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Altered expression of developmental regulators of parvalbumin and somatostatin neurons in the prefrontal cortex in schizophrenia

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ABSTRACT

Dysfunction of prefrontal cortex (PFC) inhibitory neurons that express the calcium-binding protein parvalbumin or the neuropeptide somatostatin in schizophrenia may be related to disturbances in the migration, phenotypic specification, and/or maturation of these neurons. These pre- and postnatal developmental stages are regulated in a cell type-specific manner by various transcription factors and co-activators, fibroblast growth factor receptors (FgfR), and other molecular markers. Consequently, we used quantitative PCR to quantify mRNA levels for these developmental regulators in the PFC of 62 schizophrenia subjects in whom parvalbumin and somatostatin neuron disturbances were previously reported, and in antipsychotic-exposed monkeys. Relative to unaffected comparison subjects, subjects with schizophrenia exhibited elevated mRNA levels for 1) the transcription factor MafB, which is expressed by parvalbumin and somatostatin neurons as they migrate from the medial ganglionic eminence to the cortex, 2) the transcriptional coactivator PGC-1α, which is expressed postnatally by parvalbumin neurons to maintain parvalbumin levels and inhibitory function, and 3) FgfR1, which is required for the migration and phenotypic specification of parvalbumin and somatostatin neurons. Elevations in these markers were most prominent in younger schizophrenia subjects and were not present in antipsychotic-exposed monkeys. Finally, expression levels of other important developmental regulators (i.e. Dlx1, Dlx5, Dlx6, SATB1, Sip1/Zeb2, ST8SIA4, cMaf, Nkx6.2, and Arx) were not altered in schizophrenia. The over-expression of a subset of molecular markers with distinct roles in the pre- and postnatal development of parvalbumin and somatostatin neurons might reflect compensatory mechanisms to sustain the development of these neurons in the face of other insults.

1. Introduction

Disturbances in the subpopulations of inhibitory (GABA) neurons that express the calcium-binding protein parvalbumin or the neuropeptide somatostatin have been commonly reported in the prefrontal cortex (PFC) from subjects with schizophrenia (Curley et al., 2011; Fung et al., 2010; Hashimoto et al., 2003; Mellios et al., 2009; Morris et al., 2008; Volk et al., 2012). Cortical parvalbumin and somatostatin neurons share a common site of prenatal origin in the medial ganglionic eminence in humans (Fertuzinhos et al., 2009; Hansen et al., 2013; Ma et al., 2013; Zecevic et al., 2011), and residual evidence of disruptions in early developmental processes such as phenotypic specification and migration has been reported in these neurons in postmortem brain tissue in schizophrenia (Hashimoto et al., 2003; Joshi et al., 2012). These data suggest that dysfunction of cortical parvalbumin and somatostatin neurons in schizophrenia may result, at least in part, from insults during development; such insults could occur as early as the prenatal period, initiating processes that interfere with the birth, migration, cell-type specification, and/or maturation of these neurons (Volk and Lewis, 2013, 2014). Consistent with this hypothesis, deficits in the transcription factor Lhx6, which regulates the migration and differentiation of parvalbumin and somatostatin neurons during prenatal development (Fertuzinhos et al., 2009; Georgiev et al., 2012; Jakovcevski et al., 2011; Liodis et al., 2007; Neves et al., 2013; Zhao et al., 2008), have been reported in the prefrontal cortex of two cohorts of schizophrenia subjects (Volk et al., 2012, 2014).

The various stages of pre- and postnatal development of cortical parvalbumin and somatostatin neurons are also regulated by the expression of a diverse array of transcription factors and co-activators (e.g., Dlx1, Dlx5, Dlx6, MafB, cMaf, Sox6, Nkx6.2, Zeb2/Sip1/Zfhx1b, Arx, PGC-1α), chemokine receptors (e.g., CXCR4, CXCR7), and molecular markers of other functions (e.g, Fgfr1, SATB1, and polysialyltransferases such as ST8SIA4) (Anderson et al., 1997; Azim et al., 2009; Batista-Brito et al., 2009; Cobos et al., 2005, 2006; Colasante et al., 2008; Cowell et al., 2007; Denaxa et al., 2012; Fogarty et al., 2007; Krocher et al., 2014; Lucas et al., 2010; McKinsey et al., 2013; Meechan et al., 2012; Muller et al., 2008; Sanchez-Alcaniz et al., 2011; Sousa et al., 2009; van et al., 2013; Wang et al., 2010, 2011). Consequently,

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knowledge of the status of these developmental regulators in schizophrenia may provide insight into disturbances in the molecular processes that are disrupted in early life and the resulting impact on cortical parvalbumin and somatostatin neuron development. Therefore, we quantified transcript levels of these developmental regulators in the PFC of a large cohort of schizophrenia subjects with known disturbances in parvalbumin and somatostatin neurons (Hashimoto et al., 2003; Morris et al., 2008; Volk et al., 2012, 2014).

2. Materials and methods

2.1. Human subjects

Brain specimens were obtained during routine autopsies conducted at the Allegheny County Medical Examiner's Office after consent was obtained from next-of-kin. An independent committee of experienced research clinicians made consensus DSMIV (American Psychiatric, 1994) diagnoses for each subject using structured interviews with family members and review of medical records (Volk et al., 2010); the absence of a psychiatric diagnosis was confirmed in unaffected comparison subjects using the same approach. To control for experimental variance, subjects with schizophrenia or schizoaffective disorder ($n = 62$) were matched individually to one unaffected comparison subject for sex and as closely as possible for age and RNA integrity number (RIN; Agilent Bioanalyzer) (Supplemental Table S1). Samples from subjects in a pair were processed together throughout all stages of the study. The mean age, postmortem interval, RNA integrity number (RIN), and tissue freezer storage time did not differ between subject groups ($t_{(122)} \leq 0.45$, $p \geq 0.65$) (Table 1). Mean (±standard deviation) brain pH was different between the schizophrenia (6.6 ± 0.3) and unaffected subject groups (6.7 ± 0.2; $t_{(122)} = 2.6$, $p = 0.01$), but the difference was quite small and of uncertain significance. 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.

2.2. Quantitative PCR

Standardized amounts of gray matter from PFC area 9 were collected in TRIzol reagent in a manner that ensured minimal white matter contamination and excellent RNA preservation (Volk et al., 2013). Standardized dilutions of total RNA for each subject were used to synthesize cDNA. All primer pairs (Supplemental Table S2) demonstrated high amplification efficiency (>94%) 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 ViiA-7 Real-Time PCR System (Applied Biosystems) as previously described (Volk et al., 2011). Three reference genes (beta actin, cyclophilin A, and glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) that we previously reported to be stably expressed in the present cohort of schizophrenia and comparison subjects (Volk et al., 2015). were used to normalize target mRNA levels. The difference in CT (dCT) for each target transcript was calculated by subtracting the geometric mean CT for the three reference genes from the CT of the target transcript (mean of four replicate measures). Because dCT represents the log2-transformed expression ratio of each target transcript to the reference genes, the relative level of the target transcript for each subject is reported as 2^{-dCT} (Vandesompele et al., 2002; Volk et al., 2010).

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

Parameter	Unaffected comparison	Schizophrenia
N	62	62
Sex	47 M/15F	47 M/15F
Race	52 W/10B	46 W/16B
Age (years)	48.7 ± 13.8	47.7 ± 12.7
Postmortem interval (hours)	18.8 ± 5.5	19.2 ± 8.5
Freezer storage time (months)	132.8 ± 56.8	129.0 ± 61.3
Brain pH	6.7 ± 0.2	6.6 ± 0.3
RNA integrity number	8.2 ± 0.6	8.1 ± 0.6

For brain pH, $t_{(122)} = 2.6$, $p = 0.01$. For all others, $t_{(122)} \leq 0.45$, $p \geq 0.65$. Values are group means ± standard deviation.

2.3. Antipsychotic-exposed monkeys

Young adult, male, long-tailed monkeys (*Macaca fascicularis*) received oral doses of haloperidol, olanzapine or placebo ($n = 6$ monkeys per group) twice daily for 17–27 months, as previously described (Dorph-Petersen et al., 2005). RNA was isolated from PFC area 9, and quantitative PCR was conducted for the same three reference genes and target genes described above (Supplemental Table S2), with all monkeys from a triad processed together on the same plate. All animal studies followed the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.4. Statistical analysis

The ANCOVA model we report includes mRNA level as the dependent variable, diagnostic group as the main effect, and age, postmortem interval, brain pH, RIN, and freezer storage time as covariates. Because each schizophrenia subject was individually matched to an unaffected subject to account for the parallel processing of tissue samples from a pair and to balance diagnostic groups for sex and age, a second ANCOVA model with subject pair as a blocking factor and including postmortem interval, brain pH, RIN, and freezer storage time was also used. Because both models produced similar results, only the results from unpaired ANCOVA model are reported. Subsequent analyses of differences in mRNA levels between schizophrenia subjects grouped by presence of a comorbid substance use disorder, psychotropic medications at time of death, and tobacco use at time of death and suicide as manner of death were conducted using the unpaired ANCOVA models. For the antipsychotic-exposed monkey study, an ANOVA model with mRNA level as the dependent variable, treatment group as the main effect, and triad as a blocking factor was employed.

3. Results

Among the developmental regulators of cortical parvalbumin and somatostatin neurons examined in this study (Fig. 1), transcript levels for MafB (+16%; $F_{(1,117)} = 11.2$, $p = 0.001$), FgfR1 (+16%; $F_{(1,117)} = 15.2$, $p = 0.0002$), and PGC-1α (+8%; $F_{(1,117)} = 12.6$, $p = 0.001$) were significantly higher in the PFC of schizophrenia subjects relative to unaffected comparison subjects. In contrast, none of the other transcripts examined (i.e. Dlx1, Dlx5, Dlx6, SATB1, Sip1/Zeb2, ST8SIA4, cMaf, Nkx6.2, and Arx) differed between the subject groups (for all, $F_{(1,117)} < 1.6$, $p > 0.20$).

Transcript levels for MafB, FgfR1, and PGC-1α did not differ between schizophrenia subjects as a function of use of antipsychotics (all $F_{(1,55)} \leq 3.6$, $p \geq 0.06$), antidepressants (all $F_{(1,55)} \leq 2.2$, $p \geq 0.14$), benzodiazepines and/or valproate (all $F_{(1,55)} \leq 2.6$, $p \geq 0.11$), or tobacco use (all $F_{(1,47)} \leq 1.0$, $p \geq 0.32$) at time of death, or in those with a diagnosis of a substance use disorder (all $F_{(1,55)} \leq 1.2$, $p \geq 0.28$). Although mRNA levels for MafB ($F_{(1,55)} = 0.003$, $p = 0.95$) and FgfR1 ($F_{(1,55)} = 0.1$, $p = 0.72$) did not differ between schizophrenia subjects with suicide or natural/accidental manner of death, PGC-1α mRNA levels were significantly lower (−12%; $F_{(1,55)} = 4.1$, $p = 0.048$) in schizophrenia subjects with suicide as manner of death relative to schizophrenia subjects with natural/accidental deaths.

Transcript levels for MafB ($F_{(2,10)} = 3.8$, $p = 0.06$), FgfR1 ($F_{(2,10)} = 0.5$, $p = 0.64$), and PGC-1α ($F_{(2,10)} = 0.2$, $p = 0.80$) in the PFC also

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