Archival Report

Biological Psychiatry

Reduced Brain Cannabinoid Receptor Availability in Schizophrenia

Mohini Ranganathan, Jose Cortes-Briones, Rajiv Radhakrishnan, Halle Thurnauer, Beata Planeta, Patrick Skosnik, Hong Gao, David Labaree, Alexander Neumeister, Brian Pittman, Toral Surti, Yiyun Huang, Richard E. Carson, and Deepak Cyril D'Souza

ABSTRACT

BACKGROUND: Several lines of evidence suggest the presence of abnormalities in the endocannabinoid (eCB) system in schizophrenia (SCZ). However, there are limited in vivo measures of the eCB system in SCZ.

METHODS: Twenty five male SCZ subjects (SCZs) (18 antipsychotic treated and 7 antipsychotic free) were compared with 18 age-matched male healthy control subjects (HCs). Subjects underwent one positron emission tomography scan each with the cannabinoid receptor-1 (CB_1R) selective radiotracer [^{11}C]OMAR on the high resolution research tomography scanner. Regional volume of distribution (V_T) values were determined using kinetic modeling of positron emission tomography data as a measure of CB_1R availability. Group differences in mean composite [^{11}C]OMAR V_T values were compared between SCZs and HCs. Exploratory comparisons of CB_1R availability within 15 brain regions were also conducted. All analyses were covaried for age and body mass index.

RESULTS: SCZs showed significantly ($p = .02$) lower composite [^{11}C]OMAR V_T relative to HCs (~12% difference, effect size $d = .73$). [^{11}C]OMAR V_T was significantly (all $ps < .05$) lower in SCZs in the amygdala, caudate, posterior cingulate cortex, hippocampus, hypothalamus, and insula. Composite [11]OMAR VT was HCs > antipsychotic treated SZCs > antipsychotic free SZCs. Furthermore, composite [^{11}C]OMAR V_T was greater in HCs than SCZ smokers ($n = 11$) and SCZ nonsmokers ($n = 14$).

CONCLUSIONS: CB_1R availability is lower in male SCZ subjects compared with HCs. Furthermore, antipsychotics and tobacco use may increase CB_1R availability in this population. The findings of the study provide further evidence supporting the hypothesis that alterations in the eCB system might contribute to the pathophysiology of SCZ.

Keywords: Antipsychotics, Cannabinoid, CB_1R, OMAR, Schizophrenia, Smoking

http://dx.doi.org/10.1016/j.biopsych.2015.08.021

Emerging evidence suggests the presence of abnormalities in the endocannabinoid (eCB) system in schizophrenia (SCZ) (i.e., the endogenous hypothesis). This is distinct from the better known exogenous hypothesis, according to which exogenous cannabinoids can induce transient schizophrenia-like effects in healthy individuals, exacerbate psychotic symptoms, trigger relapse, and negatively impact the course of illness in SCZ patients. Also, heavy exposure to exogenous cannabinoids in adolescence may contribute to the risk of later developing SCZ (1–6).

Several groups have reported elevated eCB levels in the blood or cerebrospinal fluid (CSF) of patients with SCZ (7–11). Furthermore, eCB levels are inversely correlated with psychotic symptoms and normalize following treatment with antipsychotics (8–10) and with clinical remission (12). The results of postmortem studies have been mixed (Supplemental Table S1) with studies reporting increases, decreases, or no changes in either cannabinoid receptor-1 (CB_1R) protein or messenger RNA (mRNA) levels in SCZ (13–23). While 4 of 6 studies that used in vitro autoradiography reported significantly increased CB_1R binding in SCZ subjects (SCZs) compared with healthy control subjects (HCs), 5 of 6 studies using immunodetection methods reported either a decrease or no change in CB_1R density. These mixed results could be due to differences in methodologies, the regions studied, or the presence of comorbidities in the patient groups.

A related issue is the effect, if any, that antipsychotic treatment may have on the eCB system. Reports on the effects of antipsychotics on CB_1R availability are mixed (24–26). Eggan *et al.* (17,18) and Volk *et al.* (23) reported that CB_1R mRNA and protein levels were significantly lower in the dorsolateral prefrontal cortex of SCZs as compared with HCs in a postmortem human study but no differences were noted between medicated ($n = 19$) and unmedicated ($n = 4$) SCZs (18). Uriguen *et al.* (19) reported reduced postmortem CB_1R density in SCZs compared with HCs that was primarily noted in antipsychotic-treated but not in drug-free SCZs. In a second cohort that also included unmedicated SCZs ($n = 6$), Eggan *et al.* (17) suggested that antipsychotic treatment might blunt the decrease in CB_1R immunoreactivity levels observed in SCZ. Others have shown in animals that treatment with haloperidol (27), risperidone (28), and clozapine (29) resulted in altered CB_1R density in various brain regions.

SEE COMMENTARY ON PAGE e97

Positron emission tomography (PET) imaging provides a means to study CB_1R availability in SCZs in vivo. Several CB_1R ligands have been developed for PET, including [^{18}F]MK-9470 and [^{11}C]OMAR (30). In the first PET study of CB_1R in SCZ, Wong *et al.* (31) reported elevated [^{11}C]OMAR binding across the brain in SCZs (n= 10), all receiving antipsychotic medications. The mean difference was small and only significant in the pons. More recently, Ceccarini *et al.* (32) reported CB_1R binding in a larger sample of SCZs on (n = 51) and off (n = 16) antipsychotics and 12 cannabis naïve, age-matched, and gender-matched control subjects (n = 12) using [^{18}F]MK-9470 PET. Of note, [^{18}F]MK-9470 shows primarily irreversible binding, while [^{11}C]OMAR shows reversible binding, a characteristic favored for quantitative receptor imaging (33). Compared with HCs, SCZs displayed increased global gray matter [^{18}F]MK-9470 uptake. Volume of interest analyses revealed significant increases of [^{18}F]MK-9470 uptake in several brain regions in SCZs. Further, cannabis or tobacco use and antipsychotic medication type did not appear to influence [^{18}F]MK-9470 uptake.

While this study has drawn attention to the possibility of CB_1R alterations in SCZ, the kinetic characteristics of [^{18}F]MK-9470 pose challenges in quantification, and furthermore, the validity of the modified standardized uptake values (mSUV) technique used has been challenged by several groups (34,35).

In the current study, we measured CB_1R availability in SCZs using the reversible ligand [^{11}C]OMAR, based on absolute quantification using arterial sampling with metabolite analysis and tracer kinetic modeling analysis that does not have the limitations associated with the mSUV method. Based on the postmortem data of Eggan *et al.* (17), SCZs were hypothesized to show lower CB_1R availability compared with HCs.

METHODS AND MATERIALS

Approvals

This study was approved by the Yale University and Veterans Affairs Connecticut Healthcare System Institutional Review Boards, the Yale Magnetic Resonance Research Center, and the Yale New Haven Hospital Radiation Drug Research Committee. All subjects signed informed consent after the study was explained to them in detail.

Subjects

Male SCZs on and off antipsychotics and age-matched (±3 years) HCs completed a comprehensive screening process that included psychiatric, medical, and neurological evaluations by a research physician as reported in the Supplement.

Assessments

Positive and negative symptoms of SCZ were assessed using the Positive and Negative Syndrome Scale (PANSS) (36).

Imaging

Magnetic resonance imaging scans were conducted before the PET scans as described in the Supplement.

Before PET scanning, urine drug toxicology was repeated and intravenous lines and an arterial catheter were placed. PET scans were acquired as subjects rested in the high-resolution research tomograph scanner (207 slices, resolution better than 3 mm full width at half maximum in three-dimensional acquisition mode) (Siemens Medical Systems, Knoxville, Tennessee). Procedures for PET imaging, metabolite analysis, and measurement of arterial input functions were the same as those previously described (37). In addition, the fraction of plasma [^{11}C]OMAR unbound to protein was determined by ultrafiltration.

Image Analysis

Summed PET images were registered to the subject's T1-weighted magnetic resonance images, which, in turn, were registered to a magnetic resonance template. Gray matter regions of interest (ROIs) were predefined on a template (Anatomical Automatic Labeling for SPM2, Cyceron, Caen, France). This process permitted direct, automatic determination of volume of distribution (V_T) values using the multilinear analysis-1 method with t^* = 30 minutes (38), the preferred kinetic modeling method for [^{11}C]OMAR. V_T is the ratio at equilibrium of total tracer to that in plasma, including free, nonspecifically bound, and specifically bound tracer. Exploratory comparisons of [^{11}C]OMAR V_T levels were examined in 15 regions, some of which are implicated in the circuitry underlying the behavioral and cognitive effects of cannabinoids. The ROIs included the amygdala, globus pallidus, caudate, putamen, hippocampus, hypothalamus, cerebellum, thalamus, and cerebral cortices (insula, anterior cingulate, posterior cingulate, temporal, frontal, parietal, and occipital).

Statistical Analyses

Whole-brain composite [^{11}C]OMAR V_T levels were compared among diagnostic groups (SCZs vs. HCs) using analysis of covariance with age and body mass index (BMI) included as covariates. Composite [^{11}C]OMAR V_T values were calculated as a mean of the regional [^{11}C]OMAR V_T values using the justification and methods in Neumeister *et al.* (39), because regional [^{11}C]OMAR V_T values were highly correlated (r values $>$ .91 in both HCs and SCZs). Group differences in regional [^{11}C]OMAR V_T values were analyzed using linear mixed models with diagnostic group (SCZs or HCs) as a between-subjects factor and region as a within-subjects factor. The interaction between group and region was modeled and age and BMI served as covariates. The best-fitting variance-covariance structure was selected based on information criteria. Additional, exploratory analyses were conducted within levels of medication and smoking. Total PANSS score was included as a covariate in all models restricted to SCZ subjects. Correlations were explored between regional CB_1R availability and PANSS total and subscale scores. Given the exploratory nature of the regional analysis and correlations, results are not corrected for multiple comparisons. All tests were significant at the alpha = .05 threshold.

RESULTS

Twenty-five male SCZs, 7 of whom were antipsychotic free (SCZ-UNMED) and 18 receiving antipsychotic treatment (SCZ-MED), and 18 age-matched (±3 years) male HCs were studied. Tables 1 and 2 show demographic and clinical characteristics of the groups. [^{11}C]OMAR injection parameters did not differ among the groups.