

## Short report

## Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis

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**Summary**

Anandamide is a bioactive lipid binding to cannabinoid receptors. A homeostatic role for anandamide has been suggested in schizophrenia. We investigated its role in initial prodromal states of psychosis. We measured the levels of anandamide and its structural analog oleylethanolamide in cerebrospinal fluid and serum of patients in the initial prodromal state ( $n=27$ ) alongside healthy volunteers ( $n=81$ ) using high-performance liquid chromatograph/mass spectrometry. Cerebrospinal anandamide levels in patients

were significantly elevated. Patients with lower levels showed a higher risk for transiting to psychosis earlier. This anandamidergic up-regulation in the initial prodromal course may suggest a protective role of the endocannabinoid system in early schizophrenia.

**Declaration of interest**

None.

We have previously shown that levels of the endocannabinoid anandamide in cerebrospinal fluid (CSF) in antipsychotic-naïve patients with schizophrenia<sup>1–3</sup> are both markedly elevated and negatively correlated with psychotic symptoms. A model of dopamine/endocannabinoid interaction in acute schizophrenia was proposed where (over-)activation of dopamine D<sub>2</sub> receptors is associated with an increased release of anandamide counterbalancing dopamine-mediated psychotic symptoms by strengthening an endogenous feedback loop. This model suggests an adaptation of endocannabinoid function over a long period to a slow, gradual increase in dopaminergic neurotransmission.<sup>4</sup> We investigated whether changes in the endocannabinoid system are already noticeable in initial prodromal states of psychosis and test the hypothesis that an elevation of anandamide in CSF is apparent in this early stage of the disease.

**Method**

The ethics committee of the University of Cologne approved the study. All patients and healthy volunteers provided written informed consent.

We consecutively recruited 108 participants, comprising 81 healthy controls with no family history of psychiatric or neurological disorders (56% male, median age 27.3 years (interquartile range (IQR) 23.9 to 29.9)) and 27 patients with initial prodromal states of psychosis (70% male, age 24.3 years (IQR 19.9 to 29.1)). Controls were recruited by word of mouth and were paid for their expenses. Out of the 27 patients, 8 (30%) received antipsychotic medication.

Controls were screened for current or previous medical and psychiatric disorders. No cannabis use within 6 weeks prior to the study was accepted. Overall, 55 controls (68%) had taken cannabis <20 times/lifetime and 26 (32%) had used cannabis >20 but <50 times/lifetime.

Patients were assessed at the Cologne Early Recognition Centre using the Positive and Negative Symptom Scale (PANSS),<sup>5</sup> the Scale of Prodromal Symptoms (SOPS),<sup>6</sup> and the Schizophrenia Prediction Instrument for Adults (SPI-A).<sup>7</sup> An initial prodromal state was assumed if any of ten cognitive–perceptive basic symptom or any attenuated or transient psychotic symptom was

present. After examination, 11 patients (41%) developed schizophrenia; 36% had already been treated with antipsychotics before transition. The 75th percentile of time from baseline to transition was 13 months; median follow-up was 61 months (IQR 56 to 80). Overall, 16 patients used cannabis <20 times/lifetime and 11 used cannabis >20 times/lifetime. All participants showed a negative urine drug screening for illicit drugs.

Cerebrospinal fluid and serum were collected around mid-day using a procedure described elsewhere.<sup>2</sup> No participants revealed pathognomonic CSF findings according to a standard examination.<sup>8</sup> We measured CSF and serum levels of anandamide along with oleylethanolamide, another endogenous fatty acid ethanolamide. These were purified and quantified by isotope dilution high-performance liquid chromatography/mass spectrometry as previously described.<sup>9</sup>

Skewed empirical distributions of continuous variables were summarised by median and IQR (25th to 75th percentile). Independent groups were compared by exact Wilcoxon rank sum test (corrected for ties). Spearman's rho was calculated to assess pairwise correlation of variables. The cumulative probability of transition to psychosis was estimated by the Kaplan–Meier method and compared overall by the log rank test (hazard ratio). Statistical analyses were performed using SPSS and R for Windows.

**Results**

In controls, the median concentration of anandamide in CSF was <0.001 pmol/ml (IQR <0.001 to 0.005) and <0.001 pmol/ml (<0.001 to 0.700) in serum. We found no significant correlation between anandamide concentrations in CSF and serum ( $r_s=0.033$ ,  $P=0.768$ ). Median CSF anandamide levels of patients were 0.006 pmol/ml (IQR <0.001 to 0.073,  $P=0.004$ ; online Fig. DS1a) and 0.216 pmol/ml (IQR <0.001 to 0.515,  $P=0.838$ ) in serum. Oleylethanolamide levels did not differ in CSF or serum between controls (CSF: 0.680 pmol/ml (IQR 0.280 to 1.100); serum: 7.700 pmol/ml (IQR 4.885 to 11.000)) and patients (CSF: 0.600 pmol/ml (IQR 0.315 to 1.026),  $P=0.896$ , online Fig. DS1b; serum: 6.641 pmol/ml (IQR 2.867 to 9.891),  $P=0.139$ ).

A significant inverse correlation between anandamide in CSF and symptoms in patients was observed for PANSS factor 3

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'cognitive syndrome' ( $r_s = -0.533$ ,  $P = 0.005$ ). No other psychopathological measures correlated with ethanolamide levels.

In this sample, no relevant differences for CSF and serum levels of anandamide and oleylethanolamide were found between patients and controls regarding smoking, cannabis use, medication or body mass index. No significant difference was found for all ethanolamide levels between medicated and antipsychotic-naïve patients. Interestingly, patients with cannabis use <20 times/lifetime showed no significantly different ethanolamide levels compared with those with >20 times/lifetime use.

By splitting patients at the median CSF anandamide value (0.006 pmol/ml) into low and high groups, patients with higher anandamide levels in CSF were found to be associated with an extended transition time into frank psychosis ( $P = 0.095$ ; online Fig. DS1c). The relative risk of transition of individuals in the high v. low anandamide groups was 0.33 (95% CI 0.09–1.29). A similar analysis for lifetime cannabis use was not associated with the likelihood of transition to psychosis.

## Discussion

This is the first study investigating levels of anandamide and oleylethanolamide in CSF and serum of patients with initial prodromal states of psychosis. We found levels of anandamide in CSF significantly elevated in patients. The anandamide elevation was comparable to that described for first-onset, antipsychotic-naïve schizophrenia<sup>2</sup> and was found in CSF exclusively, making its central origin most likely. As oleylethanolamide concentrations did not differ significantly between patients and controls, it is unlikely that changes in anandamide levels were caused by a generalised alteration in ethanolamide signalling.

Interestingly, we found a trend for an association of anandamide elevation in CSF with a longer time to psychosis transition in patients; this trend, however, did not reach statistical significance mainly owing to lack of power. This is in line with our hypothesis of an adaptive role for anandamide in schizophrenia, indicating that those patients expressing higher CSF anandamide are less likely to have psychotic symptoms. Four patients who transitioned to schizophrenia were treated with dopamine D<sub>2</sub>-antagonists at examination and showed no significantly different ethanolamide levels compared with antipsychotic-naïve patients. However, the observation that some patients did not show significant changes in CSF anandamide levels might imply a fluctuation or state-dependency of the alterations of the endocannabinoid system during the initial prodromal period. This has been recently suggested for other markers in CSF.<sup>10</sup> Alternatively, this may be related to the particular condition investigated, as not all individuals diagnosed with an initial prodromal state develop schizophrenia but may either return to a normal psychiatric status or develop another psychiatric disorder.<sup>11</sup> It is interesting to note that in this sample a history of cannabis use did not have an impact on levels of CSF anandamide as previously reported in acute antipsychotic-naïve schizophrenia.<sup>12</sup> Further, no relation between history of cannabis use and risk of transition to psychosis was observed. Both aspects may be related to the relationship between  $\Delta^9$ -tetrahydrocannabinol and cannabidiol in street cannabis consumed, which was not further evaluated.<sup>13</sup>

Our findings suggest that endogenous anandamide mobilisation may play a protective role in at least a subgroup of patients with early stage schizophrenia.<sup>4</sup> Based on this observation, it may be hypothesised that assaying the endocannabinoids in CSF may provide useful biomarkers in early schizophrenia.

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First received 16 Apr 2008, final revision 30 Jun 2008, accepted 1 Sep 2008

## Funding

The study was funded by the Stanley Medical Research Institute (01-315 and 03-NV-003 to F.M.L.), the Koeln Fortune Program (108-2000 to F.M.L.) and the Federal Ministry of Education and Research (BMBF) (01KN0706 to D.K.).

## Acknowledgements

We thank Drs Brit M. Nolden, Christoph W. Gerth, Christian Mauss and Johannes Faulhaber for collection of data.

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