Published in final edited form as:

Biol Psychiatry. 2016 November 1; 80(9): 691–701. doi:10.1016/j.biopsych.2016.04.012.

Fatty Acid Amide Hydrolase Binding in Brain of Cannabis Users: Imaging with the Novel Radiotracer [¹¹C]CURB

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Abstract

Background—One of the major mechanisms for terminating the actions of the endocannabinoid anandamide is hydrolysis by fatty acid amide hydrolase (FAAH) and inhibitors of the enzyme were suggested as potential treatment for human cannabis dependence. However, the status of brain FAAH in cannabis use disorder is unknown.

Methods—Brain FAAH binding was measured with positron emission tomography and [¹¹C]CURB in 22 healthy control subjects and ten chronic, frequent cannabis users during early

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Financial Disclosures

All authors report no biomedical financial interests or potential conflicts of interest.

abstinence. The FAAH genetic polymorphism (rs324420) and blood, urine and hair levels of cannabinoids and metabolites were determined.

Results—In cannabis users FAAH binding was significantly lower by 14–20% across the brain regions examined as compared to matched control subjects (overall Cohen's $d=0.96$). Lower binding was negatively correlated with cannabinoid concentrations in blood and urine and was associated with higher trait impulsiveness.

Conclusions—Lower FAAH binding levels in the brain may be a consequence of chronic and recent cannabis exposure and could contribute to cannabis withdrawal. This effect should be considered in the development of novel treatment strategies for cannabis use disorder that target FAAH and endocannabinoids. Further studies are needed to examine possible changes in FAAH binding during prolonged cannabis abstinence and whether lower FAAH binding predates drug use.

Keywords

Fatty acid amide hydrolase; FAAH; Endocannabinoid; Cannabis use disorder; [^{11}C]CURB; Positron emission tomography

Introduction

Cannabis (with its major pharmacologically active constituent Δ^9 -tetrahydrocannabinol (THC) (1)) is considered to be the most widely abused illicit drug (2). Similar to other psychoactive drugs, use of cannabis is associated with harm to physical and mental health (3). The lifetime prevalence of cannabis use disorder (CUD) in subjects who sampled cannabis once in their lifetime is high (e.g., 32% in females and 47% in males in one recent report (4)) and continued abstinence rates in motivated treatment seekers remain unfortunately low (2/3 relapse) (5). Despite the high demand for treatment, no pharmacotherapy options currently exist for CUD (6).

The discovery that cannabis produces its key effects (e.g., “high”) via activation of human cannabinoid receptors (CB1) prompted the discovery of the endocannabinoid system (eCS). This system consists of endogenous cannabinoid ligands (endocannabinoids) including anandamide (AEA) and 2-arachidonoyl glycerol (2-AG); the receptors mediating the functions including CB1, CB2 and other targets; the biosynthetic enzymes including N-acylphosphatidylethanolamine phospholipase D and diacylglycerol lipase; the AEA reuptake transporter and the metabolizing enzymes including monoacylglycerol lipase and fatty acid amide hydrolase (FAAH) (see ref. (7) for a review). Because of its critical role in adjusting AEA concentrations (8–11), FAAH has been the topic of a growing number of investigation and drug development (12–15) and is being actively investigated as a therapeutic target for CUD (16–18).

A growing body of evidence suggests that (“pathological”) adaptation or inherited differences in the eCS could be involved in features of CUD phenotypes (19–21). One of the most noted and replicated findings concerning adaptation to chronic cannabis exposure is CB1 function desensitization (22), which was reported in preclinical (23, 24), post-mortem human brain (25), and two independent brain imaging studies (19, 20). Although it may be

expected that development of tolerance to cannabinoids is also associated with changes in AEA concentrations and FAAH activities (26–28), few studies addressed this question in THC-dependent animals or humans. In this regard, studies by Leweke and colleagues of human cannabis users reported lower levels of AEA in cerebral spinal fluid (CSF) of frequent versus occasional users and healthy controls (a trend, (29, 30)), which is supported by some preclinical data (31, 32) and suggests that heavy chronic cannabis exposure might be associated with elevated brain FAAH activities (33, 34) and that targeting FAAH might be useful for cannabis withdrawal. Indeed, the FAAH inhibitor URB597 reduced rimonabant-precipitated withdrawal in THC-dependent mice (17). Large-scale genotyping studies showed that the functional variant of FAAH (rs324420, C385A) (35, 36) associated with lower AEA concentrations (the C allele) is related to increased CUD risks (37). Other studies showed that the C allele is associated with increased self-reported positive feeling during a marijuana challenge (38), craving during abstinence (39), cue-induced reward circuit activation (40), and negative mood symptoms linked to frontolimbic white matter abnormalities (41). Of note, we recently showed that the C/C genotype has higher levels of FAAH binding in brain using our positron emission tomography (PET) tracer [^{11}C]CURB (42).

Currently, there are no studies investigating brain FAAH in living humans chronically exposed to cannabis. The recently developed PET tracer [^{11}C]CURB ([^{11}C -carbonyl]-6-hydroxy-[1,10-biphenyl]-3-yl cyclohexylcarbamate (URB694)) (43) is an effective PET radioligand, which binds selectively and irreversibly to FAAH (44). We recently reported that FAAH binding can be estimated reliably and reproducibly (44) using a two-tissue compartment model with irreversible trapping (2-TCMi) that fitted regional time-activity curves, with the composite parameter λk_3 ($\lambda = K_1/k_2$) as the preferred index of FAAH binding (45).

The aim of present study was to use [^{11}C]CURB PET to investigate possible differences in FAAH binding in chronic cannabis users relative to healthy controls. Cannabis users were examined after acute overnight cessation of cannabis use, matching the first time point of the CB1 PET imaging study of Hirvonen (20). We hypothesized that [^{11}C]CURB λk_3 would be higher in brain of cannabis users. Quantitative measurement of cannabinoids and metabolites is highly valuable for understanding and monitoring the presence and extent of cannabis use and withdrawal (46–51). For these reasons, blood and urine samples were collected during the scan for assays of cannabinoids concentrations.

Methods and Materials

Subjects

All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board. Subjects were recruited from the local community in Toronto, Canada using Internet advertisements to participate in a single [^{11}C]CURB PET scan. After provision of written informed consent, subjects completed a comprehensive screening session to rule out past or present significant medical conditions, neurologic illnesses or head trauma, Axis I psychiatric disorders, MR and PET contraindications, use of medications that may affect the central nervous system, or positive drugs of abuse screening except for cannabis in cannabis

users. Scalp hair samples, if available, were taken for forensic drug analyses by LC/MS/MS or GC/MS at United States Drug Testing Laboratories (USDTL, Des Plaines, IL, USA), including cannabinoids [11-nor-9-carboxy-THC (THCCOOH)], cocaine, amphetamines and opiates.

Subjects were asked not to drink caffeinated beverages on the morning of the scan. Cannabis users were required not to use cannabis for 12 hours (overnight) prior to scanning (same requirement for nicotine smoking). PET intake assessments included 1) A breath alcohol concentration (BAC) measurement to ensure abstinence from alcohol (BAC=0 required for scanning); 2) A urine toxicology to rule out medication and illicit drug use (other than cannabis in cannabis users); 3) Urine pregnancy test (female subjects only); 4) Expired carbon monoxide (CO <10 ppm to rule out recent tobacco or cannabis smoking); 5) Craving and withdrawal questionnaires for cannabis users only [Severity of Dependence Scale (SDS), Obsessive Compulsive Smoking Scale (OCSS), Marijuana Craving Questionnaire-Short Form (MCQ) (52), and Marijuana Withdrawal Checklist (MWC) (53)] and the Time Line Follow Back to assess cannabis use over previous 90 days (54); and 6) the Barratt Impulsiveness Scale (BIS) to measure trait impulsivity that has been related to eCS and greater cannabis related problems in cannabis users (as a moderator of this relationship) (55, 56).

Analysis of cannabinoids and metabolites in whole blood and urine

On the day of PET scan for cannabis users, whole blood (in grey top Vacutainer containing potassium oxalate and sodium fluoride) and urine samples were collected twice (T1, upon arrival; T2, before discharge), with the interval approximately 5–6 hours. The samples were transferred to polypropylene cryotubes, frozen on dry ice, stored at –80°C and shipped within three months to Dr. Huestis' laboratory at NIH for quantification of cannabinoids and metabolites' concentrations. THC, 11-hydroxy-THC 11-OH-THC), THC-glucuronide (THC-gluc), THCCOOH, THCCOOH-glucuronide (THCCOOH-gluc), cannabidiol (CBD), and cannabinol (CBN) were quantified in blood by liquid chromatography/tandem mass spectrometry (LC-MS/MS) (46–51, 57), with limits of quantification (LOQ) of 1 µg/L for THC, 11-OH-THC, THCCOOH, CBD and CBN, 0.5 µg/L for THC-gluc, and 5 µg/L for THCCOOH-gluc. In urine, THC, 11-OH-THC, THCCOOH, CBD and CBN were quantified by two-dimensional gas chromatography mass spectrometry (2D-GC-MS) after alkaline hydrolysis, with LOQ of 2.5 µg/L for THC, 11-OH-THC, CBD and CBN and 5 µg/L for THCCOOH.

Image acquisition and reconstruction

[¹¹C]CURB radiosynthesis was described previously (43). PET was performed with a 3D HRRT brain tomograph (CPS/Siemens, Knoxville, TN, USA) (see (45) for details of image acquisition). In brief, after lying down on the scanning table with head held in place with a thermoplastic mask to reduce movement, a short transmission scan was acquired followed by injection of 370±40 MBq (10±1 mCi) (58) of [¹¹C]CURB. Brain radioactivity was measured during sequential frames of increasing duration. Scanning time was 60 min. Images were reconstructed from the 2D sinograms with a 2D filtered-back projection algorithm, with a HANN filter at Nyquist cutoff frequency. After injection, arterial samples

were manually collected from a radial artery at 3, 7, 12, 20, 30, 45 and 60 min and automatically for the first 22.25 min (automatic blood sampling system, Model PBS-101, Veenstra Instruments, The Netherlands). A metabolite corrected plasma curve was generated and used as the input function for the kinetic analysis (details in Rusjan et al. (45)). Blood-to-plasma radioactivity ratios were interpolated by a biexponential function and parent plasma fraction by a Hill function. Subjects underwent standard proton density weighted brain magnetic resonance imaging on a Discovery MR750 3T MRI scanner (General Electric, Milwaukee, WI, USA) for the purpose of region of interest (ROI) delineation and also an arterial spin labeling (ASL) sequence for cerebral blood flow (CBF) measurement (45).

Region of interest kinetic and statistical analysis

Time-activity curves acquired over 60 min (as per Rusjan et al. (45)) in each ROI were extracted using ROMI (details in Rusjan et al. (59)) and analyzed by a two-tissue compartment model with irreversible binding to the second compartment (45). The parameter of interest to quantify FAAH binding is the composite parameter λk_3 ($\lambda = K_1/k_2$). Differences in [^{11}C]CURB λk_3 in each ROI between the two groups was investigated with ANOVAs with ROI as a within subject factor and group as a between subjects factor (SPSS 21.0, SPSS Inc., Chicago, IL, USA). When appropriate, least significant difference *t*-tests, Bonferroni corrected, were applied to determine the significance of regional differences in [^{11}C]CURB binding between groups. Healthy controls (n=22) data were previously published as part of the genetic association study of FAAH rs324420 on [^{11}C]CURB binding (42).

FAAH genotyping

Given the importance of genotype on FAAH (and CURB binding) (42), all subjects were genotyped for the FAAH polymorphism (rs324420) according to published procedures (42).

Results

Subjects

Subjects' demographic information is reported in Table 1. In total 24 controls and 13 actively using cannabis users were recruited for the study. Three potential cannabis users and two controls were excluded or withdrew from the study after screening (for positive drug screens of drugs other than cannabis or relocation). In total 22 controls were matched with ten cannabis users with respect to age and gender. Education tended to be higher in controls and rate of alcohol use per week was non-significantly higher in drug users. Six cannabis users also smoked tobacco (Table 1). Cannabis users endorsed more depressive symptoms (BDI) and greater impulsiveness (BIS) (Table 1). Intake on average was 7.5 ± 4.7 grams cannabis per week and had been for 18 ± 11 years (Table 2). There were no significant differences in [^{11}C]CURB scan parameters between groups, including plasma free fraction of the tracer (Table 1). Groups also were well matched with respect to genotype, such that 70% controls and cannabis users had the C/C allele for FAAH genetic variant rs324420 (Table 1). Our healthy control data also showed that [^{11}C]CURB binding is not influenced by daytime (60) or the season of scan (Supplementary Figure S1).

Decreased [^{11}C]CURB binding in cannabis users during early abstinence

Based on our recent finding that brain binding of the FAAH probe [^{11}C]CURB is dependent on the common genetic polymorphism rs324420 (C385A) (42), genetic variant was used as a covariate in a repeated measure ANOVA (ROI [12] $\times$ Group [2]) investigating differences in λk_3 between controls and cannabis users. This analysis yielded a significant main effect of group ($F(1,29)=6.38$; $p=0.017$) and genotype ($F(1,29)=11.5$; $p=0.002$) and a non-significant ROI $\times$ group interaction ($F(4.8,140)=0.44$; $p=0.82$). The between-group difference indicated lower brain binding in cannabis users relative to controls, which ranged from -20% (amygdala and cingulate) to -14% (hippocampus) (Figure 1; overall Cohen's $d=0.96$). This effect was not accounted for by group differences in use of cigarettes per day ($F(1,28)=5.18$; $p=0.031$), weekly alcohol consumption ($F(1,28)=8.28$; $p=0.008$), or global cerebral blood flow ($F(1,28)=4.91$; $p=0.036$) (as per ASL). Removal of the cannabis user with the only A/A genotype did not change the main effect of group ($F(1,28)=5.25$; $p=0.030$). Indeed, despite overlap, most of the cannabis users (8–9 out of 10) had λk_3 values below the means of the controls (see Figure 1 for scatter plots).

Relationship between [^{11}C]CURB binding and cannabinoids and metabolites in blood and urine and trait impulsiveness

Figure 2 shows cannabinoids and metabolites concentrations in blood and urine at two time points (before and after) on the day of [^{11}C]CURB scan. The blood samples were collected 5.6 ± 0.3 h apart whereas urine samples were 6.0 ± 0.3 h apart. Concentrations of CBD, CBN and THC-gluc in blood and CBD in urine were all below LOQ. For most subjects (9 of 10), blood THC and 11-OH-THC and urine THC concentrations were below or just above the LOQ and did not show much change between time points. Blood THCCOOH ($0\text{--}165.5$ $\mu\text{g/L}$) and THCCOOH-gluc ($0\text{--}665$ $\mu\text{g/L}$) and urine 11-OH-THC ($0\text{--}77$ $\mu\text{g/L}$) and THCCOOH ($14.7\text{--}4721$ $\mu\text{g/L}$) concentrations were variable among subjects and showed only slight changes between time points for most subjects. One of ten subjects had significant blood and urine THC concentrations and had the highest metabolites concentrations that decreased sharply during the 6 h interval. This subject was also the only one with detectable amount of urine CBN at T1 (Fig. 2B), suggesting recent cannabis intake prior to arrival. Hair drug analyses confirmed THCCOOH presence in 8 of 8 cannabis users with scalp hair available; interestingly, THCCOOH concentrations in hair ($0.17\text{--}7.0$ pg/mg) and in blood and urine were significantly positively correlated with each other (Fig. 2C).

We investigated whether low brain [^{11}C]CURB binding in cannabis users correlated with blood and urine THC and metabolites concentrations. Indeed, after excluding the outlier with positive urine CBN at T1, which did not affect the main group effect on [^{11}C]CURB λk_3 ($F(1,28)=5.99$; $p=0.021$), significant negative correlations were observed between THC or metabolites blood concentrations and λk_3 in amygdala (T1: THCCOOH-gluc, $r=-0.782$, $p=0.013$; THCCOOH, $r=-0.744$, $p=0.021$; T2: THCCOOH-gluc, $r=-0.830$, $p=0.006$; THCCOOH, $r=-0.776$, $p=0.014$; THC, $r=-0.824$, $p=0.006$), medial prefrontal cortex (T2: THCCOOH-gluc, $r=-0.681$, $p=0.043$; THC, $r=-0.675$, $p=0.046$) and anterior cingulate (T1: THCCOOH-gluc, $r=-0.702$, $p=0.035$; T2: THCCOOH-gluc, $r=-0.710$, $p=0.032$). As well, urine metabolite concentrations correlated with λk_3 in amygdala (T1: THCCOOH, $r=-0.780$, $p=0.014$; T2: THCCOOH: $r=-0.670$, $p=0.048$) and hippocampus (T1: THCCOOH,

$r=-0.681$, $p=0.043$). Importantly, correlations examined between THC and metabolites and regional λk_3 were all negative with different levels of significance and similar results (not shown) were observed after accounting for C385A genotype (partial correlations). Fig 3 shows examples of correlation plots between amygdala λk_3 and blood concentrations of total THC (THC+11-OH-THC), total THCCOOH (THCCOOH+THCCOOH-gluc), and urine THCCOOH.

No significant correlation was observed between self-reported severity of cannabis intake (years of use, days used, joints/gram per week), craving, and withdrawal symptoms (as per SDS, OCSS, MWQ and MCQ) and λk_3 values in the ROIs investigated, even after accounting for genetic variability (rs324420). However, we found that λk_3 in cannabis users, controlling for rs324420 polymorphism, correlated negatively with impulsiveness (as per Attention factor from BIS, see Table 1) across brain regions ($r=-0.44$ to -0.77 , $p=0.21$ to 0.014), with significant correlations observed in ventral striatum ($r=-0.770$, $p=0.014$; see Fig. 4), caudate nucleus ($r=-0.743$, $p=0.022$), medial prefrontal cortex ($r=-0.711$, $p=0.032$), hippocampus ($r=-0.695$, $p=0.038$), prefrontal cortex ($r=-0.688$, $p=0.040$), and temporal cortex ($r=-0.668$, $p=0.049$).

Discussion

To our knowledge, this is the first report of differences in levels of the endocannabinoid metabolizing enzyme FAAH in living brain of a human with CUD. Contrary to our hypothesis, we found that FAAH binding is significantly lower in brain of chronic cannabis users during early abstinence and correlated with *chronic* and *recent* cannabis use as evidenced by concentrations of cannabinoids and metabolites in blood and urine and with trait impulsiveness, a CUD behavioural phenotype (55, 56).

Our working hypothesis of elevated brain FAAH binding in chronic cannabis users was based on limited, indirect and sometimes inconsistent data on changes in FAAH and endocannabinoids concentrations. For one, studies by Leweke and colleagues showed that CSF AEA concentrations are lower in high versus low frequency cannabis users, suggesting perhaps that frequent cannabis intake could increase brain FAAH activities (29, 30). Secondly, genetic studies showed that the FAAH variant with higher levels of the enzyme (the C allele) is associated with greater CUD risks and cannabis-related problems ((37–41), but see ref. (55)). Echoing these limited findings in humans, preclinical studies showed that mice treated with cannabinoids during adolescence have sustained, markedly increased FAAH protein concentrations in the hippocampus in adulthood (33); as well, THC-tolerant rats have significantly decreased AEA concentrations in the striatum and midbrain although opposite findings were observed in the limbic forebrain and no change was observed in other brain regions including hippocampus, suggesting possible regionally specific FAAH modulation following chronic CB1 stimulation (31, 32).

In our study, rather than the hypothesized elevated FAAH binding, we observed a significant global binding decrease (-14 – 20% ; Cohen's $d=0.96$) across examined brain regions. This finding was not explained by use of other drugs (nicotine, alcohol, stimulants) or by blood flow (ASL). One possible explanation for our unexpected finding is the presence of residual

cannabinoids in brain during the PET scan. It is well known that the highly lipophilic cannabinoids and metabolites have long elimination half-lives in the body due to redistribution and storage in the fat tissue (46–51). For chronic frequent cannabis users as compared to occasional users, low but detectable concentrations of THC, 11-OH-THC and THCCOOH, similar to those observed in our subjects (Fig. 2), can be detected in blood (46, 48, 49) and urine (50) for a prolonged time after the last intake, sometimes as long as 30–33 days for THC and THCCOOH (46), which could be related to significant psychomotor impairment documented three weeks after last use (61). One study suggested that cannabinoids may accumulate in brain for even longer than in blood (62). Currently, there are no experimental data supporting that residual cannabinoids may directly occupy FAAH binding sites for [^{11}C]CURB. In this regard, although CBD, among many constituents of cannabis, was identified as a moderate ($\text{IC}_{50}=15\text{ }\mu\text{M}$) inhibitor of rodents FAAH (63), a recent study suggested that CBD does not inhibit human FAAH at the same concentrations (64). Nevertheless, residual cannabinoids in brain may modulate FAAH indirectly, e.g., through CB1 activation (65) or through disturbance of the structure and fluidity of lipid membrane where FAAH is located and influencing the activity of the enzyme non-specifically. Therefore, we cannot exclude the possible confound of residual cannabinoids at the time of PET scan.

In the current study, we measured cannabinoids and metabolites blood and urine concentrations at two time points (~6 h apart) on the PET day. This was done to disentangle as much as possible new / residual drug exposure from chronic use (47–51). The detection of CBN in one cannabis user at T1 suggests recent cannabis intake (51) despite biochemical verification of smoking abstinence by an expired CO level <10 ppm. This subject also had the highest blood and urine cannabinoids and metabolites' concentrations at both time points. Exclusion of this subject did not influence the major finding of decreased FAAH binding in cannabis users. Interestingly, we found a relationship between cannabinoids and metabolites' concentrations and FAAH binding, suggesting that differences in FAAH binding between-groups could be related to recent and chronic cannabis exposure (46, 48–50), as opposed to CUD *per se*. However, our finding that lower FAAH binding is associated with higher impulsiveness (BIS-attention), a known CUD behavioural phenotype (55, 56), suggests that low FAAH binding may also predate drug use and be involved in CUD development (but see (37)).

A most consistent finding following chronic/repeated cannabinoid exposure in experimental animals and humans is the down-regulation of CB1 receptors in the brain that is reversible after prolonged abstinence (19, 20, 22–25). The possibility of a similar adaptive change in FAAH, an effort to increase AEA in response to low CB1 following chronic cannabis use (and withdrawal) cannot be denied. Cannabis users in our study were scanned at a time matching the baseline scan of Hirvonen CB1 report (20) and had a similar profile of drug use compared to those involved in the two CB1 imaging studies except that daily cannabis intake by our subjects (3 joints) was lower than those in the Hirvonen study (10 joints; (20)) but similar to those in the Ceccarini study (3 joints; (19)) and our subjects were slightly older and had been using cannabis for a longer duration (18 vs 10–12 years). Decreased CB1 density at early withdrawal was related to years of cannabis smoking (20) and possibly levels of cannabis consumption (19). In the present study, decreased FAAH binding was not

correlated with self-reported drug use including years/days of use, joints/gram per week, craving, and withdrawal symptoms, possibly in part because self-report has questionable reliability given widely variable strength of cannabis products and our subjects were scanned during early withdrawal. However, [^{11}C]CURB λk_3 was related to more objective measures of blood and urine cannabinoids and metabolites' concentrations, which reflect chronic and recent drug use (46, 48–50). It thus appears that decreased FAAH binding and CB1 density during early withdrawal from chronic cannabis use might be related even though the regional patterns of change are not consistent. Indeed, a recent study showed that enhanced CB1 activity by a gain of function mutation could lead to decreased FAAH expression in rat brain (65). Future studies assessing FAAH and CB1 in the same cannabis users during early and prolonged abstinence would provide additional insights on regulation of the eCS by chronic cannabis exposure and withdrawal. For example, a dual-tracer PET study can test whether similar to changes in CB1 receptors, the decreased FAAH binding is reversible after prolonged abstinence and whether CB1 and FAAH are co-regulated.

An alternate explanation is that low FAAH binding in cannabis users could be a compensatory adjustment to chronic down-regulation of AEA release (29). On the other hand, lower FAAH binding in cannabis users could reflect a suppression of microglial function or even a loss of microglia. In this regard, FAAH is expressed not only in neurons but also in microglia (66, 67), the innate immune cells in the brain. Exogenous cannabinoids (via CB2) are known to modulate brain microglia activity and have immunosuppressant effects and could induce apoptosis in immune cells (68, 69). In line with our finding, a profound FAAH protein (–80%) and peripheral blood mononuclear cell (T, B and NK) loss was observed after chronic (6–36 months) daily cannabis intake in a group of high school and university students (70).

Limitations of the study include unequal sample size in the case control study, a small sample size for cannabis users, and more tobacco smokers in cannabis users than in controls. Nevertheless, the fact that the finding of our study was opposite to our hypothesis highlights our limited knowledge about FAAH and endocannabinoid regulation, especially in humans. Currently a clinical trial with the Pfizer FAAH inhibitor PF-04457845 (71–73) is on-going for cannabis withdrawal (NCT01618656 at [ClinicalTrials.gov](https://clinicaltrials.gov)). Our recent human blocking study with the Pfizer compound and [^{11}C]CURB PET provided crucial information on the dosing regimen that produces sufficient FAAH inhibition in the human brain (44).

The current finding of decreased FAAH binding, together with literature findings of down-regulated CB1 function (19, 20, 25) and decreased AEA concentrations in CSF of heavy cannabis users (29), suggests that brain endogenous cannabinoid activities could be suppressed by chronic exposure to cannabis, which might underlie at least in part the many cannabis withdrawal effects, given the important roles of endocannabinoids in modulating many neurotransmitter functions and the report that higher CSF AEA levels are associated with lower risks of psychotic symptoms following cannabis use (29). For now, our finding on FAAH status in cannabis withdrawal is still preliminary and no data are available on FAAH changes during the course of cannabis withdrawal and its relationship to endocrine (e.g., glucocorticoids, corticotropin-releasing factor (74, 75)) and neuroinflammation. If decreased FAAH binding represents a compensatory rescuing effort of the brain to maintain

endocannabinoid activity, further inhibition with drugs such as the Pfizer compound might help boost the effect. In this scenario, it might be worth emphasizing that a partial (25–50%) FAAH loss, as evidenced by rs324420 genetics (42) and in contrast to current trial approach aiming at fully shutdown of FAAH (72, 76), is functionally significant in at least some aspects of human behaviors, e.g., mood (77), and treatment development should take this into consideration.

In conclusion, we report for the first time that FAAH binding is decreased in brain of chronic cannabis users during early withdrawal and that this is related to chronic and recent cannabis intake and to trait impulsiveness. This should be considered when developing novel FAAH and endocannabinoid targeting strategies for CUD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported in part by Canada Foundation for Innovation, the Ontario Ministry of Research and Innovation (SH), The National Institute of Health and National Institute of Drug Abuse (NIH/NIDA R21 DA036024 (IB)), CIHR TMH109787 (RFT) and an Endowed Chair in Addictions (Psychiatry Department, RFT).

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Figure 1.

Comparison of brain $[^{11}\text{C}]\text{CURB } \lambda k_3$ values, an index of fatty acid amide hydrolase (FAAH) activity, between chronic cannabis users (CU, $n=10$, triangles) during early abstinence and matched healthy control subjects (HC, $n=22$, circles). FAAH genotypes (rs324420, C385A) of the subjects are indicated (symbols open, C/C; gray, C/A; solid, A/A).

Figure 2.

Cannabinoids and metabolites' concentrations in (A) blood and (B) urine of chronic cannabis users ($n=10$) collected upon arrival (T1) and before discharge (T2) on the day of [^{11}C]CURB PET scan and (C) correlations (Pearson) between THCCOOH hair concentrations ($n=8$) and those in blood and urine. The interval between the two time points T1 and T2 was around 6 h. Dotted lines identify the limit of quantification (LOQ) for each cannabinoid. Note the one outlier in A and B identified by solid symbols, with the arrow highlighting the only one urine sample positive for CBN. Many values were 0 (below LOQ)

for THC (4 and 3), 11-OH-THC (6 and 6), THCCOOH (0 and 1) and THCCOOH-gluc (1 and 1) in blood and THC (8 and 8), 11-OH-THC (2 and 5) and CBN (9 and 10) in urine at T1 and T2, respectively, but all urine samples were positive for THCCOOH (14.7–4720.7 ng/mL). THC, ⁹-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-THC; THCCOOH, 11-nor-9-carboxy-THC; THCCOOH-gluc, THCCOOH-glucuronide; CBN, cannabinol.

Figure 3.

Correlations (Pearson) between amygdala λk_3 values and blood and urine cannabinoids and metabolites concentrations in chronic cannabis users ($n=10$) collected upon arrival (T1) and before discharge (T2) on the day of $[^{11}\text{C}]\text{CURB}$ PET scan. The outlier (arrow) was not included in the correlation analyses. Blood concentrations of total THC+11-hydroxy-THC, i.e., THC total equivalent, and total THCCOOH+THCCOOH-glucuronide, i.e., THCCOOH total equivalent, were used for the correlations after converting the concentrations of 11-

hydroxy-THC and THCCOOH-glucuronide to the equivalent of THC and THCCOOH, respectively. THC, ⁹-tetrahydrocannabinol; THCCOOH, 11-nor-9-carboxy-THC.

Figure 4. Partial correlation, controlling for FAAH genotypes (rs324420, C385A) as indicated, between $[^{11}\text{C}]\text{CURB } \lambda k_3$ values in the ventral striatum and scores of Barratt Impulsiveness Scale-Attention in chronic cannabis users ($n=10$) during early abstinence.

Table1

Demographic and clinical characteristics of healthy controls and cannabis users.

	Controls (n=22)	Cannabis users (n=10)	p	X ²
Gender	11M, 11F	7M, 3F	0.29	1.1
Age (years)	34 (11)	33 (10)	0.85	
Ethnicity	13(W), 7(A), 2(B)	7(W), 3(A)	0.59	1.0
Body Mass Index (BMI)	23.4 (3.0)	24.2 (4.8)	0.58	
Genetics (rs324420, C385A)	14(CC), 8(AC)	7(CC), 2(AC), 1(AA)	0.24	2.8
Education (years)	16.0 (2.1)	14.5 (3.4)	0.14	
Alcohol ever used (n)	18	10	0.16	
Current Alcohol use/Week	1.0 (2.2)	1.9 (2.4)	0.30	
Cigarette smokers (n)	0	6	<0.0001	
Cigarettes/day	0	5 (8)	0.003	
Barratt Impulsiveness Scale (BIS)				
-Attention	8.4 (5.0) [#]	11.7 (1.3)	0.054	
-Motor	15.8 (3.2) [#]	16.3 (1.3)	0.63	
-Self Control	16.4 (1.9) [#]	16.4 (2.2)	0.95	
-Cognitive Impairment	13.2 (3.6) [#]	12.6 (1.3)	0.60	
-Perseveration	11.4 (1.8) [#]	9.2 (1.9)	0.006	
-Cognitive Instability Impulsiveness	7.7 (2.7) [#]	6.4 (2.1)	0.19	
-Attention Impulsivity	14.1 (5.5) [#]	18.1 (2.1)	0.036	
-Motor Impulsivity	25.6 (5.9) [#]	25.5 (2.6)	0.98	
-Non plan	27.8 (2.9) [#]	29.0 (2.6)	0.30	
Marin Apathy Evaluation Scale (AES)	36.2 (21.6) [#]	36.3 (9.8)	0.99	
Becky Depression Inventory (BDI)	1.7 (2.4) [#]	5.4 (4.0)	0.004	
Amount injected (mCi)	9.3 (0.8)	9.3 (0.8)	0.96	
Specific Activity (mCi/μmol)	3270 (1264)	3206 (819)	0.88	
Mass injected (μg)	1.0 (0.5)	0.9 (0.2)	0.53	
Plasma free fraction (%)	0.81 (0.30)	0.70 (0.33)	0.33	

Data in mean (SD).

[#] data compiled using a subset of healthy controls (n=18).

Table 2

Drug use profile of cannabis users (N=10)

	mean (SD)	range (min-max)
Cannabis age of onset (years)	16.1 (3.9)	10–22
Current cannabis use/week (grams)	7.5 (4.7)	3.5–14
Current cannabis use/week (joints)	20.4 (10.2)	2.3–35
Years of cannabis use	17.5 (10.8)	5–33
Days used (last 90 days)	76.4 (15.0)	49–90
Average cannabis use days/week (last 90 days)	5.9 (1.2)	3.8–7
Estimated cannabis use days/Year ^I	305.6 (60.1)	196–360
SDS (Severity of Dependence Scale)	3.0 (2.2)	0–7
OCSS (Obsessive Compulsive Smoking Scale)	14.2 (7.1)	5–25
MWC (Marijuana Withdrawal Checklist)	6.0 (3.9)	1–12
MCQ (Marijuana Craving Questionnaire)		
-Compulsivity	1.4 (0.5)	1–2
-Emotionality	2.9 (1.3)	1–5.3
-Expectancy	4.0 (1.2)	2–6.3
-Purposefulness	4.2 (1.6)	1–6.3
-Total	12.6 (3.9)	5–19.3

^I estimation is based on Time Line Follow Back reported during the last 90 days.