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Characterization of peripheral cannabinoid receptor expression and clinical correlates in schizophrenia

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ABSTRACT

The relationship between cannabinoid receptor signaling and psychosis vulnerability requires further exploration. The endocannabinoid signaling system is extensive, with receptors exerting regulatory functions in both immune and central nervous systems. In the brain, cannabinoid receptors (CBR) directly modulate neurotransmitter systems. In the peripheral lymphocyte, CBRs mediate cytokine release, with dysregulated cytokine levels demonstrated in schizophrenia. mRNA levels of CBRs were measured in human peripheral blood mononuclear cells (PBMCs) obtained from 70 participants (35 non-clinical controls, 35 participants with schizophrenia), who were recruited for the absence of marijuana use/abuse by self-report. Changes in mRNA expression were measured using qRT-PCR. Clinical measurements collected included the MATRICS Cognitive Battery and the Positive and Negative Syndrome Scale. Levels of CB1R and CB2R mRNA in PBMCs were significantly higher in participants with schizophrenia compared to the non-clinical controls. Additionally, CB1R and CB2R mRNA levels correlated with impairments in cognitive processing and clinical symptom severity in multiple domains. These results continue to support dysregulation of particular aspects of the endocannabinoid signaling system in participants with schizophrenia selected for the self-reported absence of marijuana abuse/dependence.

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1. Introduction

The endogenous cannabinoid system (ECS) is extensive, with receptors exerting multiple regulatory functions in both immune and central nervous systems. This system can impact motor function, appetite, pain sensitivity, emotional processing, and cognition – specifically attention, learning and memory (DeRosse et al., 2010; Rabin et al., 2011; Greineisen and Turner, 2010; Bossong et al., 2014). Signaling is initiated by two subtypes of cannabinoid receptors, CB1R and CB2R. In peripheral mononuclear blood cells (PBMCs), both CB1R and CB2R are shown to either increase or suppress T-cell and B-cell functions, such as cell activation, chemotaxis, cytokine production, depending on whether the ligand is an endocannabinoid or exocannabinoid (Suarez-Pinilla et al., 2015; Klein et al., 1998; Xiu et al., 2012; Kim et al., 2009; Greineisen and Turner, 2010; Chase et al., 2015). We, and others, have reported elevated levels of cytokines in schizophrenia (Chase et al., 2015). Given these converging lines of evidence implicating both the immunological system as well as cannabinoids in schizophrenia, this signaling path becomes an opportune investigational avenue to explore in peripheral immune cells from participants with schizophrenia.

Engagement of the ECS in cannabis users, especially in early adolescence, increases risk for both developing schizophrenia at an earlier age and with greater severity of symptoms (D'Souza et al., 2005; Casadio et al., 2011; Rodriguez-Sanchez et al., 2010). Use of cannabis increases the risk of schizophrenia with an estimated odds ratio of 2.10–2.93 (Henquet et al., 2005; Moore et al., 2007), and it decreases the age of schizophrenia onset (Sugranyes et al., 2009; D'Souza et al., 2005; Casadio et al., 2011). Participants with schizophrenia have odds ratios of 1.45 for lifetime cannabis abuse and 2.72 for lifetime cannabis dependence (Davis et al., 2013). The intersection between cannabinoids and schizophrenia is complicated by this fact that it remains unclear whether increased cannabis use during the schizophrenia prodrome or symptomatic periods is a pathophysiological contributor to schizophrenia such as hastening schizophrenia onset (Gage et al., 2016), or to the contrary, as a means of self-medication of an innately. dysregulated ECS. Genetic variation of the *CB1R* gene has been suggested as a schizophrenia susceptibility locus

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(Suarez-Pinilla et al., 2015; Ho et al., 2011; Ferretjans et al., 2012) which along with the continued abuse of cannabis even long after development of psychosis (Fowler et al., 1998) supports the self-medication/innate-susceptibility hypothesis. Moreover, measuring cannabis pathway mediators is further complicated by individual factors being up- or down regulated depending upon whether the measure was taken during a period of acute intoxication, chronic use and dependence, or withdrawal.

Our goal was to examine the role of the ECS in PBMCs of a clinical schizophrenia population, who were recruited for their absence of marijuana use/abuse. It should be noted that this is an important distinction, as a considerable percentage (30–50%) of persons with schizophrenia are regular marijuana users (Green et al., 2005). As the literature indicates variant measures of the ECS system in PBMCs from participants with schizophrenia are correlated with worse cognitive performance (Bioque et al., 2013; Ferretjans, 2014; Bioque et al., 2016), we sought to replicate these cognitive associations using the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (Nuechterlein and Green, 2006; Nuechterlein et al., 2008; Kern et al., 2008). Thus, our research sought to answer the following questions: 1) Are CB1R and CB2R mRNA levels elevated in PBMC from participants with schizophrenia when compared to a non-clinical control group? 2) Are CB1R and CB2R mRNA levels associated with MATRICS Cognitive Battery domains of Attention, Processing Speed, and Working Memory in participants with schizophrenia compared to a non-clinical control group? 3) Are CB1R and CB2R mRNA levels associated with clinical symptomology as measured by the Positive and Negative Syndrome Scale (PANSS) in participants with schizophrenia compared to a non-clinical control group?

2. Method

2.1. Participant information

The sample included 70 participants, 35 non-clinical controls (17 males and 18 females) and 35 participants with schizophrenia (18 males and 17 females) between the ages of 21–62. Exclusion criteria included current substance abuse/dependence (specifically alcohol, opiates, cocaine, methamphetamine and marijuana/cannabinoids), seizure disorders, and neurological conditions. Illicit substance use was obtained as part of the SCID interview to determine current and past cannabis abuse/dependence although we did not confirm this with urine or blood drug-toxology. History and patterns of cannabis use were documented as part of the SCID interview and participants were selected for the absence of cannabis use within the last 12 months. For control participants, additional exclusionary criteria included a major Axis I disorder and a known first-degree familial history of psychosis. Demographic characteristics, clinical metrics, and peripheral blood sample were obtained at study participation. The study was approved by the Institutional Review Board at the University of Illinois at Chicago and all participants provided written informed consent before participating in any study procedures. The DSM-IV-TR and the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, research version, patient edition (SCID) were used in the diagnostic procedure (First et al., 2002; American Psychiatric Association, 2000). The SCID interview was administered by experienced diagnosticians (MD or PhD) and a consensus diagnoses was determined prior to diagnostic group assignment.

At the time of sampling, 43% (n=15) of the participants with schizophrenia were evaluated while hospitalized on the inpatient psychiatric unit and 57% (n=20) were evaluated in the psychiatric outpatient clinic. Prescribed antipsychotic medication was coded on all participants as follows: typical antipsychotic 9% (n=3), atypical antipsychotic 83% (n=29) and unmedicated 9% (n=3). Due to this heterogeneity, all antipsychotic use was converted to Chlorpromazine (CPZ) units (Danivas and Venkatasubramanian, 2013; Gardner et al., 2010).

2.2. Clinical measures

The measures of the MATRICS Consensus Cognitive Battery utilized in this study consisted of the Trails, Symbol coding, HVLT, WMS spatial span, Letter number span, Mazes, BVMT, Verbal fluency, and Continuous performance test (Nuechterlein and Green, 2006; Nuechterlein et al., 2008). The Trails A subtest is a simple measure of sustained attention, visual scanning, and psychomotor processing speed. The Symbol coding subtest assesses visual-motor coordination as well as psychomotor and mental speed. The HVLT subtest is a measure of verbal learning, while the BVMT subtest is a measure of visual learning, both of which are based on three trials. The WMS spatial span subtest is a measure of visual working memory, while the Letter number span subtest measures verbal working memory. The Mazes subtest is a measure of executive functioning and assesses problem-solving abilities. The Verbal fluency subtest is a test of semantic fluency, using animals as the category. The Continuous performance test is a test of sustained attention and measures impulsive response styles. For the present study, emphasis was placed on the domains of Attention, Processing Speed, and Working Memory, as these domains are particularly affected in the psychopathology of schizophrenia (Kern et al., 2008; Kern et al., 2011; Palmer et al., 2010; Dickinson et al., 2007; Reichenberg et al., 2010). The MATRICS Consensus Cognitive Battery discriminative analyses of persons with schizophrenia spectrum disorder compared to community residents on the seven cognitive domains showed that Processing Speed and Working Memory are most impaired in persons with schizophrenia (Kern et al., 2011). Additionally, lower scores in the Attention domain further demonstrated a discriminative function within schizophrenia. Lower MATRICS scores indicate worse performance.

PANSS was scored along a continuum of severity between one (asymptomatic) to seven (extreme symptom severity) (Kay et al., 1987). Scores were calculated for five-factors: Positive symptoms (delusions, grandiosity, suspiciousness/persecution, unusual thought content), Negative symptoms (blunted affect, emotional withdrawal, poor rapport, passive/apathetic social withdrawal, lack of spontaneity and flow of conversation, and active social avoidance), Cognitive Disorganization (conceptual disorganization, difficulty in abstract thinking, mannerisms and posturing, disorientation, and poor attention), Depression (somatic concern, anxiety, guilt feelings, depression, and preoccupation) and Excitement (excitement, hostility, tension, and poor impulse control). Items were pooled in this way based on previous factor analytic findings (Lindenmayer et al., 1994; Lehoux et al., 2009; Wallwork et al., 2012).

2.3. Peripheral blood mononuclear cells (PBMCs) isolation

A blood sample was obtained by sterile venipuncture and collected in 0.5 M EDTA. PBMCs were extracted utilizing the Ficoll-Paque® method (GE Healthcare Life Sciences). Subsequent washing of the cream-colored interlayer was performed using a Hanks Balanced Salt Solution (Gibco #14170-161) to remove any remaining platelets, plasma or other contaminants. PBMC samples were pelleted at 2000 RPM for ten minutes at 10 °C and frozen in −80 °C until further processing (Gavin et al., 2009; Jayaraman et al., 1999).