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Cannabinoids Influence Lipid–Arachidonic Acid Pathways in Schizophrenia

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Increasing evidence suggests modulating effects of cannabinoids on time of onset, severity, and outcome of schizophrenia. Efforts to discover the underlying pathomechanism have led to the assumption of gene × environment interactions, including premorbid genetical vulnerability and worsening effects of continuing cannabis use. The objective of this cross-sectional study is to investigate the relationship between delta-9-tetrahydrocannabinol intake and niacin sensitivity in schizophrenia patients and healthy controls. Intensity of niacin skin flushing, indicating disturbed prostaglandin-mediated processes, was used as peripheral marker of lipid–arachidonic acid pathways and investigated in cannabis-consuming and nonconsuming schizophrenia patients and in healthy controls. Methylnicotinate was applied in three concentrations onto the forearm skin. Flush response was assessed in 3-min intervals over 15 min using optical reflection spectroscopy. In controls, skin flushing was significantly decreased in cannabis-consuming as compared to nonconsuming individuals. When comparing the nonconsuming subgroups, patients showed significantly decreased flush response. The populations as a whole (patients and controls) showed an inverse association between skin flushing and sum scores of Symptom Check List 90-R. Results demonstrate an impact of long-term cannabis use on lipid–arachidonic acid pathways. Considering pre-existing vulnerability of lipid metabolism in schizophrenia, observed effects of cannabis use support the notion of a gene × environment interaction.
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INTRODUCTION

There is a high prevalence of substance misuse in people suffering first-episode psychosis (Cantwell *et al*, 1999; Hambrecht and Hafner, 2000; Sevy *et al*, 2001). Cannabis is by far the most often used substance in people developing psychosis (Duke *et al*, 2001; Menezes *et al*, 1996). The Swedish long-term follow-up study by Andreasson and coworkers (Andreasson *et al*, 1989; Zammit *et al*, 2002) reported a six-fold increased risk for developing psychosis after using cannabis 50 times or more. Two recent meta-analyses concluded that cannabis use confers an overall two-fold increase in the relative risk for later schizophrenia, depending on age of onset and frequency of cannabis use (Arseneault *et al*, 2004; Henquet *et al*, 2005a). If schizophrenia occurs, cannabis-using patients seem to be prone to develop more severe positive symptoms, to suffer a more continuous course of disorder and more frequent relapses (Caspari, 1999; Cleghorn *et al*, 1991; D'Souza *et al*, 2005; Grech *et al*, 2005; Johns, 2001).

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However, still the vast majority of cannabis users do not develop psychosis causing a controversial debate about the importance of cannabis as risk factor for developing psychosis, its influence on symptom presentation, treatment response, and long-term outcome. It was postulated that some people must be genetically vulnerable to the deleterious effects of cannabis, whereas others are not (McGuire *et al*, 1995; Verdoux *et al*, 2003; Arseneault *et al*, 2004; Zammit and Lewis, 2004; Henquet *et al*, 2005b; Caspi *et al*, 2005; Rosa *et al*, 2006).

The main psychoactive compound of herbal cannabis preparations, delta-9-tetrahydrocannabinol (Δ^9-THC), displays an extraordinary affinity to biological membranes (Dingell *et al*, 1973; Seeman *et al*, 1972), causing its ability to interact directly with membrane lipids (Kalofoutis and Koutselinis, 1979, 1980; Kalofoutis *et al*, 1978). This feature of Δ^9-THC is crucial, as first-episode schizophrenia patients (Fukuzako, 2001; Keshavan *et al*, 2000; Stanley *et al*, 2000) and nonaffected twin siblings and offsprings of schizophrenia patients (Klemm *et al*, 2001) show alterations of cerebral phospholipid metabolites, suggesting abnormalities of lipid metabolism as part of a genetic vulnerability to develop psychosis. If Δ^9-THC has the potential to interact with a pre-existing vulnerability of cerebral lipid metabolism, this could represent a biochemical basis for gene × environment interaction.

Evidence of disturbed lipid metabolism in schizophrenia is based on a variety of findings, including altered cerebral phospholipids (Fukuzako, 2001; Keshavan *et al*, 2000; Stanley *et al*, 2000), upregulation of phospholipase A_2 (PLA_2) activity (Gattaz *et al*, 1987, 1990, 1995; Ross *et al*, 1997; Smesny *et al*, 2005a), diminished arachidonic acid levels (Berger *et al*, 2002, 2003; Fenton *et al*, 2000; Reddy *et al*, 2004; Reddy and Yao, 2003), and impaired function of bioactive lipids (eg prostaglandins) (Smesny, 2004b; Ward, 2000). Dysfunction of prostaglandin-mediated processes—measured as attenuation of niacin skin flushing—was associated with cerebral lipid metabolites (Puri *et al*, 2006), increased PLA_2 activity (Tavares *et al*, 2003), and decreased arachidonic acid levels (Glen *et al*, 1996; Messamore, 2003) and was also reported in nonaffected parents of schizophrenia patients (Waldo, 1999). Therefore, and owing to its easy bedside applicability, the niacin patch test was introduced as clinical test of a schizophrenia endophenotype characterized by abnormal lipid biochemistry, and as clinical test to assess the respective genetical vulnerability in risk populations (Ward, 2000; Gottesman and Gould, 2003).

We assume that the ability of Δ^9-THC to influence onset, severity, and long-term course of psychosis might be related to its lipophilic behavior and its potential to interact with a pre-existing vulnerability of lipid metabolism. To investigate metabolic effects of Δ^9-THC, we applied niacin patch tests to groups of neuroleptic-treated cannabis-consuming and nonconsuming schizophrenia patients and consuming and nonconsuming healthy controls.

PATIENTS AND METHODS

Subjects

Niacin skin tests were performed on 64 acutely ill consecutively admitted schizophrenia patients having suffered not more than two psychotic episodes and not overlapping with the sample investigated in our previous studies (Smesny *et al*, 2003, 2005b). All met DSM-IV criteria for paranoid schizophrenia. Diagnosis was made by two independent experienced psychiatrists (SS and HS) and further supported by structured clinical interview (SCID IV) (Wittchen *et al*, 1997). Majority of patients were treated mostly with atypical neuroleptic drugs (demographic data in Table 1). The patient population was subdivided into one group having used cannabis on a regular basis ($\geqslant$0.5 g/day, $\geqslant$3 month) before admission (consuming patients (cp), $n=35$), and another group (nonconsuming patients (ncp), $n=29$) having never used cannabis apart from unique trials. Cannabis-consuming patients did not use any other drug or alcohol on a regular basis. Psychiatric symptoms were assessed using Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962), Scale of Assessment of Positive Symptoms (SAPS) (Andreasen, 1984), Scale of Assessment of Negative Symptoms (SANS) (Andreasen, 1983), and Symptom Check List 1990 Revised (SCL 90-R) (Kaplan *et al*, 1998) (mean scores in Table 1).

Patients were compared to 53 healthy volunteers recruited by newspaper advertisement including one group of cannabis users (consuming controls (cc), $n=22$, duration and dose of cannabis use as in patients) and one group without any cannabis experience (nonconsuming controls (ncc), $n=31$). Controls were interviewed in depth to rule out a current psychiatric diagnosis or personal or family psychiatric history. As in patients, SCL 90-R was also applied in controls.

Table 1 Demographics of Schizophrenia Patients and Control Subjects

	cp	cc	ncp	ncc
Gender ♀/♂	5/30	3/19	14/15	11/20
Age (±SD)	21.6 (3.1)	24.1 (4.4)	28.8 (9.03)	27.9 (7.6)
Nicotine use	32 (91.4%)	22 (100%)	23 (79.3%)	3 (10%)
Cannabis use	100%	100%	0%	0%
Antipsychotic medication (*n*)				
Neuroleptic-naive	2	∅	5	∅
Atypic NL	32	∅	20	∅
Haloperidol	1	∅	4	∅
Psychopathological ratings (±SD)				
SAPS total	39.7 (21.8)	∅	38.7 (14.9)	∅
SANS total	44.5 (24.97)	∅	45.5 (22.3)	∅
BPRS total	44.6 (10.8)	∅	47.6 (13.3)	∅
SCL 90-R	81.06 (74.4)	37.5 (26.01)	60.6 (63.9)	14.4 (12.9)
Educational state				
Years at school (%)				
>10 years	58	87	56	83
<10 years	42	13	44	7

cc = consuming controls, $n=22$; cp = consuming patients, $n=35$; ncc = nonconsuming controls, $n=31$; ncp = nonconsuming patients, $n=29$; SD, standard deviation; NL, neuroleptic drugs; SAPS, Scale of Assessment of Positive Symptoms; SANS, Scale of Assessment of Negative Symptoms; BPRS, Brief Psychiatric Rating Scale; SCL 90-R, Symptom Check List 1990 Revised, given as sum scores.

All cannabis-using participants were tested positive for cannabinoids in urine at the time of niacin testing. Subjects with any current or history of skin disorders (eczema, atopical dermatitis, psoriasis) or recent treatment with steroids or nonsteroidal anti-inflammatory drugs (eg acetylsalicylic acid) were excluded from the study before niacin testing. The study was approved by the Ethics Committee of Friedrich-Schiller-University Jena. All participants gave written informed consent to participate in the study.

Niacin Skin Test Protocol

Methylnicotinate ($C_7H_7NO_2$, 99%; Sigma-Aldrich Chemie GmbH, Germany) was applied simultaneously in three dilutions (0.001, 0.01, 0.1 M) of 50 µl each to the skin at the inner side of the forearm using chambered plaster for epicutaneous testing. After 90 s the plaster was removed. Skin flushing was quantified before and up to 15 min after methylnicotinate exposure in 3-min intervals, starting 90 s after removal of the methylnicotinate patches. Methylnicotinate solutions were freshly prepared for each test to prevent any influence of sunlight.

Reflection Spectroscopy

Optical reflection spectroscopy was applied as described in more detail in a methodological paper (Smesny *et al*, 2001). Skin content of oxygenated blood was assessed with a handheld optical reflection spectrometer (spectral range: 400–700 nm, area of measurement: ∅ 5 mm), using the oxyhemoglobin (HbO_2)-absorption double peak at 542 and 577 nm. Each measurement was repeated three times (within 10 s) and then averaged.

Spectroscopic data were processed automatically creating difference spectra by subtraction of prestimulation reflection intensities (also measured three times) from test intensities. Two Gaussian curves were fitted to the HbO_2-absorption double peak. The area under the resulting sum curve was taken as measure of current skin flushing (measured in arbitrary units (a.u.)).

Data Analysis

We first conducted a repeated measure analysis of variance (ANOVA) with TIME (3, 6, 9, 12, 15 min) and methylnicotinate CONCENTRATION (0.001, 0.01, 0.1 M) as within-subject factors and GROUP (patients, controls) and CANNABIS (cannabis user, cannabis nonuser) as between-subject factors. GENDER and AGE were treated as covariates. For repeated measure ANOVAs, a Greenhouse–Geisser correction was performed where necessary and is indicated in the text by quotation of ε values. For an easier understanding, only uncorrected degrees of freedom are reported. For *post hoc* comparison of single values, Mann–Whitney *U* tests were calculated.

Psychopathological ratings of the SCL 90-R were compared between groups using an univariate ANOVA with the same between group factors (GROUP, CANNABIS) and covariates (AGE, GENDER) as above. Furthermore, effects of cannabis use on SAPS, SANS, and BPRS were investigated within the patient group. To explore a possible association between age or psychopathological ratings and skin flushing, Spearman correlation coefficients were calculated. Owing to the high number of calculated coefficients the significance level was set on $p<0.01$.

RESULTS

Niacin Flushing in the Total Group of Patients and Controls

Initial ANOVA for repeated measures revealed significant effects of GROUP ($F_{1,111}=35.287$, $p<0.001$) and GENDER ($F_{1,111}=11.558$, $p<0.001$), as well as a significant interaction of CANNABIS by GROUP ($F_{1,111}=5.328$, $p=0.023$). Patients exhibited generally a less intensive flushing after methylnicotinate exposure, but the extent of group differences was also modulated by TIME and CONCENTRATION (TIME × CONCENTRATION × GROUP interaction $F_{8,888}=7.012$, $p<0.001$, $\varepsilon=0.326$). AGE had neither alone ($F_{1,111}=0.000$, NS) nor in interaction with other factors any influence on results and was, therefore, not used as covariable for following analyses. As consequence of the significant CANNABIS × GROUP interaction, the niacin responses of cannabis users and nonusers were compared for healthy subjects and patients separately.

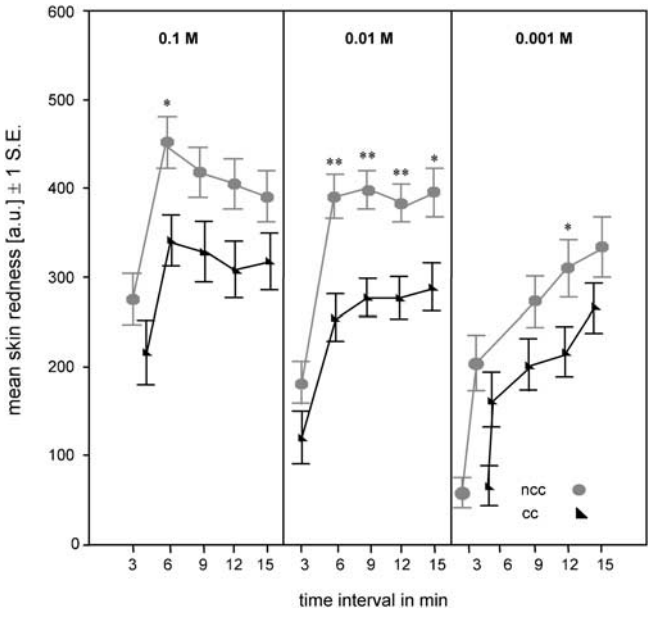

Figure 1 Mean skin redness in arbitrary units (a.u.) ± standard error (SE), separated for nonconsuming ($n=31$) and consuming ($n=22$) healthy controls, at each time interval for each methylnicotinate concentration if possible (* significant differences $p<0.05$, ** highly significant differences $p<0.01$).

Effects of Cannabis Use

Within healthy controls, cannabis-using subjects generally exhibited a less intense skin flushing as compared to nonconsuming subjects ($F_{1,49}=4.528$, $p=0.038$, Figure 1). Interactions between CANNABIS and TIME/CONCENTRATION/GENDER were not significant. Within patients, CANNABIS had neither alone ($F_{1,62}=0.059$, NS) nor in interaction with TIME/CONCENTRATION/GENDER a significant influence on skin flushing. Thus, cannabis use had an impact on niacin response only in healthy subjects.

Effects of Schizophrenia

The interaction between GROUP and CANNABIS might also indicate that differences between patients and healthy controls are modified by cannabis use. Within the nonconsumers, healthy subjects showed a much more intense skin flushing as compared to patients ($F_{1,57}=39.678$, $p<0.001$, Figure 2). In contrast, within the cannabis-consuming group, the differences between patients and healthy controls were less pronounced and less significant ($F_{1,54}=6.746$, $p=0.017$, Figure 3), as compared to nonconsumers. Within the nonconsumers, differences between patients and healthy controls were also influenced by CONCENTRATION and TIME ($F_{1,57}=7.069$, $p<0.001$, $\varepsilon=0.299$). Results of the pairwise comparisons are provided within Figures 2 and 3.

Psychopathological Ratings

Comparing psychiatric symptoms of all participants (patients and controls), highly significant differences of total

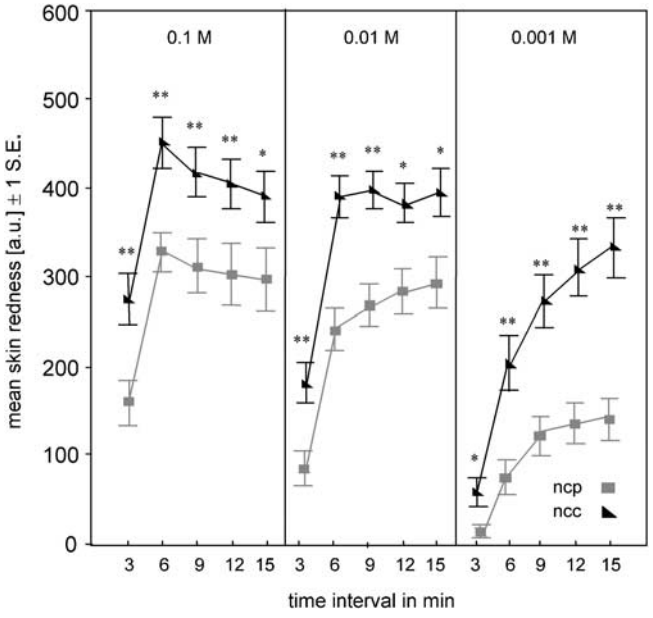

Figure 2 Mean skin redness in arbitrary units (a.u.) ± standard error (SE), separated for nonconsuming controls ($n = 31$) and nonconsuming schizophrenia patients ($n = 29$), at each time interval for each methylnicotinate concentration. (* significant differences $p < 0.05$, ** highly significant differences $p < 0.01$).

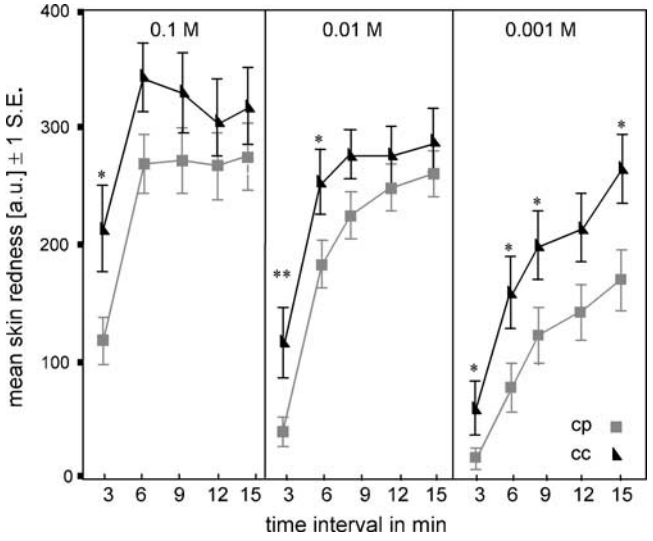

Figure 3 Mean skin redness in arbitrary units (a.u.) ± standard error (SE), separated for consuming patients ($n = 35$) and consuming controls ($n = 22$), at each time interval for each methylnicotinate concentration. (* significant differences $p < 0.05$, ** highly significant differences $p < 0.01$).

SCL 90-R values were found between patients and controls ($F_{1,99} = 12.545$, $p < 0.001$), as well as significant differences between cannabis-consuming and nonconsuming subjects ($F_{1,99} = 6.436$, $p = 0.013$). Patients had higher values than controls, and cannabis consumers had higher values than nonconsumers (Figure 4). There were neither significant effects of AGE ($F_{1,99} = 1.402$, NS) and GENDER ($F_{1,99} = 2.540$, NS) nor a significant GROUP × CANNABIS interaction ($F_{1,99} = 0.039$, NS). Comparison of BPRS, SAPS, and SANS total scores between the two patient groups revealed no significant differences (BRPS: $F_{1,49} = 0.156$; SAPS: $F_{1,49} = 1.590$; SANS: $F_{1,49} = 0.165$, all NS).

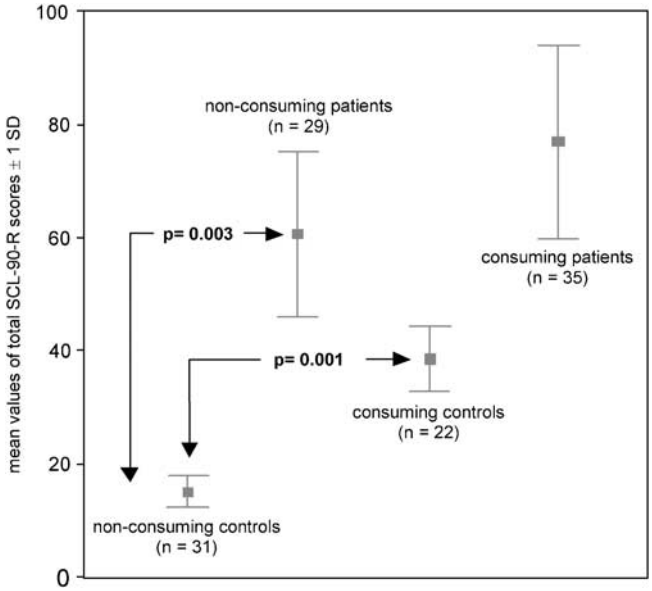

Figure 4 Mean values of SCL 90-R total scores ± standard deviation, separated for nonconsuming and consuming subgroups of patients and controls.

Investigating the entire population of patients and controls, SCL 90-R total scores showed some associations between a reduced skin flushing and high SCL 90-R ratings. The skin flushing at the highest and the medium niacin concentration was negatively correlated to SCL 90-R ratings at nearly all time points (0.1 M: $r_{3\,min} = -0.214$, NS; $r_{6\,min} = -0.323$, $p < 0.001$; $r_{9\,min} = -0.283$, $p < 0.01$; $r_{12\,min} = -0.268$, $p < 0.01$; $r_{15\,min} = -0.252$, $p < 0.01$; 0.01 M: $r_{3\,min} = -0.276$, $p < 0.01$; $r_{6\,min} = -0.303$, $p < 0.01$; $r_{9\,min} = -0.321$, $p < 0.001$; $r_{12\,min} = -0.318$, $p < 0.001$; $r_{15\,min} = -0.313$, $p < 0.001$). No significant correlations were found between skin flushing and BRPS, SANS, and SAPS scores within the patient sample.

DISCUSSION

The key finding of this study is the evidence that regular use of cannabinoids influences the function of bioactive lipids (ie prostaglandins) in healthy individuals, suggesting the potential of cannabinoids to modulate an alteration of the lipid–arachidonic acid cascade. If alterations of the lipid and arachidonic acid metabolism characterize an endophenotype of schizophrenia, as being presumed but not proven as yet, it would explain an increased susceptibility of some, but not all schizophrenia patients for the psychotogenic effects of cannabinoids. Evidence for interactions between cannabis use and abnormalities of the lipid–arachidonic acid pathway might also provide a biochemical basis for the gene–environment hypothesis of cannabis effects on schizophenia pathophysiology (Ferdinand *et al*, 2005; Henquet *et al*, 2005b; van Os *et al*, 2005).

The influence of long-term cannabis use on niacin skin flushing has not been investigated in a systematic manner as

yet, especially not in healthy subjects. We assume that duration of use (month to years before psychosis), regularity (daily use), and dose ($\geqslant$0.5 g/day) may have an impact on findings.

Results in nonconsuming patients and controls corroborate the often replicated finding of attenuated niacin flushing occurring in schizophrenia (Smesny, 2004b; Ward, 2000). But, our study could not reveal decreased skin response in cannabis-consuming patients as compared to nonconsuming patients. Statistically, there is a limited chance to detect an additional alteration of skin flushing due to cannabis use in a patient population with preexisting attenuation of niacin sensitivity. Otherwise, the acceptance of a null hypothesis as the result of a statistical comparison does not prove the assumption that an additional alteration of niacin response caused by frequent cannabis use is not apparent.

We highlight the finding in cannabis-consuming and nonconsuming control subjects, as it implies, that niacin flushing may generally allow to detect lipid metabolic abnormalities caused by constituents of herbal cannabis. The pathways of cannabis action are rather complex and still not fully understood. Of particular importance, however, seem to be the lipophilic behavior of Δ^9-THC and its interaction with membrane turnover, phospholipase activity, arachidonic acid release, and prostaglandin function (Burstein *et al*, 1994; Hunter *et al*, 1986; Reichman *et al*, 1991). That basic feature of Δ^9-THC may explain its ability to interact with many membrane-embedded receptor systems, including the dopamine, glutamate, and certainly the endocannabinoid system.

The influence of Δ^9-THC on phospholipases is mediated by G-protein-coupled receptors, most likely cannabinoid receptors (Audette *et al*, 1991; Burstein *et al*, 2000; Wartmann *et al*, 1995). Furthermore, Δ^9-THC modulates the availability of free arachidonic acid, the precursor of endogenous cannabinoid (CB_1) ligands (eg anandamide). CB_1 receptors are expressed in basal ganglia, cortex, hippocampus and cerebellum, brain areas involved in the control of emotion, cognition, and motivation, all crucial in schizophrenia (Giuffrida *et al*, 2004). In the course of illness, the endocannabinoid system could play an adaptive role. During an initial 'protective' phase the demand for arachidonic acid precursors of anandamide seem to be increased, as anandamide seem to counterbalance psychotic symptoms being upregulated (Giuffrida *et al*, 2004; Glass, 2001). Longer lasting and sufficiently frequent exposure to Δ^9-THC may cause an exhaustion of arachidonic acid reserves followed by downregulation of the endocannabinoid system, weakening of its protective power, and increasing severity of psychosis. This is supported by findings of CB_1 receptor downregulation and lower levels of anandamide in CSF in schizophrenia patients frequently using cannabis (Di Marzo, 1999; Di Marzo *et al*, 2000; Leweke *et al*, 2006). Following this model, attenuated skin flushing in healthy individuals and schizophrenia patients (as reported here) may be taken as peripheral correlate of a systemic exhaustion of arachidonic acid and its derivates after continuous exposure to Δ^9-THC. Provided that this metabolic deficit is directly linked to psychopathology, it may also underlie the reported inverse overall association between niacin skin flushing and SCL 90-R scores.

In general, the finding of considerable mental disturbance in 'healthy' cannabis-using participants was not unexpected. 'Positive'- and 'negative'-like symptoms close to psychosis have been reported after long-lasting daily cannabis use, which are apparently dose-dependent (Patton *et al*, 2002; Reilly *et al*, 1998; Skosnik *et al*, 2001; Verdoux *et al*, 2003). In fact, unreliability in keeping appointments and increased mistrust was the main obstacle in recruiting our group of healthy consumers. These difficulties caused a recruitment time of more than 2 years. However, within a follow-up interval of 2 years since the end of recruitment of healthy volunteers, none of the healthy cannabis-using individuals has been admitted to psychiatric services. Therefore, findings in cannabis-using healthy participants are unlikely to be due to undiscovered prodromal schizophrenia.

The investigation of an unmatched population has caused some limitations of this study in terms of age and gender effects on flush response. Possible reasons for age and gender effects on niacin skin flushing were extensively discussed in a previous study of our group (Smesny *et al*, 2004a). Considering possible confounding effects, data analysis was repeated for gender-separated subgroups (results not shown in detail). In general, similar results as in the entire group were found on a trend level in the male subgroups. Subgroups of females did not reach the appropriate sample size for reliable statistics. The influence of other demographic covariates cannot be completely ruled out.

Most of the patients were on neuroleptic medication at the time of niacin testing. This raises the question of confounding medication effects. In general, attenuation of niacin flushing was reported in both schizophrenia patients treated with neuroleptics and those without any neuroleptic medication (Hudson *et al*, 1997; Shah *et al*, 2000). Thus, our findings are very likely not due to medication for two reasons. First, the specific effect of cannabinoids on skin flushing has been shown in healthy individuals. Second, neuroleptic drugs and doses were comparable in the cannabis-consuming and nonconsuming patient group.

In summary, our results support the assumption that Δ9-THC may aggravate a premorbid vulnerability of lipid metabolism in schizophrenia, interfering with maintenance of membrane structure and the release of arachidonic acid, and modulating metabolic steps downward the arachidonic acid cascade. This biochemical influence also provides a biochemical basis for the deregulation of endogenous CB_1 ligands in psychosis, in turn modulating dopaminergic and glutamatergic neurotransmission. Finally, it causes alterations in peripheral prostaglandin signaling associated with the possibility to assess metabolic Δ^9-THC effects by easy to apply, standardized niacin skin testing. Results introduce lipid biochemistry as promising field to study Δ^9-THC involving gene $\times$ environment interactions with an impact on psychosis liability, age of onset, severity of symptoms, and long-term outcome of schizophrenia.

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