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Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: Impact of cannabis use

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Abstract

Background: Previous studies have shown that cerebrospinal fluid (CSF) from schizophrenic patients contains significantly higher levels of the endogenous cannabinoid anandamide than does CSF from healthy volunteers. Moreover, CSF anandamide levels correlated inversely with psychotic symptoms, suggesting that anandamide release in the central nervous system (CNS) may serve as an adaptive mechanism countering neurotransmitter abnormalities in acute psychoses. In the present study we examined whether cannabis use may alter such a mechanism.
Methods: We used liquid chromatography/mass spectrometry (LC/MS) to measure anandamide levels in serum and CSF from first-episode, antipsychotic-naïve schizophrenics ($n=47$) and healthy volunteers ($n=81$). Based on reported patterns of cannabis use and urine Δ^9-tetrahydrocannabinol (Δ^9-THC) tests, each subject group was further divided into two subgroups: 'low-frequency' and 'high-frequency' cannabis users (lifetime use ≤ 5 times and >20 times, respectively). Serum Δ^9-THC was investigated to determine acute use and three patients were excluded from the analysis due to detectable Δ^9-THC levels in serum.
Results: Schizophrenic low-frequency cannabis users ($n=25$) exhibited >10-fold higher CSF anandamide levels than did schizophrenic high-frequency users ($n=19$, $p=0.008$), healthy low-frequency ($n=55$, $p<0.001$) or high-frequency users ($n=26$, $p<0.001$). In contrast, no significant differences in serum anandamide levels were found among the four subgroups. CSF anandamide levels and disease symptoms were negatively correlated in both user groups.
Conclusions: The results indicate that frequent cannabis exposure may down-regulate anandamide signaling in the CNS of schizophrenic patients, but not of healthy individuals. Thus, our findings suggest that alterations in endocannabinoid signaling might be an important component of the mechanism through which cannabis impacts mental health.

Keywords: Anandamide; Cannabis; Endocannabinoids; First episode; Schizophrenia

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1. Introduction

Cannabis use is highly prevalent among schizophrenic patients (Kovasznay et al., 1997) and is considered a risk factor for development (Andreasson et al., 1987; Arseneault et al., 2002; Henquet et al., 2005; van Os et al., 2002; Zammit et al., 2002) and relapse of psychotic symptoms and schizophrenia in vulnerable subjects (Linszen et al., 1994; Veen et al., 2004). However, the neurobiological mechanism underlying these clinical observations remains largely unknown. Investigations aimed at addressing this question have adopted three distinct approaches. Firstly, clinical pharmacological experiments have highlighted possible similarities between the effects of cannabis' psychoactive principle, Δ^9-THC, and certain symptoms of psychosis. For example, it has been observed that Δ^9-THC may cause perceptual alterations and induce psychotic symptoms and cognitive alterations in healthy individuals that are reminiscent of those observed in prodromal states of psychosis and first-episode schizophrenia (D'Souza et al., 2004; Koethe et al., 2006; Leweke et al., 1999b; Semple et al., 2003). Moreover, it has been demonstrated that administration of Δ^9-THC is associated with transient exacerbation in core psychotic and cognitive deficits in schizophrenic patients (D'Souza et al., 2005). Secondly, neuroanatomical studies have sought to identify alterations in the properties of brain CB_1 cannabinoid receptors, the molecular target of Δ^9-THC (Glass et al., 1997; Herkenham et al., 1990; Matsuda et al., 1993; Piomelli, 2003), associated with schizophrenia. These efforts have led to reveal a significant association between disorganized (hebephrenic) schizophrenia and a cannabinoid CB_1 receptor polymorphism (Ujike et al., 2002) as well as increases in CB_1 receptor densities in the dorsolateral prefrontal cortex (Dean et al., 2001) in *post mortem* brains from schizophrenic patients, while respective findings in anterior cingulate cortex are controversial (Koethe et al., 2007; Zavitsanou et al., 2004). Finally, biochemical analyses have focused on the impact of schizophrenia on serum and CSF levels of endocannabinoid mediators such as anandamide (De Marchi et al., 2003; Giuffrida et al., 2004; Leweke et al., 1999a). These studies concord in suggesting that anandamide signaling may be hyperactive in schizophrenic patients. For example, CSF levels of anandamide were found to be approximately 8-fold higher in first-episode antipsychotic-naïve schizophrenic patients than in healthy controls (Giuffrida et al., 2004).

Despite these advances, no data are available yet on the possible impact of cannabis use on endocannabinoid function in schizophrenia. In the present study we have begun to address this question by determining whether 'high frequency' or 'low frequency' cannabis use (lifetime exposure ≤ 5 times and >20 times, respectively) alters anandamide levels in the CSF of first-episode, antipsychotic-naïve schizophrenics compared with age- and gender-matched healthy volunteers. In addition, psychopathology was correlated to anandamide levels in CSF to prove the relation of our findings to disease specific symptoms.

2. Methods

2.1. Study outline and primary hypothesis

The Ethical committee of the Medical Faculty of the University of Cologne and the Institutional Review Board of the University of California, Irvine, reviewed and approved the protocol of this study and the procedures for sample collection and analysis. All study participants gave their written informed consent and investigations were conducted according to the principles expressed in the Declaration of Helsinki. Psychiatric inpatients and healthy volunteers were enrolled in the study following a protocol designed to test the hypothesis that lifetime frequency of cannabis use alters CSF and serum anandamide levels in patients suffering from schizophrenia-spectrum disorders, when compared to healthy volunteers. CSF and serum anandamide levels were the primary endpoint of the study. The reliability of self-reported, long term, retrospective estimates of cannabis use is subject to certain errors by users (Morral et al., 2003; Weinfurt and Bush, 1996). Thus, we decided to simplify the categorization of frequency of lifetime cannabis use into low frequency (less than 5 times in life) and high frequency (more than 20 times in life for patients and more than 20 but less than 50 times in healthy volunteers) based on the observation of Andreasson et al. (1987) that more than 20 times of cannabis use in life more than doubles the risk to suffer from schizophrenia later on while cannabis use of up to five times in life does not have such an effect.

2.2. Patients

Schizophrenic patients fulfilled pertinent diagnostic criteria, as defined by the IV edition of the Diagnostic and Statistical Manual (DSM-IV) (American Psychiatric Association, 1994). They received lumbar punctures as part of a routine diagnostic procedure recommended by the German Society of Psychiatry, Psychotherapy and Nervous Diseases (Gaebel et al., 2006). Due to detectable Δ^9-THC levels in serum, 3 patients were excluded from the study, resulting in a sample of 44

remaining patients. Thirty-six first-episode (first time clinical diagnosis) antipsychotic-naïve (no known previous or current treatment with antipsychotic medications, though tranquilizers were allowed) schizophrenics met DSM-IV criteria for paranoid schizophrenia (295.30), while eight additional antipsychotic-naïve patients met DSM-IV criteria for schizophreniform psychosis (295.40) due to duration of illness at the time of lumbar puncture. All patients will be referred to as "schizophrenic patients" in the results and discussion section. All patients were caucasians. For demographic details, history of cannabis, current nicotine use, and use of benzodiazepines see Table 1. Cannabis use was quantified retrospectively on admission. No patients fulfilling criteria of cannabis dependence were included in our trial. Moreover, there was no clinical indication for any kind of cannabis withdrawal symptom in our sample. Based on this screening, the patients were grouped into two categories: those who reported to have used cannabis less than 5 times in their life and those who reported to have used cannabis more than 20 times in their life (see Table 1). Within the latter category, 10 patients reported more recent cannabis use (not within the 3 days preceding the lumbar puncture), which was confirmed by urine test on admission. None of the patients included in the final analysis of our study did show CSF or serum detectable levels of Δ^9-THC. Out of 8 patients suffering from schizophreniform psychosis, 6 were low frequency cannabis users with a negative urine drug screening, whereas 2 patients were high frequency cannabis users with a positive urine drug screening for cannabinoids. Beside the slightly higher rate of schizophreniform patients in the low frequency cannabis patients group, there was no indication for differences between groups in terms of duration of untreated psychosis. Trained clinical psychiatrists evaluated ongoing psychotic symptoms in schizophrenic patients on the day of lumbar puncture using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987).

2.3. Control subjects

Healthy subjects ($n=81$) serving as controls were recruited by word of mouth in the premises of the University of Cologne and received a compensation for their participation in the study. They received complete neurological and physical examinations and were screened for psychiatric disorders using the SCID-I and SCID-II clinical interviews for DSM-IV. Subjects taking medications other than oral contraceptives or hormone substitution or resulting positive in a urine test for illicit drugs were excluded from the study. Other exclusion criteria included family history of psychiatric or neurological disorders and previous reported use of illicit drugs other than cannabis. For demographic details, history of cannabis, and current nicotine use see Table 1. Cannabis

Table 1
Basic demographic data of patients and healthy controls

	All ($n=125$)	HC-LFC ($n=55$)	HC-HFC ($n=26$)	SZ-LFC ($n=25$)	SZ-HFC ($n=19$)
Age (years)	28.4 (7.2)	28.8 (±6.6)	26.2 (±3.4)	28.4 (±8.8)	30.3 (±9.8)
Education (years)	14.5 (±2.8)	14.9 (±2.5)	15.9 (±1.0)	14.4 (±2.9)	11.4 (±2.3)
Weight (BMI)	23.1 (±3.6)	23.2 (±3.5)	22.9 (±3.0)	23.1 (±5.0)	22.5 (±3.1)
Gender (male/female)	76/ 49	29/ 26	16/ 10	16/ 9	16/ 3
Current Smoking state (yes/no)	64/ 125	17/ 38	19/ 7	15/ 10	14/ 5
Cannabis use history					
Never		28	–	17	–
Less than 5 times		27	–	8	–
6–20 times		–	–	–	–
21–50 times		–	26	–	1
51–100 times		–	–	–	2
101–500 times		–	–	–	9
>500 times		–	–	–	7
Use of Benzodiazepines					
Lorazepam		–	–	11	11
None		55	26	14	8

HC-LFC = Healthy volunteers with low frequency cannabis use (lifetime use ≤5 times), HC-HFC = Healthy volunteers with high frequency cannabis use (lifetime use >20 times, respectively), SZ-LFC = schizophrenic patients with low frequency cannabis use, SZ-HFC = schizophrenic patients with high frequency cannabis use.

use was quantified retrospectively: 55 subjects reported to have used cannabis no more than 5 times in their life and not to have used it during the 12 months preceding the study. The remaining 26 subjects reported to have used cannabis 20 to 50 times during their life and not to have used it during the 6 weeks preceding the study. All healthy controls were Caucasians. No matching for gender has been applied since no gender specific differences of anandamide levels have been found in a recent study of our laboratories (Giuffrida et al., 2004).

2.4. CSF investigations

CSF samples were collected at approximately 12:00 PM using a non-traumatic lumbar puncture procedure. Samples for analysis were processed, deep-frozen and stored at −80 °C immediately after the lumbar puncture. Routine CSF analyses included total cell count, total protein, CSF/serum albumin and IgG quotients, and determination of oligoclonal bands by isoelectric focusing and silver staining. Extensive virological and microbiological testing of the CSF was also performed. All CSF samples revealed no pathognomonic cell counts, CSF/serum albumin ratios or oligoclonal bands, indicating no pathognomonically disturbed blood-brain barrier function and lack of intrathecal immunoglobulin G synthesis. Anandamide was measured in 1 ml aliquots of CSF samples (total volume, 15–20 ml). The aliquots were spiked with 25 pmol of [$^2H_4$]-anandamide (used as an internal standard) and subjected to acetone precipitation of proteins. The supernatants were collected and their volumes reduced under a stream of nitrogen gas. Lipids were extracted with chloroform/methanol (2:1, vol/vol), and chloroform phases were evaporated to dryness under nitrogen and reconstituted in methanol and chloroform (80 µl total). Anandamide was quantified by isotope dilution LC/MS (Giuffrida et al., 2000) using an HP 1100 Series LC/MS system equipped with an octadecylsilica (ODS) Hypersil column (100×4.6 mm, i.d. 5 µm) (Agilent Technologies). MS analyses were performed with an electrospray ion source as previously described with a detection limit of 0.025 pmol/ml for anandamide (Giuffrida et al., 2004, 2000). Palmitoylethanolamide and oleoylethanolamide were also analysed. As previously reported (Giuffrida et al., 2004), no significant alteration in the CSF levels of either compound was found (data not shown).

2.5. Chemicals

[$^2H_4$]-anandamide was prepared as described (Giuffrida et al., 2000) using as reagents 5,8,11,14-eicosatetraenoylchloride (Nu-Check Prep, Elysian, MN) and [$^2H_4$]-ethanolamine (Cambridge Isotope Laboratories, Andover, MA). All solvents were from Burdick and Jackson (Muskegon, MI).

2.6. Data analysis

To account for apparent non-normality, the distributions of CSF anandamide levels in subject groups were compared for location differences by non-parametric rank tests, *i.e.* the Kruskal–Wallis rank sum test (3 or more groups) and the Wilcoxon rank sum test (2 groups, exact and corrected for ties). For example, the Shapiro–Wilk normality test yielded a p-value smaller than 0.0003 in patients with low cannabis use. The overall error rate of the test family (6 pairwise comparisons) on the primary variable was controlled at level $\alpha = 0.05$ by means of a Bonferroni correction. Thus only raw p-values lower than or equal to 0.0083 were considered statistically significant. Moreover pairwise correlation between variables was assessed by Spearman's Rho (r_S), a rank correlation coefficient. Fisher's z transformation was used to compare any two of them. Statistical analyses were performed using "SPSS" software, SPSS Inc., Illinois, USA and the free software "R", R Foundation for Statistical Computing, Vienna, Austria.

3. Results

CSF anandamide levels were markedly altered in one of the schizophrenic patients subgroups: patients who reported low frequency cannabis use exhibited >10-fold higher CSF anandamide levels than did healthy low-frequency users ($p<0.001$), or healthy high-frequency users ($p<0.001$) as well as schizophrenic high-frequency users ($p=0.008$), (see Fig. 1). Two points are noteworthy, suggesting that frequent exposure to cannabis may not affect anandamide signaling in healthy individuals. Firstly, no difference in CSF anandamide levels was observed between the two healthy subject subgroups. Secondly, the lower anandamide levels in schizophrenic high-frequency users may not be attributed to recent cannabis use. Indeed, the subgroup of schizophrenic high-frequency users who had tested positive for urinary Δ^9-THC (10 out of 19 patients) displayed higher, but not significantly different CSF anandamide levels than did schizophrenic high-frequency users who had tested negative for urinary Δ^9-THC. Confirming and extending a prior study from our labs (Giuffrida et al., 2004), we observed no significant differences in serum anandamide levels among the four

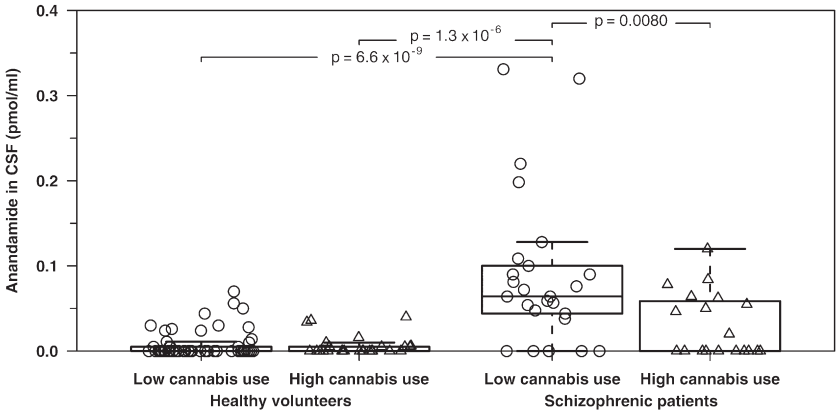

Fig. 1. Box-whiskers-plots (box shows 25th, 50th and 75th percentile of the empirical distribution; whiskers extend to smallest and largest value excluding outliers) of anandamide levels in cerebrospinal fluid (CSF) of schizophrenic patients and healthy volunteers. Left panel, anandamide levels in CSF of healthy volunteers with low-frequency cannabis use (≤5 times in life; column with circles, left panel; $n=55$) or with high-frequency cannabis use (>20 and <50 times in life; column with triangles, left panel; $n=26$). Right panel, anandamide levels in CSF of acute antipsychotic-naïve patients suffering from paranoid schizophrenia or schizophreniform psychosis with ≤5 times of cannabis use in life (column with circles, right panel; $n=25$) or >20 times of cannabis use in life (column with triangles, right panel; $n=19$). Data points were randomly dithered along the horizontal axis. A Kruskal–Wallis rank sum test was performed ($p=8.2\times10^{-8}$) followed by Wilcoxon rank sum tests. *P*-values lower or equal 0.0083 were considered statistical significant (Bonferroni correction for 6 comparisons).

subject subgroups ($p=0.053$; Fig. 2). There was no statistically significant influence of current nicotine smoking on anandamide levels in CSF.

Rank correlation analysis between CSF anandamide levels and disease symptoms as assessed by the PANSS Scores revealed a negative correlation between these two variables in the schizophrenic patient subgroup with low-frequency cannabis use (PANSS Positive: $r_S=-0.459$, $p=0.021$; PANSS General: $r_S=-0.474$, $p=0.017$; $n=25$). Most prominent in the same subgroup was a negative correlation between CSF anandamide levels and negative disease symptoms, as determined by the PANSS Negative Score ($r_S=-0.526$, $p=0.007$; $n=25$; Fig. 3). This particular correlation remained significant after Bonferroni correction ($\alpha=0.0083$). In contrast, for schizophrenic patients with high-frequency cannabis use the respective correlations were weaker (PANSS Positive: $r_S=-0.153$, $p=0.532$; PANSS General: $r_S=-0.360$, $p=0.130$; $n=19$)

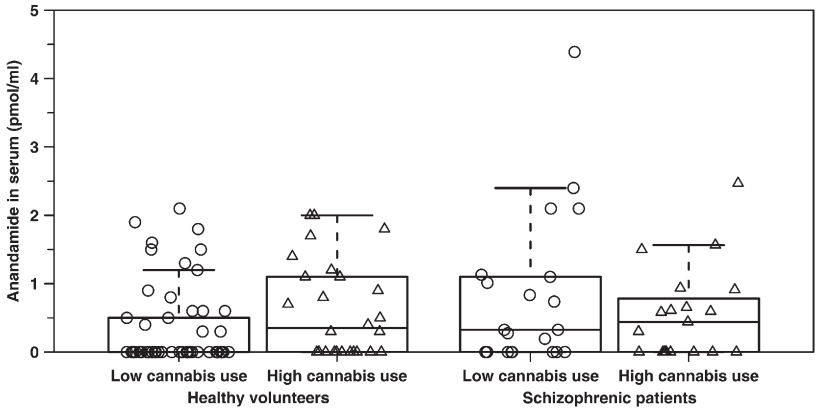

Fig. 2. Box-whiskers-plots (box shows 25th, 50th and 75th percentile of the empirical distribution; whiskers extend to smallest and largest value excluding outliers) of anandamide levels in serum of healthy volunteers with low-frequency cannabis use (≤5 times in life; column with circles, left panel; $n=55$) or with high-frequency cannabis use (>20 and <50 times in life; column with triangles, left panel; $n=26$) as well as of acute antipsychotic-naïve patients suffering from paranoid schizophrenia or schizophreniform psychosis with ≤5 times of cannabis use in life (column with circles, right panel; $n=25$) or >20 times of cannabis use in life (column with triangles, right panel; $n=19$). Data points were randomly dithered along the horizontal axis. A Kruskal–Wallis rank sum test just failed to show a significant difference between groups ($p=0.053$). Thus, for the time being, there is insufficient evidence in support of a relevant trend hypothesis. Note that the *p*-value may require adjustment for multiple testing (*e.g.* Bonferroni).

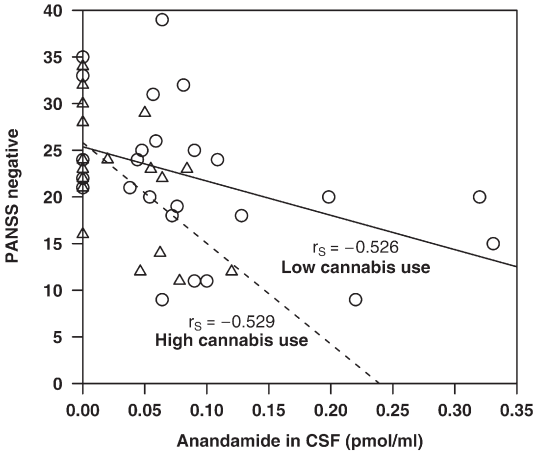

Fig. 3. Scatterplot with least-squares regression lines and rank correlation coefficients of PANSS negative score and anandamide levels (pmol/ml) in CSF for schizophrenic patients reporting low- (circles and line, $r_S = -0.526$, $p = 0.007$; $n = 25$) and high-frequency (triangles and broken line, $r_S = -0.529$, $p = 0.020$; $n = 19$) cannabis use, respectively. Noteworthy, the rank correlation for the high-frequency group excluding patients with more recent cannabis use (positive urine drug screening for cannabis) was much weaker ($r_S = -0.297$, $p = 0.438$; $n = 9$).

or on a comparable level (PANSS Negative: $r_S = -0.529$, $p = 0.020$; $n = 19$), though not reaching statistical significance (Fig. 3). There was also no statistically significant difference between both the correlations in both groups of patients ($p > 0.298$, *via* Fisher's z transformation).

4. Discussion

In a previous report, we have shown that CSF levels of the endocannabinoid anandamide are markedly higher in first-episode antipsychotic-naïve schizophrenic patients than in age-and gender-matched healthy volunteers (Giuffrida et al., 2004). Importantly, in the same patient sample, CSF anandamide levels were found to be negatively correlated with psychotic symptoms (Giuffrida et al., 2004), suggesting that anandamide release in the brain may serve as a compensatory mechanism engaged during acute psychoses. Animal experiments documenting a link between dopamine D_2 receptor activation and anandamide release support this hypothesis. Direct agonists of D_2-like dopamine receptors, such as quinpirole or apomorphine, or indirect dopamine agonists, such as cocaine, stimulate anandamide formation in the dorsal striatum and other basal ganglia structures of the rat brain (Centonze et al., 2004; Ferrer et al., 2003; Giuffrida et al., 1999; Steffens et al., 2004). As acute psychoses may be associated in humans with region-specific alterations in dopamine neurotransmission (Frankle et al., 2003; Laruelle et al., 1999, 2003), it is reasonable to hypothesize that the increased CSF anandamide concentrations observed in first-episode schizophrenics may be a consequence, albeit not necessarily a direct one, of aberrant dopaminergic activity. In agreement with this idea, we found that treatment with 'typical' antipsychotic drugs, which antagonize D_2-type dopamine receptors, normalize CSF anandamide levels in schizophrenic patients (Giuffrida et al., 2004). The possibility that endogenous anandamide may act as a downstream signal regulating dopaminergic transmission is further supported by animal experiments showing that the anandamide reuptake inhibitor AM404 attenuates certain behavioral effects exerted by D_2-type receptor agonists, such as motor hyperactivity, whereas the CB_1 antagonist rimonabant exacerbates such effects (Bortolato et al., 2006; Fegley et al., 2004; Giuffrida et al., 1999).

The hypothesis that anandamide and its attending CB_1 receptors serve an adaptive function in acute schizophrenia is in apparent contrast with the association between cannabis use and precipitation of psychotic episodes, which has been documented by numerous clinical studies (Arseneault et al., 2002, 2004; Henquet et al., 2005; van Os et al., 2002; Veen et al., 2004; Zammit et al., 2002). If the above-stated idea is correct, the question arises as to why CB_1 receptor activation by exogenous Δ^9-THC exerts a deleterious effect in schizophrenics. In our study we found that schizophrenic patients who have consumed cannabis more than 20 times in their life exhibit significantly lower CSF anandamide levels than do schizophrenics who have used the drug 5 times or less. A plausible interpretation of these results is that cannabis use, when it exceeds a certain threshold, may cause a down-regulation of anandamide signaling in the CNS — for example, a decrease in anandamide biosynthesis or an increase in anandamide degradation (see for review Piomelli, 2003). Animal studies showing that repeated treatment with Δ^9-THC reduces anandamide levels in the rat striatum corroborate this possibility (Di Marzo et al., 2000). Interestingly, the lifetime frequency of cannabis exposure associated with lowered CSF anandamide levels, though relatively modest, is generally considered to be a risk factor for the development of psychotic symptoms (Henquet et al., 2005) and schizophrenia (Arseneault et al., 2004) in susceptible individuals. However, similar levels of cannabis use do not modify CSF anandamide concentration in healthy volunteers, suggesting that cannabis use may down-regulate anandamide signaling only when the latter is pathologically hyperactive.

In addition to modulating anandamide release, cannabis exposure may affect other components of the

endocannabinoid signaling system. Thus, cannabis use in schizophrenic patients has been linked to increased CB_1 receptor densities in the caudate putamen, as assessed by *in situ* binding experiments with the radioactively labeled cannabinoid agonist [^{3}H]CP-55940 (Dean et al., 2001). This finding is difficult to reconcile with available animal studies, which show that repeated administration of Δ^9-THC causes a loss of CB_1 receptor function (Breivogel et al., 1999), but is consistent with the present results. Indeed, the down-regulation in brain anandamide signaling suggested by our study is expected to be accompanied by a compensatory increase in CB_1 receptor density in certain brain areas (Dean et al., 2001).

In conclusion, evidence indicates that cannabis use may facilitate the precipitation of psychotic episodes in susceptible individuals, but the neurobiological bases for this phenomenon remain elusive. Our results, showing that frequent cannabis use in schizophrenic patients may be associated with reduced CSF anandamide levels, suggest that alterations in anandamide signaling secondary to cannabis exposure must be taken into consideration when constructing models of the impact of cannabis on human health.

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Contributors

Author Leweke designed the study, wrote the protocol, raised funding, contributed to the collection of data and its analysis. Author Giuffrida developed the method and contributed to the collection of the data. Authors Koethe, Schreiber, Nolden, Kranaster, Neatby and Gerth contributed substantially to the collection of data. Authors Koethe and Schreiber contributed to the analysis of the data and managed the literature searches and analyses. Author Hellmich undertook the statistical analysis, and author Leweke wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript

Conflict of interest

All other authors declare that they have no conflicts of interest.

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