Abstract

Objective: Schizophrenia is a psychiatric disorder whose mechanisms have remained only partially elucidated. The current proposals regarding its biological basis, such as the dopaminergic hypothesis, do not fully explain the diversity of its symptoms, indicating that other processes may be involved. This paper aims to review evidence supporting the involvement of the endocannabinoid system (ECS), a neurotransmitter group that is the target of Cannabis sativa compounds, in this disorder. Methods: A systematic review of original papers, published in English, indexed in PubMed up to April, 2012. Results: Most studies employed genetics and histological, neuroimaging or neurochemical methods - either in vivo or post-mortem - to investigate whether components of the ECS are compromised in patients. Overall, the data show changes in cannabinoid receptors in certain brain regions as well as altered levels in endocannabinoid levels in cerebrospinal fluid and/or blood. Conclusions: Although a dysfunction of the ECS has been described, results are not entirely consistent across studies. Further data are warrant to better define a role of this system in schizophrenia.
Introduction

Schizophrenia is a major psychiatric disorder consisting of a diversity of clinical features, which have been grouped as positive, negative and cognitive symptoms.\(^6,7\) The pharmacological approach for its treatment is quite limited and consists mainly of antipsychotic compounds, which are not effective in all the dimensions of this disorder. Almost all of these drugs share a common mechanism of action, which is the antagonism of dopamine receptors.\(^3\)

The biological basis of schizophrenia has been extensively studied and discussed. Based on the mechanisms of antipsychotic medications and other pieces of evidence, the prevalent view is that its symptoms could result from a dysfunction in the dopaminergic neurotransmission, the so-called dopaminergic hypothesis.\(^4,5\) There are, however, clear limitations for this hypothesis, as it does not properly explain the complexity of symptoms and its clinical heterogeneity. Apart from dopamine, there are other neurotransmitters that are also in focus, such as serotonin and glutamate.\(^6,7\)

More recently, there has been investigation as to whether the endocannabinoid system (ECS) might be involved in schizophrenia. This neurotransmitter system is named after the herb Cannabis sativa (“marijuana”), known as one of the most consumed drugs of abuse. Its main active compound is delta-9-tetrahydrocannabinol (THC), the prototype of a class of compounds called cannabinoïds. Other major natural cannabinoïds are cannabidiol (CBD) and cannabinoïn. The ECS comprises the receptors for the cannabinoïns, thus termed cannabinoïd type-1 and type-2 receptors (CB1-R and CB2-R); their endogenous ligands, such as arachydonoyl ethanolamide (AEA, also known as anandamide), 2-arachydonoyl glycerol (2-AG), palmitoyl ethanolamide (PEA) and oleoyl ethanolamida (OEA), collectively termed endocannabinoïnds (eCBs); and the enzymes responsible for their synthesis and catabolism. Anandamide and 2-AG are metabolized by the enzymes fatty acid amide hydrolase (FAAH) and monoacyl glycerol lipase (MAGL), respectively.\(^8,9\) A schematic view of the proposed functioning of the ECS is depicted in Figure 1.

The chronic use of cannabis has been pointed as a possible factor leading to psychosis, more specifically schizophrenia. Other comprehensive reviews have focused on this possible link.\(^10,11,12\) The aim of the present paper is to review the literature addressing a putative role of the ECS in the pathophysiology of schizophrenia.

Method


The inclusion criteria were: original papers; English language; studying changes of the ECS in schizophrenia (genetic variations in the components of the ECS, changes in cannabinoïd receptors in the brain and changes in eCB levels in liquor or blood). Abstracts from scientific meetings were also included. There was no limit for the year of publication, and the search included papers until April, 2012.

The search retrieved 90 articles, from which 22 were included. An Additional 9 articles were included based on references from these articles, totalling 31 articles on which the review was based. The remaining 68 articles were excluded for the following reasons: review papers (n = 19); studies with new radioligands for the cannabinoïd receptor (n = 15); studies in animals (n = 7); studies investigating the effects of cannabis in healthy volunteers or schizophrenic patients (n = 10); studies evaluating the link between Cannabis sativa use and schizophrenia (n = 3); studies evaluating other outcomes from therapeutic interventions (n = 2); case report (n = 1); comment on an original paper (n = 1); and studies focusing on other disorders and conditions (n = 10).

Results

The studies were divided according to three main strategies to approach the ECS in schizophrenia: investigation of polymorphisms, detection of cannabinoïd receptors in brain regions and measurement of eCB levels in CSF or blood.

Genetic variations in the components of the ECS

Genetic variations related to the components of the ECS have been investigated in several studies. Most of them focused on the relationship between polymorphisms of the CNR1 gene, which encodes for CB1-R, and schizophrenia. This gene is located in the 6q14-q15 chromossomic region, which has been identified as a locus for schizophrenia susceptibility.\(^13\)

The first studies evaluating the relationship between CNR1 variations and schizophrenia obtained negative results (Table 1). Tsai et al.\(^14\) did not find any link between the (AAT)n triple repeat (AL136096) polymorphism and schizophrenia.
schizophrenia in a study comparing 127 Chinese patients with schizophrenia and 146 healthy controls. Leroy et al.15 evaluated a distinct polymorphism from the same gene, 1359 G/A (rs1049353). These authors also did not find any difference in the allele frequency or genotypic distribution between 102 patients with schizophrenia or schizoaffective disorder and 63 controls in a French Caucasian population. Likewise, Zammit et al.16 did not find any relation between this same polymorphism and schizophrenia in 750 patients as compared to 688 controls in a British population. Seifert et al.17 evaluated the association of three polymorphisms from the CNR1 (1359 G/A (rs1049353), (AAT)n triple repeat (AL136096) and rs6454674) with schizophrenia in 104 patients and 140 controls in a German population, but did not find differences between these groups. There was a tendency towards a lower frequency of the (AAT)9 allele in patients, although the result did not reach statistical significance, possibly due to the low sample size. Hamdani et al.18 also studied the 1359 G/A (rs1049353) polymorphism and, again, did not find any association with schizophrenia in 133 patients as compared to 141 controls in a French population. Despite the negative result, this work did find a higher frequency of the G allele in patients with refractory schizophrenia, which could mean that the 1359 G/A polymorphism would not be related to vulnerability for this disorder, but rather to a response to antipsychotic drugs. In addition, the differences between three other polymorphisms (rs806368, rs806379 and rs806380) were analysed between patients refractory or responsive to antipsychotic treatment, but no association was found. Finally, Morita et al.19 investigated a possible relationship...
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<td>Genetic association study</td>
<td>113 patients with Scz; 111 controls; Spanish population</td>
<td>Allelic frequency of (AAT)n repeat was different between patients and controls (lower frequency of allele 4 – (AAT)10).</td>
<td>Supports the hypothesis that the triple repeat (AAT)n polymorphism is associated with the pathophysiology of Scz. Allele 4 could be the protective variant for Scz of the CNR1 gene.</td>
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<td>Zammit et al.</td>
<td>Assess the involvement of CNR1 and CHRNA7 genes in the pathogenesis of Scz.</td>
<td>Genetic association study</td>
<td>750 patients with Scz; 688 controls; British population</td>
<td>Genotypic distribution of 1359 G/A did not differ between patients and controls.</td>
<td>Does not support the hypothesis that the 1359 G/A polymorphism is associated with the pathophysiology of Scz.</td>
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<tr>
<td>Seifert et al.</td>
<td>Assess the involvement of CNR1 gene in the pathogenesis of Scz.</td>
<td>Genetic association study</td>
<td>104 patients with Scz; 140 controls; German population</td>
<td>Allelic frequency of (AAT)n was lower in Scz patients than in controls, but it was not statistically significant. There was no difference in allelic frequency of 1359 G/A (rs1049353) and rs6454674 polymorphisms between patients and controls.</td>
<td>Does not support the hypothesis that the 1359 G/A (rs1049353) and rs6454674 polymorphisms are associated with the pathophysiology of Scz. There was a tendency of lower frequency of the (AAT)n allele in Scz, maybe not confirmed due to the small sample size.</td>
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<tr>
<td>Chavarria-Siles et al.</td>
<td>Assess the involvement of CNR1 gene in the pathogenesis of Scz.</td>
<td>Family-based genetic association analyses</td>
<td>66 patients with hebephrenic Scz; 244 patients with Scz (broad phenotype); Costa Rican population</td>
<td>There was an association between the triple repeat (AAT)n polymorphism and patients with hebephrenic Scz (lower frequency of the allele 4 – (AAT)n). There was no association between the polymorphism and patients with Scz (broad phenotype).</td>
<td>Supports the hypothesis that the triple repeat (AAT)n polymorphism is associated with the pathophysiology of hebephrenic Scz. Supports the hypothesis that different genetic and pathophysiological mechanisms can relate to different subtypes of Scz.</td>
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<tr>
<td>Hamdani et al.</td>
<td>Assess the involvement of CNR1 gene in the pathogenesis of Scz and in the APs response.</td>
<td>Genetic association study</td>
<td>133 patients with Scz on atypical APs (responders: 74; non-responders: 59); 141 controls; French population</td>
<td>There was no difference in allelic and genotypic frequencies of 1359 G/A (rs1049353) polymorphism between patients and controls. The allelic frequency of the G allele of 1359 G/A (rs1049353) polymorphism was higher in non-responders Scz patients.</td>
<td>1359 G/A (rs1049353) polymorphism would not be related to vulnerability to Scz, but to atypical antipsychotic response.</td>
</tr>
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### Tiwari et al.23
Assess the involvement of CNR1 gene in the AP-induced weight gain in Scz.

**Genetic association study.**
- 183 patients with Scz or Schizoaffective disorder on antipsychotic treatment
  - European (n=117) and African (n=55) ancestry population
- Allelic frequencies of rs806368, rs806379 and rs806380 polymorphisms between responders and non-responders.

### Ishiguro et al.25
Assess the involvement of CNR2 gene in the pathogenesis of Scz.

**Genetic association study.**
- 1920 patients with Scz
  - 1920 controls
  - Japanese population
- Allelic frequencies of rs12744386 and rs2501432 (R63Q) polymorphisms were higher in Scz patients.

### Ho et al.24
Assess interactions between CNR1 gene polymorphisms, cannabis use, cerebral volume and cognitive function in Scz.

**Cross-sectional with neuroimaging (MRI) and cognitive battery.**
- 52 patients with Scz or Schizoaffective disorder with cannabis abuse/dependence.
- 183 patients with Scz or Schizoaffective disorder without cannabis abuse/dependence.
- Allelic frequencies did not differ between users and non-users.
- Cannabis users had smaller WM frontotemporal volumes than non-users.
- Allelic frequencies of rs12720071 (G allele) carriers had smaller frontotemporal WM volumes than A allele carriers. Cannabis users had even smaller parietal WM volumes.
- Supports the hypothesis that the rs806378 polymorphism relates to atypical AP-induced weight gain.
- Suggests that cannabis use associated with specific CNR1 genotypes can contribute to WM alterations and cognitive deficits in a subgroup of Scz patients.
- Supports the hypothesis that genetic and environmental influences work together to determine the phenotypic expression in Scz.
between the Pro129Thr (rs3244420) polymorphism of the FAAH gene and schizophrenia. No difference was found in a group of 260 patients with schizophrenia (127 paranoïds, 127 hebephrenics and 6 not classified) as compared to 63 controls in a Japanese population, regardless of the disorder subtype.

Contrasting these negative results, other studies point to an association between variations in the CNR1 gene and schizophrenia. Ujike et al.,20 compared 242 patients (110 paranoïds and 128 hebephrenics) with 296 healthy controls in a Japanese population, in relation to the (AAT)n triple repeat polymorphism (AL136096), and found a difference in the allelic frequency in hebephrenics versus controls (higher frequency for the (AAT)9 allele and lower for (AAT)17). In the same study, another group of 116 patients and 137 controls were evaluated for differences in 1359 G/A (rs1049353) polymorphism, but no differences were found. Some of these results were replicated by Martínez-Gras et al.,21 that found a lower frequency of the (AAT)10 allele (allele 4) in 113 patients with schizophrenia in comparison to 111 controls in a Spanish population. Chavarría-Siles et al.,22 compared 244 patients with schizophrenia, without subtype classification, to 66 patients of the hebephrenic subtype and did not find an association between the (AAT)n triple repeat polymorphism (AL136096) and patients with schizophrenia in general, but, similar to Ujike et al.,20 they observed an effect for patients of the hebephrenic subtype (lower frequency of the (AAT)10 allele). These data reflect the pathophysiologic heterogeneity of schizophrenia and suggest that variations in the CNR1 gene may contribute to the pathogenesis of specific subtypes of this disorder.

Tiwari et al.,23 evaluated 20 polymorphisms of the CNR1 gene in 183 patients with schizophrenia or schizoaffective disorder that were on antipsychotic treatment and found higher allelic frequency (allele T) of the rs806378 polymorphism on those patients that gained more weight while using clozapine or olanzapine, which suggests that this genetic variation relates to susceptibility to antipsychotic-induced weight gain.

Ho et al.,24 evaluated interactions between CNR1 polymorphisms, cannabis use, cerebral volume and cognitive function in an interesting study. They compared 52 patients with schizophrenia or schizoaffective disorder with cannabis abuse/dependency and 183 patients without cannabis use and observed smaller frontotemporal white matter (WM) volumes in those that smoked cannabis. Besides that, patients with rs12720071 polymorphism (G allele) had lower WM volumes than those with the A allele. Those with the G allele that used cannabis had even lower WM volumes. Patients with rs7766029 (C allele) and rs9450898 (C allele) had lower WM volumes than those with the T allele. In the cognitive battery, patients with rs12720071 (G allele) had worse results on processing speed/attention and problem-solving tests. Results on problem-solving tests were even worse in those G allele carriers that smoked cannabis. Those results suggest that the use of cannabis in association with specific CNR1 genotypes can contribute to alterations in WM and cognitive deficits in a subgroup of schizophrenic patients, which favors the hypothesis that genetic and environmental factors work together to determine the phenotypic expression in schizophrenia.

Only one study focused on variations in the CNR2 gene (which encodes CB2-R) in the pathogenesis of schizophrenia. Ishiguro et al.,25 evaluated differences in the allelic frequencies of five CNR2 polymorphisms (rs9424339, rs2502959, rs2501432 (R63Q), rs2229579 (H316T) and rs12744386), comparing 1920 patients with schizophrenia to 1920 controls in a Japanese population. The authors found an association of the polymorphisms rs2501432 (R63Q) and rs12744386 with the disorder. This result supports the hypothesis that variations in the CNR2 gene may participate in the pathophysiology of schizophrenia.

To summarize, most studies refer to (AAT)n triple repeat (AL136096) and 1359 G/A (rs1049353) polymorphisms of the CNR1 gene. Among the studies evaluating the (AAT)n triple repeat (AL136096) polymorphism, one found an association with schizophrenia,21 two found associations with schizophrenia,21 14,17 two did not find any associations between the polymorphism and the disorder.14,17 Among those evaluating the 1359 G/A (rs1049353)15-18,20 polymorphism, no study found any association. The only study evaluating variations in the CNR2 gene20 observed a relationship between two polymorphisms with schizophrenia. The polymorphisms Pro129Thr (rs3244420) of the FAAH gene; rs6454674 of the CNR1 gene; as well as rs9424339, rs2502959 and rs2229579 (H316T) of the CNR2 gene did not seem to have any association with the disorder.

Changes in cannabinoid receptors in the brain

Another strategy employed by several authors to investigate the role of the ECS in the pathophysiology of schizophrenia focuses on the determination of the levels of CB1-R in certain brain regions possibly related to this disorder. This has
been performed either in post-mortem or in vivo studies. Post-mortem studies evaluated the density of CB1-R through three main methods: radio-ligand binding assays, immunohistochemistry or polymerase chain reaction (PCR), whereas in vivo studies employed neuroimaging techniques. These studies are summarized in Table 2.

The first post-mortem study with a radioligand was conducted by Dean et al.,26 who investigated differences in the levels of [³H] CP-55940 binding (a CB1-R agonist) in area 9 of the dorsolateral prefrontal cortex (dlPFC), caudato-putamen and hippocampus of 14 patients with schizophrenia and 14 controls. The authors detected an increase of CB1-R density in the dlPFC of the patients, a result not related to cannabis consumption. There was no difference in other brain regions. In addition, Dalton et al.27 evaluated the density of CB1-R in another area of the dlPFC (area 46) with the same ligand and found an increase in the density of this receptor in patients with paranoid schizophrenia (n = 16) as compared to controls (n = 37). Zavitsanou et al.28 focused on the anterior cingulate cortex (ACC) using the CB1-R antagonist [³H]-SR141716A in 10 patients with schizophrenia versus 10 controls, describing an increase in CB1-R density. Newell et al.29 also found an increase in CB1-R expression in posterior cingulate cortex (PCC), as revealed by the CB1-R agonist [³H]-CP-55940 in eight patients and eight controls. Finally, Deng et al.30 evaluated differences in [³H]-SR141716A binding in the superior temporal gyrus (STG), a brain region proposed to be particularly involved in the auditory hallucinations. However, they did not find any difference between patients (n = 8) and controls (n = 8).

Four post-mortem studies employed different techniques to measure CB1-R density. Through immunohistochemistry, Koethe et al.31 did not find differences in the ACC of patients with schizophrenia in relation to their controls (n = 15 per group). However, Eggan et al.32 observed a reduction in CB1-R in the dlPFC (area 9), as revealed by protein and mRNA expression of 23 patients and equal number of controls. Likewise, Urigüen et al.33 found reduced CB1-R protein expression (though not mRNA) in this same region in a sample of 31 young patients as compared to 33 controls. Finally, Eggan et al.34 also evaluated CB1-R density in dlPFC, area 46, in two cohorts of patients and controls. In the first,

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| Dean et al.26    | Evaluate the density of CB1-R (through the level of the [³H] CP-55940 radioligand binding, CB1 agonist) in brain areas involved in Scz. | Observational, transversal, post-mortem analysis of brain tissue using radioligand and autoradiography. | • 14 patients with Scz  
• 14 controls | • dlPFC, area 9  
• CP  
• Temporal lobe | • Increase in the density of CB1-R in the CP (independent of recent cannabis ingestion).  
• No difference in the density of CB1-R in the CP and in the hippocampus when comparing patients with Scz to controls.  
• Increase in the density of CB1-R in the CP from subjects who had recently ingested cannabis (independent of diagnoses). | Favors the hypothesis that changes in ECS in the dlPFC are associated with the pathology of Scz. |
| Zavitsanou et al.28 | Evaluate the density of CB1-R (through the level of the [³H] SR141716A, radioligand binding, CB1 antagonist) in brain areas involved in Scz. | Observational, transversal, post-mortem analysis of brain tissue using radioligand and autoradiography. | • 10 patients with Scz  
• 10 controls | • ACC | Increase in the density of CB1-R in the ACC from subjects with Scz (independent of recent cannabis ingestion). | Favors the hypothesis that changes in ECS in the ACC are associated with the pathology of Scz (mainly negative and cognitive symptoms). |
| Newell et al.29 | Evaluate the density of CB1-R (through the level of the [³H] CP-55940 radioligand binding, CB1 agonist) in brain areas involved in Scz. | Observational, transversal, post-mortem analysis of brain tissue using radioligand and autoradiography. | • 8 patients with Scz  
• 8 controls | • PCC | Increase in the density of CB1-R in the PCC (superficial layers) from subjects with Scz. | Favors the hypothesis that changes in ECS in the PCC are associated with the pathology of Scz. |
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<td>Deng et al.</td>
<td>Evaluate the density of CB1-R (through the level of the [3H] SR141716A, radioligand binding, CB1 antagonist) in brain area involved in Scz.</td>
<td>Observational, transversal, post-mortem analysis of brain tissue using immunohistochemistry.</td>
<td>• 8 patients with Scz&lt;br&gt;• 8 controls&lt;br&gt;• STG</td>
<td>No difference in the density of CB1-R in the STG when comparing patients with to controls.</td>
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<td>Koethe et al.</td>
<td>Evaluate the density of CB1-R in brain area involved in Scz, BD and MDD.</td>
<td>Observational, transversal, post-mortem analysis of brain tissue using immunohistochemistry.</td>
<td>• 15 patients with Scz&lt;br&gt;• 15 patients with BD&lt;br&gt;• 15 patients with MDD&lt;br&gt;• 15 controls</td>
<td>No difference in the density of CB1-R in the ACC when comparing patients with Scz to controls.</td>
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<tr>
<td>Eggan et al.</td>
<td>Evaluate the density of CB1-R (protein and mRNA expression) in brain area involved in Scz.</td>
<td>Observational, transversal, post-mortem analysis of brain tissue using immunohistochemistry and in situ hybridization.</td>
<td>• 23 patients with Scz&lt;br&gt;• 23 controls&lt;br&gt;• diPFC, area 9</td>
<td>Reduction of protein and mRNA expression of CB1-R in the diPFC (area 9) when comparing patients with Scz to controls</td>
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<tr>
<td>Urigüen et al.</td>
<td>Evaluate the density of CB1-R, dopamine D2 receptor and adenosine A2a receptor (protein and mRNA expression) in brain area involved in Scz.</td>
<td>Observational, transversal, post-mortem analysis of brain tissue using immunoblot and PCR.</td>
<td>• 31 young patients with Scz that committed suicide (11 treated with atypical AP)&lt;br&gt;• 13 non-Scz suicide victims&lt;br&gt;• 33 non-suicide controls&lt;br&gt;• diPFC, area 9</td>
<td>• Reduction of protein expression of CB1-R in the diPFC (area 9) when comparing patients with Scz to controls (independent of suicide).&lt;br&gt;• No difference in the mRNA expression of CB1-R in the diPFC (area 9) when comparing patients with Scz to controls</td>
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<td>Wong et al.</td>
<td>Evaluate the density of CB1-R in brain area involved in Scz.</td>
<td>Observational, transversal, in vivo neuroimaging (PET scan) with radioligand.</td>
<td>• 10 patients with Scz on APs&lt;br&gt;• 10 controls&lt;br&gt;• frontal, temporal, parietal, occipital and cingulate cortex, fusiform gyrus, hippocampus, para-hippocampus, insula, putamen, caudato, globus pallidus, thalamus, cerebelum and pons.</td>
<td>• Increase in the density of CB1-R in the pons from subjects with schizophrenia.</td>
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<td>Eggan et al.</td>
<td>Evaluate the density of CB1-R in brain area involved in Scz.</td>
<td>Observational, transversal, post-mortem analysis of brain tissue using immunocytochemistry.</td>
<td>Cohort nº 1:&lt;br&gt;• 12 patients with Scz&lt;br&gt;• 12 controls Cohort nº 2:&lt;br&gt;• 14 patients with Scz&lt;br&gt;• 14 patients with MDD&lt;br&gt;• 14 controls&lt;br&gt;• diPFC, area 46</td>
<td>• Reduction of CB1-R density in diPFC (area 46) from subjects with Scz. Cohort nº 2:&lt;br&gt;• Reduction of CB1-R density in diPFC (area 46) when comparing patients with Scz to MDD patients and controls.&lt;br&gt;• No difference in the density of CB1-R in the diPFC when comparing patients MDD to controls.</td>
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</table>
comprising the same group from their previous study, they found reduction in CB1-R density in this brain region. In the second cohort, comprising 14 patients with schizophrenia, 14 with major depression and 14 controls, there was also a reduction in CB1-R density in schizophrenia as compared to controls, as well as to the major depression group.

Regarding in vivo studies, there were two investigating the levels of CB1-R in brain through neuroimaging methods. Wong et al.\textsuperscript{35} employed PET scan to evaluate receptor expression in several brain areas (frontal, temporal, parietal, occipital, and cingulated cortices; fusiform gyrus, hippocampus, insula, putamen, caudate nucleus, glob pallidium, thalamus, cerebellum, and pons) of 10 patients and equal number of controls. They found a significant increase in receptor expression only in the pons. There was also a tendency in this direction in most of the other regions (except for the fusiform gyrus and the cerebellum). In addition, they found that CB1-R expression correlated directly with positive symptoms and inversely with negative. Ceccarini et al.\textsuperscript{36} also employed PET scan to evaluate the levels of the receptor in three brain areas (nucleus accumbens, insula, and ACC) of 49 patients and equal number of controls. They found an increase in CB1-R expression in the mRNA analysis of brain tissue using radioligand and autoradiography and quantitative PCR.

### Changes in endocannabinoid levels in CSF and blood

In addition to changes in the ECS in brain regions, studies described altered levels of eCBs in the CSF and blood collected from patients. The eight studies retrieved by our search are summarized in Table 3. Four of them measured the levels of eCBs in the CSF. Leweke et al.\textsuperscript{37} focused on the levels of AEA and PEA in 10 patients with schizophrenia and 11 controls and found increased levels of AEA in patients. In another study, Giuffrida et al.\textsuperscript{38} measured AEA, PEA, and OEA in four groups: 47 patients with untreated first episode of paranoid schizophrenia; 71 paranoid patients undergoing antipsychotic treatment (36 typical and 35 atypical); 22 patients with mood disorders and 13 patients with dementia syndrome. The levels of AEA were increased, whereas PEA was reduced in the first group of patients. Both eCBs were increased in patients under treatment with atypical antipsychotics. In patients with schizophrenia treated with typical antipsychotics, in those with mood disorders or those with dementia there were no changes in the levels of AEA as compared to controls. The levels of OEA in patients with untreated first episode of paranoid schizophrenia did not differ from controls. Noteworthy, there was a negative correlation between the levels of AEA in patients with schizophrenia and the symptoms (as revealed by the Positive and Negative Syndrome Scale, PANSS), suggesting that AEA may be associated with the pathology of schizophrenia.

In order to evaluate the effects of cannabis consumption on the levels of eCBs, in the CSF, Leweke et al.\textsuperscript{39} measured the levels of AEA, PEA, and OEA in 44 patients with schizophrenia and 81 controls, both being divided in subgroups accordingly to high or low cannabis consumption. The authors detected a higher level of AEA in patients who consumed less cannabis as compared to those who used it frequently as well as to the controls. The levels of other eCBs were not altered. There was a negative correlation between the levels of AEA in

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| Ceccarini et al.\textsuperscript{36} | Evaluate the density of CB1-R in brain area involved in Scz. | Observational, transversal, in vivo neuroimaging (PET scan) with radioligand. | • 49 patients with Scz with APs  
  • 9 patients with Scz without APs  
  • 12 controls  
  • NA  
  • Insula  
  • ACC  
  • Increase in the density of CB1-R in NA from subjects with Scz.  
  • Increase in the density of CB1-R in insula and ACC from subjects with Scz. on APs.  
  • Favors the hypothesis that changes in ECS in NA, insula and ACC are associated with the pathology of Scz. |
| Dalton et al.\textsuperscript{27}  | Evaluate the density of CB1-R in brain area involved in Scz. | Observational, transversal, post-mortem analysis of brain tissue using radioligand and autoradiography and quantitative PCR. | • 16 patients with paranoid Scz  
  • 21 patients with non-paranoid Scz  
  • 37 controls  
  • dlPFC, area 46  
  • Increase in the density of CB1-R in dlPFC (area 46) from subjects with paranoid Scz.  
  • No difference in the mRNA expression of CB1-R when comparing patients with schizophrenia to controls.  
  • Favors the hypothesis that changes in ECS in the dlPFC (area 46) are associated with the pathology of paranoid Scz. |
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<td>Leweke et al.</td>
<td>Assess the hypothesis that Scz is related to ECS alterations in CSF.</td>
<td>eCBs quantification through gas-chromatography/mass-spectrometry.</td>
<td>• 10 patients with Scz</td>
<td>CSF</td>
<td>• AEA</td>
<td>No</td>
<td>N.A.</td>
<td>Higher AEA and PEA levels in Scz.</td>
<td>Supports the hypothesis that CSF ECS alterations are involved in the pathophysiology of Scz.</td>
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<td></td>
<td></td>
<td></td>
<td>• 11 controls</td>
<td></td>
<td>• PEA</td>
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<td>Yao et al.</td>
<td>Assess the hypothesis that Scz is related to ECS alterations in blood.</td>
<td>eCBs quantification through gas-chromatography/mass-spectrometry.</td>
<td>• 17 patients with Scz FENN</td>
<td>Blood (plasma)</td>
<td>• AEA</td>
<td>No</td>
<td>N.A.</td>
<td>• Higher AEA levels in patients with Scz FENN than in controls.</td>
<td>Supports the hypothesis that blood ECS alterations are involved in the pathophysiology of Scz.</td>
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<td>• 20 chronic stable Scz patients (with and without APs)</td>
<td></td>
<td>• 2-AG</td>
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<td>• Lower 2-AG levels in patients with Scz FENN than in chronic stable Scz patients (without APs).</td>
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<td>• 20 controls</td>
<td></td>
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<td></td>
<td>• 2-AG levels did not differ between Scz FENN and controls.</td>
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<tr>
<td>De Marchi et al.</td>
<td>Assess the hypothesis that Scz is related to ECS alterations in blood.</td>
<td>eCBs quantification through liquid-chromatography/mass-spectrometry and mRNA through PCR.</td>
<td>• 12 acute Scz patients without treatment</td>
<td>Blood</td>
<td>• AEA (in all groups) • FAAH, CB1-R and CB2-R mRNA (patients in acute phase and remission)</td>
<td>Antipsychotic treatment (olanzapine)</td>
<td>Elevated AEA levels in acute Scz patients.</td>
<td>In the remitted Scz patients subgroup (post-treatment): • Decrease in AEA levels • Decrease in FAAH and CB2-R mRNA • There was no difference in CB1-R mRNA levels.</td>
<td>Supports the hypothesis that blood ECS alterations are involved in the pathophysiology of Scz.</td>
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<td>• Subgroup of 5 remitted Scz patients (post-treatment)</td>
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<td>• 20 controls</td>
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<td>Giuffrida et al.</td>
<td>Assess the hypothesis that eCBs are altered in Scz, but not in other psychiatric disorders.</td>
<td>eCBs quantification through liquid-chromatography/mass-spectrometry.</td>
<td>• 47 patients with paranoid Scz FENN</td>
<td>CSF and blood (serum)</td>
<td>• AEA</td>
<td>No</td>
<td>N.A.</td>
<td>• Higher AEA CSF levels and lower PEA CSF levels in patients with Scz FENN than in controls.</td>
<td>Supports the hypothesis that CSF ECS alterations are involved in the pathophysiology of Scz.</td>
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<td>• 71 patients with paranoid Scz on APs (typical: 36; atypical: 35)</td>
<td></td>
<td>• PEA</td>
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<td>• Higher AEA and PEA CSF levels in patients with Scz on atypical antipsychotic treatment than in controls.</td>
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<td></td>
<td>• 22 patients with affective disorders</td>
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<td>• OEA</td>
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<td></td>
<td></td>
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<td>• 13 patients with dementia</td>
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<td></td>
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<td>• 84 controls</td>
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## The Endocannabinoid System and Its Role in Schizophrenia

The endocannabinoid system (eCBs) plays a crucial role in the pathology of schizophrenia (Scz). Several studies have explored the levels of endocannabinoids such as anandamide (AEA) and palmitoylethanolamide (PEA), and their role in the pathophysiology of Scz.

### Leweke et al. (2011)
- **Assess the hypothesis that the frequency of cannabis use alters CSF and blood AEA levels in Scz.**
- **eCBs quantification through liquid-chromatography/mass-spectrometry.**
- **44 patients with Scz FENN (2 subgroups: high and low-frequency cannabis use)**
- **81 controls (2 subgroups: high and low-frequency cannabis use)**
- **CSF and blood (serum)**
- **AEA, PEA, OEA**
- **No**
- **N.A.**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Details</th>
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<tbody>
<tr>
<td>AEA CSF levels did not differ between Scz patients on typical APs, patients with affective disorders, dementia patients and controls.</td>
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<td>OEA CSF levels did not differ between patients with Scz FENN and controls.</td>
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<td>AEA blood levels did not differ between all patients groups and controls.</td>
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<td>Negative correlation between AEA CSF levels and PANSS in patients with Scz FENN.</td>
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- Supports the hypothesis that CSF ECS alterations are involved in the pathophysiology of Scz.
- Heavy cannabis use in individuals with a hyperactive ECS could down-regulate the central eCB signaling.

### Potvin et al. (2011)
- **Assess the hypothesis that quetiapine reduces drug use in Scz through ECS modulation.**
- **Intervention study. eCBs quantification through liquid-chromatography/mass-spectrometry.**
- **27 patients with Scz and substance use**
- **17 controls**
- **Blood (plasma)**
- **AEA, 2-AG, PEA, OEA**
- **Antipsychotic treatment (quetiapine)**

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<thead>
<tr>
<th>Outcome</th>
<th>Details</th>
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<tbody>
<tr>
<td>Elevated AEA, PEA and OEA levels in Scz.</td>
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<td>2-AG levels did not differ between Scz and controls.</td>
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<td>Results do not support the hypothesis that quetiapine reduces drug use in Scz through ECS modulation.</td>
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### Summary
- Increased central AEA levels would be a modulatory response to the increase of dopamine in psychosis (inhibitory feedback).
- Higher AEA CSF levels in patients with low-frequency cannabis use than patients with high-frequency cannabis use and controls.
- AEA, PEA and OEA blood levels did not differ between patients and controls.
- Negative correlation between AEA CSF levels and PANSS in patients with Scz FENN and low-frequency cannabis use.
| Koethe et al. | Assess the hypothesis that the increase in CSF AEA levels is present in the initial phases of Scz. | eCBs quantification through liquid-chromatography/mass-spectrometry. | 27 patients with initial prodromal states of psychosis | CSF and blood (serum) | AEA | No | N.A. | Patients improved substance use severity scores and positive, negative and depressive symptoms. |

| Schwarz et al. | Assess alterations in pFAA levels in Scz. | pFAA quantification through liquid-chromatography/mass-spectrometry. | 70 patients with paranoid Scz FENN | Blood (serum) | pFAA | No | N.A. | Higher AEA CSF levels in patients with initial prodromal states of psychosis than in controls. AEA blood levels did not differ between patients and controls. OEA CSF and blood levels did not differ between patients and controls. Negative correlation between CSF AEA levels and PANSS (cognitive syndrome). Results support the hypothesis that central AEA signaling would protect against psychosis development. |

Supports the hypothesis that ECS alterations are involved in the pathophysiology of Scz.
the CSF and the PANSS score, but only in the low consumption patient subgroup. The authors suggested that heavy cannabis use by subjects with a hyperactive ECS may down-regulate AEA signalling in the central nervous system and disrupt the eCB modulation over the dopaminergic system.39

Koethe et al.40 tested the hypothesis that the increase in eCBs in schizophrenia could be detected in the early stages of the disorder. Thus, they measured the levels of AEA and OEA in 27 psychotic patients in the prodromic phase and 81 controls. The levels of AEA, but not OEA were increased in the patients. Again, there was an inverse correlation between the PANSS score, although only with the cognitive dimensions. Patients in prodromatic stage with higher levels of AEA in the CSF tend to develop less psychosis, supporting the hypothesis that the ECS might exert a modulatory role upon the dopaminergic system that, in turn, protect against positive symptoms. Giusfrida et al.,38 Leweke et al.39 and Koethe et al.40 also measured the levels of AEA, PEA and OEA in the blood (serum), but did not observe differences in relation to controls. By contrast Yao et al.41 measured AEA and 2-AG in 17 untreated first episode patients with schizophrenia, 20 stable patients and 20 controls. They observed an increase in AEA in the first group in relation to controls, and reduced levels of 2-AG in relation to stable patients. Shwarz et al.42 measure fatty acid amides (FAAs, a class of lipids that include the eCBs) in 70 untreated patients with paranoid schizophrenia, 74 patients with paranoid schizophrenia undergoing antipsychotic therapy (34 with atypical and 40 with typical antipsychotic), 37 patients with mood disorders and 59 controls. They observed that the levels of FAAs were increased in untreated patients with schizophrenia in relation to controls and that these levels were normalized in patients treated with typical, but not atypical antipsychotic.

Finally, two studies evaluated the blood levels of eCBs before and after treatment with antipsychotics. De Marchi et al.43 measured AEA and mRNA for FAAH, for CB1-R and for CB2-R in the blood of 12 patients with schizophrenia presenting acute psychosis and 20 controls. Before treatment, AEA levels were higher in patients, but after olanzapine treatment and improvement in positive symptoms in 5 patients, AEA levels were similar to controls. There was also a reduction in FAAH and CB2-R mRNA, but not CB1-R mRNA. Potvin et al.44 tested the hypothesis that quetiapine could help to reduce drug abuse in patients with schizophrenia through modulation of the ECS. They quantified the levels of AEA, 2-AG, PEA and OEA in 27 addicted patients with schizophrenia and 17 controls. Before treatment, the levels of AEA, PEA and OEA, but not 2-AG, were increased. After 12 weeks on quetiapine treatment, there was a reduction in drug abuse and improvement in positive, negative and depressive symptoms, but no reduction in AEA, PEA or OEA levels.

In summary, four studies detected an increase in the levels of AEA in the CSF.37-40 Regarding measures in the blood, three also reached this result,38,41-43 whereas other three did not find any difference.39,40 The levels of 2-AG in the blood were found to be reduced in one study41 and unchanged in another.44 The levels of OEA in the CSF did not differ from controls in two studies,38,39 although in the blood they were either higher44 or unchanged.39,40 One study found that the levels of PEA in the CSF were increased in patients receiving atypical antipsychotics and reduced in the untreated.38 Other studies found that the blood levels were either increased44 or unchanged.43 The treatment with atypical antipsychotics was inversely correlated with the levels of AEA in the blood in two studies;38,43 but another one did not find any change.44 As for typical AP, their use changed AEA and FAAs to levels similar to controls.38,42

Conclusion

The present systematic review described the literature investigating changes in the ECS in patients suffering from schizophrenia. The original papers reviewed here studied genetic polymorphisms, the expression of cannabinoid receptors in specific brain regions and levels of eCBs in CSF or blood.

So far, it is difficult to draw any consistent theory on the role of the ECS in this major psychiatric disorder. Taking into account the acute effects of Cannabis sativa and cannabinoids, which induce psychotomimetic effects, and the epidemiologic evidence suggesting that chronic consumption of Cannabis may be a predisposing factor to schizophrenia,10,11,12 there is a rationale to link changes in the ECS to symptoms in this disorder. Indeed, an endocannabinoid hypothesis of schizophrenia has been proposed.44 Nonetheless, from the studies retrieved in our review, no clear picture has emerged.

This topic is relevant not only for theoretical reasons. The current pharmacological therapy of schizophrenia is limited to the antagonism of dopamine receptors, which presents limited efficacy and significant side effects. Thus, alternative pharmacological strategies must be pursued and one approach involves the characterization of other neurotransmitters systems affected in this disorder. Such a strategy has been put into practice, for instance, with the glutamate system. Based on the theory that schizophrenia might be related to a low functioning of glutamate, there have been attempts to develop drugs that enhance this neurotransmitter.6,7 As for the ECS, it has been investigated whether CB1-R antagonism induces antipsychotic effects, as a corollary to the psychotomimetic effects of cannabinoids, which activate this receptor. However, results to date have been mixed.47 Studies in experimental animals have also been inconsistent.48

In conclusion, despite several studies investigating changes in the ECS in schizophrenia, it remains uncertain whether a malfunctioning of this system would be consistently related to the disorder.

Disclosures

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The authors have no conflict of interest related to the topic of this article.

* Modest
** Significant
*** Significant. Amounts given to the author’s institution or to a colleague for research in which the author has participation, not directly to the author.
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