant administration.

The delay in therapeutic onset of antidepressants has been attributed to gradual

neuroplastic adaptations at the presynaptic and postsynaptic levels that result from the progressive augmentation of 5-HT activity. These modifications include desensitization of autoinhibitory 5-HT1A receptors and sensitization or increased tonic activation of postsynaptic 5-HT1A receptors (Besson et al., 2000; Haddjeri et al., 1998; Szabo & Blier, 2001). The hippocampal pyramidal response to the 5-HT1A receptor antagonist WAY 100635 indicates enhanced tonus on the hippocampal 5-HT1A heteroreceptors, a hallmark of antidepressant-like action. $FAAH^{-/-}$ mice, compared to their wild-type littermates, showed increased tonic activity of 5-HT1A receptors, as tested with ip administration of WAY 100635 (0.5 mg/kg), which potently disinhibited hippocampal pyramidal neural activity (Bambico et al., 2010). Together, these results suggest that genetic deletion or pharmacological inhibition of FAAH (systemically or intra-mPFCv) elicits antidepressant-like effects, paralleled by increased 5-HT transmission and augmented postsynaptic 5-HT1A and 5-HT2A/2C receptor function.