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Reciprocal alterations in cortical cannabinoid receptor 1 binding relative to protein immunoreactivity and transcript levels in schizophrenia

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ABSTRACT

The deleterious effects of cannabis use in schizophrenia have been linked, in part, to underlying disturbances in endogenous cannabinoid signaling in the prefrontal cortex. However, while receptor autoradiography studies of the primary cannabinoid receptor (CB1R) have consistently found higher CB1R binding in the prefrontal cortex in schizophrenia, deficits in CB1R mRNA levels and protein immunoreactivity have also been reported in the illness. To investigate this apparent discrepancy, we quantified CB1R binding using receptor autoradiography with the selective CB1R ligand [^{3}H]-OMAR in the prefrontal cortex of 21 subjects with schizophrenia who were previously found to have lower levels of both CB1R mRNA using in situ hybridization and CB1R protein using radioimmunocytochemistry relative to matched healthy comparison subjects. We observed higher levels of [^{3}H]-OMAR binding in the prefrontal cortex of schizophrenia subjects that did not appear to be attributable to psychotropic medications or substance abuse. The combination of lower levels of CB1R mRNA and immunoreactivity with higher CB1R receptor binding may reflect 1) altered trafficking of the receptor resulting in higher levels of membrane-bound CB1R or 2) higher CB1R affinity. In either case, greater CB1R receptor availability may contribute to the increased susceptibility of schizophrenia subjects to the deleterious effects of cannabis use.

1. Introduction

Cannabis use during early adolescence has been linked to a higher risk of developing schizophrenia, an earlier age of onset, and greater illness severity (D'Souza et al., 2005; Compton et al., 2009; Foti et al., 2010; Casadio et al., 2011; Galvez-Buccollini et al., 2012). These associations may indicate that the disease process of schizophrenia involves disturbances in endogenous cannabinoid signaling which may in turn predispose at-risk adolescents to greater debilitating effects of cannabis use. However, at the present time, studies of the primary endogenous cannabinoid receptor (CB1R) in the brains of individuals with schizophrenia have yielded apparently conflicting results. Receptor autoradiography studies using a CB1R agonist ([^{3}H]-CP55940) or antagonists/inverse agonists ([^{3}H]-SR141716 and [^{3}H]-MePPEP) have consistently found higher CB1R binding to receptor protein across multiple brain regions, including the prefrontal cortex, in schizophrenia subjects (Dean et al., 2001; Zavitsanou et al., 2004; Newell et al., 2006; Dalton et al., 2011; Jenko et al., 2012). By contrast, we previously reported lower CB1R mRNA levels by in situ hybridization and CB1R protein levels using radioimmunocytochemistry in the prefrontal cortex of subjects with schizophrenia (Eggan et al., 2008, 2010b). Furthermore, other groups have reported either lower or unchanged levels of CB1R immunoreactivity and mRNA in the prefrontal cortex and the anterior cingulate cortex (Koethe et al., 2007; Uriguen et al., 2009). The reason for this discrepancy in CB1R measures in schizophrenia has remained unclear.

Interestingly, binding of an allosteric modulation site has been reported to induce a conformational change in CB1R, which in turn increases the affinity of ligands such as [^{3}H]-CP55940 for the orthosteric binding site on CB1R (Price et al., 2005). Thus, reports of higher CB1R binding in the prefrontal cortex in schizophrenia may reflect greater receptor affinity, perhaps even in the presence of fewer CB1Rs. The recent development of a novel analog of the selective CB1R inverse agonist rimonabant, OMAR (JHU75528; 4-cyano-1-(2,4-dichlorophenyl)-5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide), that displays a high binding affinity for CB1R (Fan et al., 2006) provides an opportunity to examine this idea. Indeed, a pilot PET study employing radiolabeled [^{11}C]-OMAR reported evidence of higher brain CB1R receptor binding in some

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subjects with schizophrenia (Wong et al., 2010) and of an inverse correlation between CB1R receptor binding and negative symptoms assessed using the Brief Psychiatric Rating Scale (Wong et al., 2012). Consequently, we conducted a receptor autoradiography study using [^{3}H]-OMAR in the same schizophrenia subjects in whom we had previously found lower CB1R mRNA levels by in situ hybridization and protein levels by radioimmunocytochemistry. Employing a highly similar film-based quantification approach across studies, we sought to determine the relationship between CB1R binding and CB1R mRNA and immunoreactivity levels in nearby tissue sections from the same subjects with schizophrenia.

2. Materials and methods

2.1. [^{3}H]-OMAR ([methoxy-3H]JHU75528)

The radiotracer was purchased from PerkinElmer (Shelton, Connecticut). The specific radioactivity was >80 Ci/mmol and radiochemical purity was 97.0%.

2.2. Human subjects

Brain specimens were obtained during routine autopsies conducted at the Allegheny County Office of the Medical Examiner (Pittsburgh, PA) after consent was obtained from next-of-kin. An independent committee of experienced research clinicians made consensus DSM-IV (American Psychiatric Association, 1994) diagnoses for each subject using structured interviews with family members and review of medical records, and the absence of a psychiatric diagnosis was confirmed in healthy comparison subjects (Volk et al., 2011, 2012). To control for experimental variance, subjects with schizophrenia or schizoaffective disorder ($n = 21$) were matched individually to one healthy comparison subject for sex and as closely as possible for age and postmortem interval (Table 1; Supplemental Table S1) as previously described (Eggan et al., 2008), and samples from subjects in a pair were processed together throughout all stages of the study. The mean age, postmortem interval, brain pH, and tissue freezer storage time did not differ between subject groups ($t_{(40)} \leq 0.67$, $p \geq 0.51$) (Table 1). In the right hemisphere of all subject pairs, CB1R mRNA and protein levels were previously quantified by in situ hybridization and radioimmunocytochemistry, respectively (Eggan et al., 2008). (Two subject pairs from this previous study of CB1R mRNA and protein levels were not included in the present study due to lack of tissue availability.) All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research Involving the Dead and Institutional Review Board for Biomedical Research.

2.3. Tissue processing

For each subject, coronal blocks through the right prefrontal cortex were frozen and stored at −80 °C. Cryostat sections (20 μm) from the middle portion of the superior frontal sulcus were thaw mounted on Superfrost slides (VWR Scientific, West Chester, Pennsylvania) and stored at −80 °C. Cytoarchitectonic criteria (Rajkowska and Goldman-Rakic, 1995) were used to identify the location of area 9 in Nissl-stained sections (Volk et al., 2000). For each subject, 3 sections separated by at least 320 μm and at a similar rostral–caudal level to the matched subject within the pair were processed for quantitative receptor autoradiography. The selected sections were located within the same tissue block as the tissue sections previously studied for CB1R mRNA using in situ hybridization and protein using radioimmunocytochemistry.

2.4. CB1R autoradiography with [^{3}H]-OMAR

To our knowledge, [^{3}H]-OMAR has not been employed previously in radiolabeled ligand binding studies of human prefrontal cortex tissue. Consequently, we tested a wide range of concentrations of [^{3}H]-OMAR

Table 1
Summary of demographic and postmortem characteristics of human subjects.

Parameter	Healthy comparison	Schizophrenia
N	21	21
Sex	16M/5F	16M/5F
Race	18W/3B	14W/7B
Age (years)	49.0 ± 14.9	48.9 ± 14.0
Postmortem interval (hours)	18.6 ± 5.5	18.5 ± 9.2
Freezer storage time (months)	179.9 ± 22.9	184.1 ± 23.3
Brain pH	6.87 ± 0.24	6.81 ± 0.35

For all, $t_{(40)} \leq 0.67$, $p \geq 0.51$. Values are group means ± standard deviation.

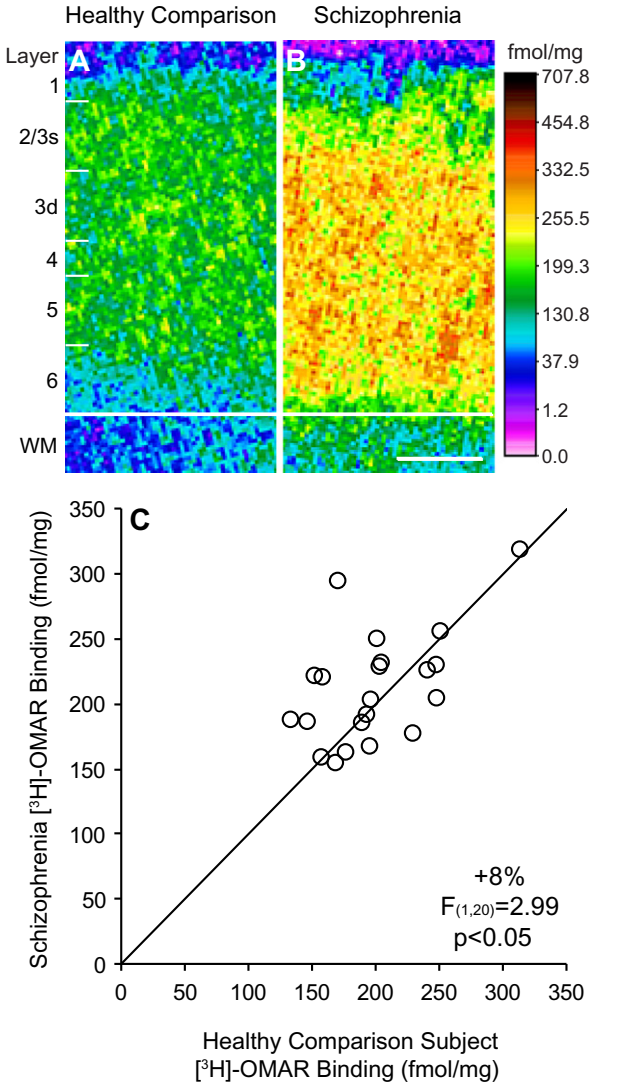

Fig. 1. Receptor autoradiography for [^{3}H]-OMAR binding in the prefrontal cortex in schizophrenia. A–B. Pseudocolored film autoradiographs of prefrontal cortical sections processed by receptor autoradiography demonstrate higher [^{3}H]-OMAR binding in a schizophrenia subject (B) relative to the matched comparison subject (A). Solid white line indicates the layer 6/white matter (WM) border; white distance calibration bar = 1 mm. C. Average [^{3}H]-OMAR binding levels across the gray matter of prefrontal cortical area 9 for schizophrenia subjects relative to matched healthy comparison subjects in a pair are indicated by open circles. Data points to the left of the unity line indicate higher [^{3}H]-OMAR binding levels in the schizophrenia subject relative to the healthy comparison subject and vice versa. Mean [^{3}H]-OMAR binding was 8% higher in schizophrenia subjects relative to matched healthy comparison subjects.