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Chemokine receptors and cortical interneuron dysfunction in schizophrenia

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ABSTRACT

Alterations in inhibitory (GABA) neurons, including deficiencies in the GABA synthesizing enzyme GAD67, in the prefrontal cortex in schizophrenia are pronounced in the subpopulations of neurons that contain the calcium-binding protein parvalbumin or the neuropeptide somatostatin. The presence of similar illness-related deficits in the transcription factor Lhx6, which regulates prenatal development of parvalbumin and somatostatin neurons, suggests that cortical GABA neuron dysfunction may be related to disturbances in utero. Since the chemokine receptors CXCR4 and CXCR7 guide the migration of cortical parvalbumin and somatostatin neurons from their birthplace in the medial ganglionic eminence to their final destination in the neocortex, we sought to determine whether altered CXCR4 and/or CXCR7 mRNA levels were associated with disturbances in GABA-related markers in schizophrenia. Quantitative PCR was used to quantify CXCR4 and CXCR7 mRNA levels in the prefrontal cortex of 62 schizophrenia and 62 healthy comparison subjects that were previously characterized for markers of parvalbumin and somatostatin neurons and in antipsychotic-exposed monkeys. We found elevated mRNA levels for CXCR7 (+29%; $p < .0001$) and CXCR4 (+14%, $p = .052$) in schizophrenia subjects but not in antipsychotic-exposed monkeys. CXCR7 mRNA levels were inversely correlated with mRNA levels for GAD67, parvalbumin, somatostatin, and Lhx6 in schizophrenia but not in healthy subjects. These findings suggest that higher mRNA levels for CXCR7, and possibly CXCR4, may represent a compensatory mechanism to sustain the migration and correct positioning of cortical parvalbumin and somatostatin neurons in the face of other insults that disrupt the prenatal development of cortical GABA neurons in schizophrenia.

1. Introduction

Alterations in inhibitory (GABA) neurons in the prefrontal cortex in schizophrenia, including lower transcript levels for the GABA synthesizing enzyme glutamate decarboxylase (GAD67) (Akbarian et al., 1995; Guidotti et al., 2000; Volk et al., 2000, 2014; Straub et al., 2007; Duncan et al., 2010; Curley et al., 2011; Kimoto et al., 2014), are among the most consistently reported postmortem findings in schizophrenia. Specific subpopulations of GABA neurons, in particular those that contain the calcium-binding protein parvalbumin or the neuropeptide somatostatin, have been reported to be severely affected in the illness (Hashimoto et al., 2003; Morris et al., 2008; Mellios et al., 2009; Fung et al., 2010; Volk et al., 2012; Glausier et al., 2014). Recent evidence suggests that the disease process that leads to dysfunction of cortical parvalbumin and somatostatin neurons may have a prenatal origin. For example, approximately 50% of parvalbumin neurons have been reported to fail to complete their phenotypic specification into fully functioning GABA neurons (Hashimoto et al., 2003), while disrupted migration of cortical somatostatin neurons has also been reported (Yang et al., 2011). Furthermore, we recently reported disease-related deficits in the transcription factor Lhx6 (Volk et al., 2012, 2014), which is important for the prenatal development of cortical parvalbumin and somatostatin neurons as they tangentially migrate out of the medial ganglionic eminence and toward the cerebral cortex (Liodis et al., 2007; Zhao et al., 2008; Neves et al., 2013; Vogt et al., 2014). However, subsequent animal models revealed that a partial loss of Lhx6 similar to that seen in schizophrenia was not sufficient in isolation to reproduce the pattern of disturbances in cortical GABA neurons seen in schizophrenia (Neves et al., 2013; Volk et al., 2014). These findings indicate the need to explore whether additional factors that regulate the prenatal ontogeny of cortical GABA neurons may also be altered in schizophrenia and perhaps may interact with deficits in Lhx6 to produce GABA-related disturbances similar to those seen in schizophrenia.

Interestingly, the chemotaxic cytokine (i.e. chemokine) receptors CXCR4 and CXCR7, which bind the ligand CXCL12 (also known as stromal cell-derived factor-1; SDF-1) (Balabanian et al., 2005), are expressed by migrating cortical interneurons, including future cortical parvalbumin and somatostatin neurons (Stumm et al., 2003, 2007; Li et al., 2008; Lopez-Bendito et al., 2008; Tanaka et al., 2010; Sanchez-Alcaniz et al., 2011; Wang et al., 2011). CXCR4 and CXCR7 play critical

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roles in sustaining the tangential migration of cortical parvalbumin and somatostatin neurons from their birth in the medial ganglionic eminence to their final destination in the neocortex, including the timing of their radial migration through the cortical plate (Li et al., 2008; Tanaka et al., 2010; Sanchez-Alcaniz et al., 2011; Wang et al., 2011; Vogt et al., 2014). Furthermore, Lhx6 can affect the expression of CXCR7 by binding to an intronic enhancer element of CXCR7, while transduction of CXCR7 can, in turn, partially compensate for the deleterious effects of a loss of Lhx6 on cortical GABA neuron migration (Vogt et al., 2014). These findings raise the question of whether alterations in CXCR4 and/or CXCR7 in the prefrontal cortex in schizophrenia may act in concert with, or may instead compensate for, deficits in Lhx6 to affect prenatal ontogeny of cortical parvalbumin and somatostatin neurons in schizophrenia.

2. Materials and methods

2.1. Human subjects

Brain specimens were obtained during routine autopsies conducted at the Allegheny County Office of the Medical Examiner (Pittsburgh, Pennsylvania) after consent was obtained from next-of-kin. An independent committee of experienced research clinicians made consensus DSMIV (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 using the same approach (Volk et al., 2011). To control for experimental variance, subjects with schizophrenia or schizoaffective disorder ($n = 62$) were matched individually to one healthy comparison subject for sex and as closely as possible for age (Supplementary Table S1). Tissue 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 healthy 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

Frozen tissue blocks containing the middle portion of the right superior frontal sulcus were confirmed to contain prefrontal cortex area 9 using Nissl-stained, cryostat tissue sections for each subject (Volk et al., 2000). The gray-white matter boundary of prefrontal cortex area 9 in a tissue block from each subject was carefully scored with a scalpel blade where the gray matter had uniform thickness and the gray-white matter boundary was easily delineated. The scored gray matter region of the tissue block was then digitally photographed, and the number of tissue sections (40 μm) required to collect ~30 mm^3 of gray matter was determined for each subject. The calculated number of required tissue sections for each subject was then cut by cryostat, and gray matter was separately collected into a tube containing 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 (Supplementary Table S2) demonstrated high amplification efficiency (>98%) across a wide range of cDNA dilutions and specific single products in dissociation curve analysis, and control studies in which the cDNA template was not included in the quantitative PCR reaction resulted in a complete lack of amplification. 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., 2014). Three reference genes (beta actin, cyclophilin A, and glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) were used to normalize target mRNA levels (Hashimoto et al., 2008). 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). The mean coefficient of variance (±SD) of the replicate measures for CXCR4 was 0.048 (±0.031) and for CXCR7 was 0.053 (±0.036). Furthermore, the mean relative expression level for each reference gene relative to the two other reference genes did not differ between schizophrenia and healthy comparison subjects, respectively (beta actin: 1.36 ± 0.25 versus 1.35 ± 0.17; cyclophilin: 0.41 ± 0.05 versus 0.43 ± 0.04; GAPDH: 1.85 ± 0.24 versus 1.78 ± 0.15).

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

Parameter	Healthy comparison	Schizophrenia
N	62	62
Sex	47M/15F	47M/15F
Race	52W/10B	46W/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)	131.8 ± 56.2	128.1 ± 60.7
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 prefrontal cortical area 9, and qPCR was conducted for the same three reference genes and CXCR4 and CXCR7 (Supplementary 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 a healthy 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, and both models produced similar results. Subsequent analyses of differences in mRNA levels between schizophrenia subjects grouped by predictors and indicators of disease severity, psychotropic medications, and smoking 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.

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