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Endocannabinoid metabolism in the prefrontal cortex in schizophrenia

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ABSTRACT

Adolescent cannabis use is associated with greater relative risk, increased symptom severity, and earlier age of onset of schizophrenia. We investigated whether this interaction may be partly attributable to disease-related disturbances in metabolism of the major cortical endocannabinoid 2-arachidonoylglycerol (2-AG). Transcript levels for the recently discovered 2-AG metabolizing enzyme, α-β-hydrolase domain 6 (ABHD6), were assessed using quantitative PCR in the prefrontal cortex of schizophrenia and healthy subjects (n = 84) and antipsychotic- or tetrahydrocannabinol-exposed monkeys. ABHD6 mRNA levels were elevated in schizophrenia subjects who were younger and had a shorter illness duration but not in antipsychotic- or tetrahydrocannabinol-exposed monkeys. Higher ABHD6 mRNA levels may increase 2-AG metabolism which may influence susceptibility to cannabis in the earlier stages of schizophrenia.

1. Introduction

Cannabis use, particularly during adolescence, has been associated with increased symptom severity in schizophrenia, a higher risk of developing the disorder, and an earlier age of illness onset (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 be partly attributable to preexisting disturbances in endogenous cannabinoid signaling in the prefrontal cortex (PFC) (Eggan et al., 2008). Unfortunately, the major endocannabinoid in the PFC, 2-arachidonoylglycerol (2-AG), cannot be measured in postmortem human brain (Palkovits et al., 2008). We previously reported that mRNA levels for synthesizing (diacylglycerol lipase α and β) and metabolizing (monoglyceride lipase) enzymes for 2-AG were not altered in the PFC in schizophrenia (Volk et al., 2010). However, the serine hydrolase α-β-hydrolase domain 6 (ABHD6) was recently discovered to metabolize 2-AG and to tightly regulate 2-AG signaling in the PFC (Marrs et al., 2010). Furthermore, in vitro studies have demonstrated that overexpression of ABHD6 leads to higher levels of 2-AG metabolism, while RNA silencing of ABHD6 mRNA and selective inhibitors of ABHD6 lower 2-AG metabolism (Marrs et al., 2010, 2011; Navia-Paldanius et al., 2012). Given the ability of ABHD6 to regulate 2-AG levels, we sought to further investigate the status of 2-AG metabolism in schizophrenia by quantifying ABHD6 mRNA levels in the PFC.

2. Methods

2.1. Human subjects

Brain specimens were obtained during autopsies conducted at the Allegheny County Medical Examiner's Office after consent was obtained from next-of-kin. Independent, experienced research clinicians made consensus DSMIV diagnoses for each subject using structured interviews with family members and review of medical records (Volk et al., 2010). To control for experimental variance, 42 subjects with schizophrenia or schizoaffective disorder were matched individually to one healthy comparison subject for sex and as closely as possible for age (Supplemental Table S1) as previously described (Volk et al., 2011), and samples from subjects in a pair were processed together throughout all stages of the study. The mean age, postmortem interval, freezer storage time, brain pH, and RNA integrity number (RIN; Agilent Bioanalyzer) did not differ between subject groups (Table 1), and each subject had a RIN ≥7.0. All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research Involving the Dead and Institutional Review Board.

2.2. Quantitative PCR

Frozen tissue blocks containing the middle portion of the right superior frontal sulcus were confirmed to contain PFC area 9 using Nissl-stained, cryostat tissue sections for each subject (Volk et al., 2000). Standardized amounts of cortical gray matter from tissue blocks were collected in TRIzol in a manner that ensured minimal white matter contamination and excellent RNA preservation (Volk

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

Parameter	Healthy comparison	Schizophrenia
N	42	42
Sex	31M/11F	31M/11F
Race	34W/8B	29W/13B
Age (years)	48 ± 13	47 ± 13
Postmortem interval (hours)	17.8 ± 5.9	18.1 ± 8.7
Freezer storage time (months)	128 ± 44	129 ± 46
Brain pH	6.8 ± 0.2	6.6 ± 0.4
RNA integrity number	8.3 ± 0.6	8.2 ± 0.7
Medications at time of death		
Antipsychotic	–	36/42
Antidepressant	–	17/42
Benzodiazepine/valproic acid	–	15/42
Cause of Death		
Cardiopulmonary-related	35/42	17/42
Suicide	–	11/42
Other	7/42	14/42

For age, postmortem interval, freezer storage time, brain pH, and RNA integrity number, values are group means ± standard deviation ($t_{(82)} < 2.0$, $p > 0.05$). For medications at time of death and cause of death, the number of subjects in each applicable category is provided.

et al., 2012). cDNA was synthesized from standardized dilutions of total RNA for each subject. All primer pairs (Supplemental Table S2) demonstrated high amplification efficiency (>96%) across a wide range of cDNA dilutions and specific single products in dissociation curve analysis. Quantitative PCR was performed using the comparative cycle threshold (CT) method with Power SYBR Green dye and the StepOnePlus Real-Time PCR System (Applied Biosystems). Based on their stable relative expression levels between schizophrenia and comparison subjects (Hashimoto et al., 2008), three reference genes (beta actin, cyclophilin A, and glyceraldehyde-3-phosphate dehydrogenase) were used to normalize ABHD6 mRNA levels. The difference in CT (dCT) was calculated by subtracting the geometric mean CT for the three reference genes from the CT for ABHD6 (mean of four replicates). Because dCT represents the log2-transformed expression ratio of ABHD6 to the reference genes, the relative ABHD6 mRNA level is reported as 2^{-dCT} (Vandesompele et al., 2002; Hashimoto et al., 2008).

2.3. Antipsychotic-exposed monkeys

Young adult, male monkeys (*Macaca fascicularis*) received oral doses of haloperidol, olanzapine or placebo (n = 6 monkeys per group) twice daily for 17–27 months (Dorph-Petersen et al., 2005). RNA was isolated from PFC area 9, and qPCR was conducted for the same three reference genes and ABHD6 (Supplemental Table S2). All animal studies were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

2.4. Tetrahydrocannabinol-exposed monkeys

As described previously (Verrico et al., 2012), tetrahydrocannabinol (THC) or vehicle was administered intravenously via vascular access port to adolescent male monkeys (*Macaca mulatta*; n = 7 monkeys per group) once daily for 5 days per week at doses gradually titrated up to 180–240 μg/kg which induced signs of acute intoxication and impairments in spatial working memory. After 12 months of THC administration followed by one month of withdrawal (Verrico and Lewis, 2011), monkeys were euthanized. qPCR for ABHD6 mRNA in PFC area 9 was conducted as described earlier.

2.5. Statistical analysis

An analysis of covariance (ANCOVA) was employed with ABHD6 mRNA level as the dependent variable; diagnostic group as the main effect; postmortem interval, brain pH, RIN, and storage time as covariates, and subject pair as a blocking factor to account for the matching of subjects in a pair for sex and age and for the parallel processing of tissue samples from a pair. Analyses of differences in mRNA levels between schizophrenia subjects grouped by indicators of disease severity, substance abuse and psychotropic medications were conducted using the same ANCOVA model (substituting age for subject pair) with $\alpha = .05$. For the monkey studies, an ANOVA model was employed with mRNA level as the dependent variable and treatment as the main effect.

3. Results

Mean ABHD6 mRNA levels did not differ ($F_{(1,37)} = 0.71$, $p = .41$) between schizophrenia and healthy comparison subjects (Fig. 1A). A second primer set designed against a different ABHD6 mRNA region (Supplemental Table 2) confirmed the absence of a between-group difference in ABHD6 mRNA levels ($F_{(1,37)} = 0.13$, $p = .72$). ABHD6 mRNA levels from the two primer sets were highly correlated across all subjects ($r = .92$, $p < 0.0001$), demonstrating the specificity and reproducibility of the quantification technique.

The coefficient of variation in ABHD6 mRNA levels was strikingly higher in schizophrenia subjects (20.1%; Fig. 1A) than in comparison subjects (6.7%), and we examined several factors that might contribute to this variability. Among schizophrenia subjects, ABHD6 mRNA levels did not differ as a function of factors that predict a more severe course of illness (male sex, a diagnosis of schizophrenia rather than schizoaffective disorder, first-degree relative with schizophrenia, early age at illness onset [≤18 years of age]) or measures of illness severity (suicide, no history of marriage, low socioeconomic status as measured by the Hollingshead Index of Social Position, living dependently at the time of death) (all $F_{(1,35)} < 2.7$, $p > .11$). We also found no relationship between history of cannabis use, comorbid diagnosis of substance abuse or dependence, use of antipsychotic, antidepressant, or benzodiazepine medications at time of death, or cause of death (categorized as cardiopulmonary-related, suicide, or other; Table 1; Supplemental Table 1) and ABHD6 mRNA levels in schizophrenia subjects (all $F \leq 1.6$, $p \geq 0.23$). ABHD6 mRNA levels also did not differ in the PFC of monkeys chronically exposed to haloperidol, olanzapine, or placebo (Fig. 1B; $F_{(2,15)} = 0.12$, $p = .89$) or to THC (Fig. 1C; $F_{(1,12)} = 0.003$, $p = .96$).

Interestingly, ABHD6 mRNA levels were positively correlated with age in healthy subjects ($r = 0.43$, $p = .004$) but not in schizophrenia subjects ($r = -0.18$, $p = .25$; Fig. 2A). Consequently, we subdivided the schizophrenia subjects into three similarly-sized age groups using natural break points (<40 years n = 13, 40–49 years n = 13, ≥50 years n = 16; Fig. 2B) and found that schizophrenia subjects under 40 years of age had higher ABHD6 mRNA levels (+18.4%; $F_{(1,8)} = 8.0$, $p = .02$) relative to age-matched comparison subjects (all other age groups: $F \leq 1.0$, $p \geq 0.33$). To assess whether higher ABHD6 mRNA levels in younger individuals with schizophrenia might also reflect a shorter duration of illness, we subdivided the schizophrenia subjects into three similarly-sized groups based on illness duration (<15 years n = 12, 15–25 years n = 14, ≥26 years n = 16; Fig. 2C) and found that subjects with illness duration of less than 15 years had higher ABHD6 mRNA levels (+16.1%; $F_{(1,7)} = 8.0$, $p = .03$) relative to age-matched comparison subjects (all other groups: $F \leq 2.9$, $p \geq 0.11$). Furthermore, age and illness duration were strongly correlated in schizophrenia subjects ($r = .79$, $p < .0001$) which made it difficult to differentiate between their effects on ABHD6 mRNA levels.

4. Discussion

ABHD6 mRNA levels did not differ overall in the PFC in schizophrenia. However, the markedly high variability in ABHD6 mRNA levels in