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Elevated endogenous cannabinoids in schizophrenia

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EVIDENCE suggests that cannabinoid receptors, the pharmacologcial target of cannabis-derived drugs, and their accompanying system of endogenous activators may be dysfunctional in schizophrenia. To test this hypothesis, we examined whether endogenous cannabinoid concentrations in cerebrospinal fluid of schizophrenic patients are altered compared to non-schizophrenic controls. Endogenous cannabinoids were purified from cerebrospinal fluid of 10 patients with schizophrenia and 11 non-schizophrenic controls by high-performance liquid chromatography, and quantified by isotope dilution gas-chromatography/mass-spectrometry. Cerebrospinal concentrations of two endogenous cannabinoids (anandamide and palmitylethanolamide) were significantly higher in schizophrenic patients than non-schizophrenic controls ($p < 0.05$). By contrast, levels of 2-arachidonylglycerol, another endogenous cannabinoid lipid, were below detection in both groups. The findings did not seem attributable to gender, age or medication. Elevated anandamide and palmitylethanolamide levels in cerebrospinal fluid of schizophrenic patients may reflect an imbalance in endogenous cannabinoid signaling, which may contribute to the pathogenesis of schizophrenia. *NeuroReport* 10:1665–1669 © 1999 Lippincott Williams & Wilkins.

Key words: Anandamide; palmitylethanolamide; 2-arachidonylglycerol; cannabinoid receptors; cerebrospinal fluid; gas chromatography/mass spectrometry

Introduction

Progress in the treatment of schizophrenia has been hindered by an inadequate understanding of the pathogenesis of this disease and of the mechanisms by which drugs that block D_2-family dopamine receptors mitigate its symptoms. Although dopamine neurotransmission is thought to be abnormal in schizophrenia, D_2-blocking drugs are only partially effective in preventing its diverse manifestations [1]. Thus alterations in other brain signaling pathways have been postulated, and hypotheses on the causation of schizophrenia extended to include interactions among multiple neurotransmitter systems in addition to dopamine (for review see [2]). However, despite the urgent need for improved antipsychotic therapies, the nature of these transmitter interactions is still largely unknown. Several lines of evidence suggest that schizophrenia may be associated with anomalies in the function of cannabinoid receptors and their attendant system of endogenous activators. Cannabinoid receptors are the pharmacological target of marijuana and hashish, cannabis-derived drugs that contain Δ^9-tetrahydrocannabinol (Δ^9-THC) (for review see [3]). Prevalence of cannabis consumption is significantly higher among schizophrenics than normal individuals [4], and prolonged abuse of large quantities of cannabis may trigger relapse of psychotic symptoms in schizophrenic patients [5,6]. These phenomena, though unexplained at the molecular level, are consistent with the neuroanatomical distribution of cannabinoid receptors. Indeed some of the highest densities of these receptors are found in regions of the human brain that have been implicated in schizophrenia, including prefrontal cortex, basal ganglia, hippocampus and anterior cingulate cortex [7]. Similarities between certain cognitive impairments occurring in psychoses and the pharmacological effects of Δ^9-THC have also been documented [8,9].

Additional support for a role of cannabinoid signaling in schizophrenia comes from the existence of functional interactions between dopamine and anandamide (arachidonylethanolamide), an endogenous cannabinoid compound [10,11]. Microdialysis experiments have shown that anandamide release in rat dorsal striatum is dramatically stimulated by activation of D_2-family, but not D_1-family dopamine receptors [12]. The physiological significance of this effect is still unresolved, but behavioral

experiments indicate that anandamide may act as a local modulatory signal to offset dopamine-induced psychomotor activation [12]. Along with anandamide, brain neurons produce two additional lipids that have been involved in cannabinoid signaling: 2-arachidonylglycerol (2-AG) [13–15] and palmitylethanolamide (PEA) [16]. Neither compound appears to be released by D_2-family receptor activation *in vivo*, however, pointing to a specific role for anandamide in this response [12].

To reveal possible abnormalities of endogenous cannabinoid signaling in schizophrenia, we have measured endogenous cannabinoid levels in cerebrospinal fluid (CSF) of schizophrenic patients and non-schizophrenic controls by using a combination of high-performance liquid chromatography (HPLC) and gas-chromatography/mass spectrometry (GC/MS) techniques, which provide the high degree of sensitivity and selectivity needed to accomplish this task.

Materials and Methods

Patients and controls: Ten patients with acute schizophreniform psychotic symptoms were included in our study. Nine patients fulfilled the diagnostic criteria from DSM-IV [17] for schizophrenia and one for schizophreniform psychosis (due to limited observation time). Patient characteristics are summarized in Table 1. Four patients had a history of intermittent consumption of cannabis resin and/or alcohol, but did not match the diagnostic criteria for acute intoxication, drug dependence or withdrawal syndrome. Schizophrenic symptoms lasted at least 4 months in all patients. Psychopathological disturbance was additionally quantified using the seven step Brief Psychiatric Rating Scale (BPRS) [18]. BPRS scores reflecting the psychopathological condition at the time of lumbar puncture are provided in Table 1. All CSF samples were collected for diagnostic purposes. Patients were informed that the rest of the samples would be stored and used for further research investigations. The mean age of the patients (seven male, three female) at the time of examination was 27.7 years (s.d. 9.6). Control subjects were a group of 11 age-matched subjects free of diagnosable psychopathology according to DSM-IV criteria. CSF was obtained for neurological differential diagnoses, revealing no significant pathological findings. All patients and subjects gave informed consent according to the Declaration of Helsinki.

CSF investigations: Lumbar punctures were done around noon, with the subjects in lying position and the needle inserted in the lumbar 4–5 interspace. An

Table 1. Clinical characteristics of patients participating in the study

No.	Age	Sex	DSM-IV	Man.	Neuroleptics at time of lumbar puncture	Benzodiazepines at time of lumbar puncture	Substance abuse[a]	AEA (pmol/ml)	PEA (pmol/ml)	OEA (pmol/ml)	BPRS (total)
1	22	m	295.90	1st	Never	Lorazepam 6 mg/day	No	0.0	1.9	0.4	52
2	32	f	295.30	2nd	Perazine 150 mg/day	Oxazepam 50 mg/day	No	0.7	1.5	0.7	49
3	19	m	295.10	1st	None for 7 days	Lorazepam 8 mg/day	No	1.1	5.0	1.3	71
4	28	m	295.30	1st	Never	Diazepam 10 mg/day	No	0.5	6.1	1.4	58
5	19	f	295.40	1st	Never	Lorazepam 2 mg/day	No	0.4	1.6	0.2	73
6	25	m	295.30	2nd	Perazine 400 mg/day	None	Cannabis up to 0.5 g/day; Alcohol up to 60 g/day	1.0	5.3	1.0	53
7	23	m	295.30	1st	None for 7 days	Lorazepam 8 mg/day	Cannabis up to 1.0 g/day	0.2	1.6	0.2	67
8	38	m	295.30	1st	Never	None	Alcohol up to 180 g/day	1.6	4.2	1.1	61
9	49	f	295.20	2nd	Never	None	No	1.0	4.3	1.1	70
10	22	m	295.30	1st	Risperidone 4 mg/day	None	Cannabis up to 1.5 g/day	0.6	3.5	0.8	70

AEA, anandamide; PEA, palmitylethanolamide; OEA, oleylethanolamide.
Man. = Manifestation of the disease.

atraumatic Sprotte needle was used to collect 15–30 ml CSF. Routine CSF investigations were performed according to a recent European consent [19], including total cell count, total protein, measurement of the concentrations of albumin and IgG in CSF and serum by kinetic nephelometry, and determination of oligoclonal bands by isoelectric focusing and silver staining. An extensive virological and microbiological testing of the CSF was also performed. Samples were stored at −80°C for various lengths of time (4 years to 2 months) before analysis.

Endogenous cannabinoid analyses: [$^2H_4$]anandamide, [$^2H_4$]PEA and [$^2H_4$]oleylethanolamide were synthesized by the reaction of the corresponding fatty acyl chlorides with [$^2H_4$]ethanolamine, as described previously [20]. [$^2H_8$]-2-AG was custom-synthesized at Deva Biotech (Hartboro, PA). These standards were added to CSF samples (1.2 nmol in 15 ml) to improve recovery and allow for quantitative measurements. The CSF samples were divided into three aliquots of 5 ml, subjected to acetone precipitation of proteins and centrifuged for 10 min at room temperature ($800 \times g$). The supernatants were collected and their volumes reduced under a stream of N_2. Lipids were extracted with chloroform/methanol (2:1, vol/vol). Chloroform phases were evaporated to dryness, reconstituted in chloroform (150 µl) and fractionated by normal-phase HPLC [20]. Analytes of interest were collected in glass reaction vessels, brought to dryness and converted to trimethylsilylethers by treatment with BSTFA (30 min at room temperature). Details on the isotope dilution GC/MS methods are provided elsewhere [20]. Concentrations of analytes in CSF samples are expressed as pmol/ml and were calculated by averaging three separate determinations for each 5 ml sample. Statistical significance was determined by Student's *t*-test. Sample storage did not affect analysis, as no significant differences in endogenous cannabinoid concentrations were found between two groups of non-schizophrenic CSF samples kept at −80°C for 4 years ($n = 5$) or 2 months ($n = 5$) (data not shown).

Results

CSF investigations: Normal cell counts were found for all subjects except for one schizophrenic with a marginal elevation to 6.0 cells/µl, and one control with 6.3 cells/µl. The CSF/serum albumin quotient, which is considered a measure of the blood–CSF barrier function, was also normal (< 7.4), apart from one control with a slight increase to 8.6. The IgG index was always below 0.700 and no oligoclonal bands were found in either group by isoelectric focusing, indicating the absence of intrathecal IgG synthesis. Extensive virological and microbiological testing gave negative results.

Endogenous cannabinoid analyses: Figure 1A illustrates the electron-impact mass spectrum of the trimethylsilylether derivative of synthetic anandamide. The most abundant fragment observed in the high-mass range of the spectrum has a mass/charge ratio (m/z) of 404 ($[M\text{-}15]^+$), corresponding to the loss of one methyl radical from the molecular ion ($[M]^+$). Analogous fragmentation patterns are seen with synthetic PEA, which yields an $[M\text{-}15]^+$ ion of m/z 356, and oleylethanolamide, which yields an $[M\text{-}15]^+$ ion of m/z 382 (data not shown). Additional properties of these spectra are discussed elsewhere [21].

We fractionated CSF samples of schizophrenic patients and non-schizophrenic controls by HPLC and analyzed them by GC/MS for the presence of $[M\text{-}15]^+$ fragments eluting at the retention times

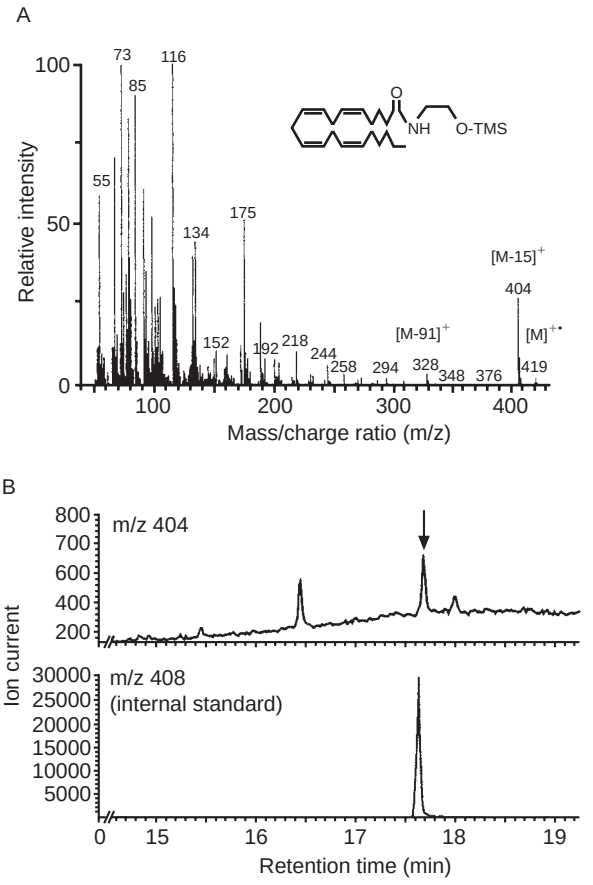

FIG. 1. Identification of anandamide in human CSF by GC/MS. (**A**) Electron-impact mass spectrum of synthetic anandamide, analyzed as trimethylsilylether (TMS). The chemical structure of anandamide-TMS is also shown. (**B**) Selective ion monitoring tracings of a representative sample from the CSF of a schizophrenic patient. Upper panel: native anandamide (fragment of mass/charge ratio m/z 404); Lower panel: synthetic [$^2H_4$]anandamide (m/z 408). The arrow indicates the retention time of authentic anandamide.

expected for anandamide and other fatty acylethanolamides. As shown in Fig. 1B, an m/z 404 fragment co-eluted with authentic anandamide, while m/z 356 and m/z 382 fragments co-eluted with PEA and oleylethanolamide, respectively (data not shown). These results indicate that anandamide and other fatty acylethanolamides are present in human CSF.

We quantified these lipid compounds using an isotope dilution method [21]. Deuterium-containing acylethanolamide standards were included in CSF samples and both native and deuterated $[M\text{-}15]^+$ ions were monitored by GC/MS (Fig. 1B). CSF concentrations were calculated from the native-to-deuterated ion ratio by using calibration curves generated with synthetic standards. In schizophrenic patients, average anandamide and PEA levels were ~2-fold higher than in controls (Fig. 2A,B; $p < 0.05$, Student's *t*-test). Eleven control subjects had anandamide levels of 0.30 ± 0.29 pmol/ml and PEA levels of 1.80 ± 0.83 pmol/ml (mean ± s.d.). Levels in 10 schizophrenic patients were 0.70 ± 0.47 pmol/ml and 3.50 ± 1.73 pmol/ml, respectively. By contrast, no difference was observed between the two groups in the levels of oleylethanolamide, an acylethanolamide that does not activate central or peripheral cannabinoid receptors (controls 0.90 ± 0.42 pmol/ml; schizophrenics 0.80 ± 0.40 pmol/ml; Fig. 2C). In this limited patient sample, we found no obvious correlation between endogenous cannabinoid levels and age, gender, medication or drug abuse (Table 1). The limited number of schizophrenic patients examined prevented the identification of possible clusters of individuals differing in endogenous cannabinoid levels.

We also attempted to determine CSF levels of 2-AG, an endogenous cannabinoid that has been implicated in hippocampal neurotransmission [13–15]. We monitored a diagnostic $[M\text{-}90]^+$ fragment (m/z 432) and its deuterated counterpart (m/z 440) produced by standard $[^2H_8]$-2-AG. Although the limit of detection for 2-AG (1 pmol) was similar to that for anandamide (0.4 pmol) and the recoveries of internal standard were generally satisfactory, we could not detect significant levels of the m/z 432 fragment in any of the samples analyzed (data not shown). These results are consonant with the lack of 2-AG release observed in rat striatum by microdialysis [12] and suggest that 2-AG concentrations are exceedingly low in CSF of both normal and schizophrenic subjects.

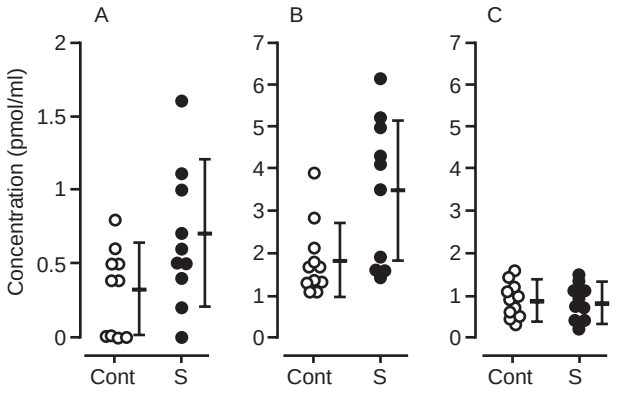

FIG. 2. Concentrations of anandamide (**A**), palmitylethanolamide (**B**) and oleylethanolamide (**C**) in CSF of schizophrenic (S) and non-schizophrenic (Cont) subjects. Bars indicate mean ± s.d. for each group (A and B, $p < 0.05$; Student's *t*-test).

Discussion

We have found that CSF concentrations of anandamide and PEA, two lipid compounds that have been implicated in different aspects of endogenous cannabinoid signaling, are markedly elevated in individuals with schizophrenia compared with controls.

Since anandamide is released in rat brain by activation of D_2-family dopamine receptors [12], our findings may reflect a homeostatic adaptation of the endogenous cannabinoid system to neurotransmitter imbalances that involve dopamine. Such imbalances have been postulated to occur in schizophrenia [1,2,22]. Alternatively, increased anandamide concentrations in CSF may reflect a primary 'hypercannabinergic' state, which may occur in schizophrenia or in a subgroup of schizophrenic syndromes [8,9].

Although anandamide and PEA are produced in brain through a common biochemical mechanism [11], PEA does not bind to CB1 cannabinoid receptors [10] and it is not released *in vivo* by neural activity or D_2-receptor occupation [12]. Pharmacological evidence indicates, however, that PEA may reduce excitotoxicity in cerebellum and activate CB2-like receptors in peripheral tissues [23]. Our findings, showing elevated PEA levels in schizophrenia, emphasize the need to investigate further the biochemical and pharmacological properties of this putative signaling molecule.

There are several limitations and possible confounders in our study. First, like other phospholipid metabolites, anandamide and other acylethanolamides accumulate post mortem in the central nervous system [24]. Although this phenomenon may confuse quantitative analyses in autopsy tissues, it is unlikely to account for the differences observed in CSF of schizophrenic and control subjects. In agreement with this conclusion, we found that the levels of 2-AG and oleylethanolamide, which are also increased following ischemia [24,25], are not changed in schizophrenia. Second, after release from neurons, anandamide is rapidly inactivated by high-affinity uptake and enzymatic hydrolysis [11,26–28].

PEA may also be a substrate for hydrolase enzymes [26]. Thus differences in acylethanolamide levels in CSF may reflect changes in either release or inactivation of these compounds. Third, the neuronal origin of the acylethanolamides found in CSF remains conjectural. It is important to point out, however, that the predominant cannabinoid compound released by vascular cells is 2-AG [29–31], which was undetectable in CSF. Finally, although the apparent lack of correlation between antipsychotic drug exposure and acylethanolamide levels suggests that medication does not account for our observations, further studies will be needed to establish this point on a firmer basis.

Conclusion

CSF concentrations of anandamide and PEA are significantly elevated in schizophrenic patients compared with non-schizophrenic controls, suggesting that functional abnormalities in endogenous cannabinoid signaling may participate in the pathogenesis of this disease. Confirmation of our results in a larger and more diverse patient population will be instrumental not only to construct specific hypotheses on the possible roles of the endogenous cannabinoid system in schizophrenia, but also to suggest novel therapeutic interventions for the treatment of this disease.

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