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REDUCED *CHRNA7* EXPRESSION IN C3H MICE IS ASSOCIATED WITH INCREASES IN HIPPOCAMPAL PARVALBUMIN AND GLUTAMATE DECARBOXYLASE-67 (GAD67) AS WELL AS ALTERED LEVELS OF $GABA_A$ RECEPTOR SUBUNITS

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Abstract—Decreased expression of *CHRNA7*, the gene encoding the α7* subtype of nicotinic receptor, may contribute to the cognitive dysfunction observed in schizophrenia by disrupting the inhibitory/excitatory balance in the hippocampus. C3H mice with reduced *Chrna7* expression have significant reductions in hippocampal α7* receptor density, deficits in hippocampal auditory gating, increased hippocampal activity as well as significant decreases in hippocampal glutamate decarboxylase-65 (GAD65) and γ-aminobutyric acid-A ($GABA_A$) receptor levels. The current study investigated whether altered *Chrna7* expression is associated with changes in the levels of parvalbumin, GAD67 and/or $GABA_A$ receptor subunits in the hippocampus from male and female C3H *Chrna7* wildtype, C3H *Chrna7* heterozygous and C3H *Chrna7* knockout (KO) mice using quantitative Western immunoblotting. Reduced *Chrna7* expression was associated with significant increases in hippocampal parvalbumin and GAD67 and with complex alterations in $GABA_A$ receptor subunits. A decrease in α3 subunit protein was seen in both female C3H *Chrna7* Het and KO mice while a decrease in α4 subunit protein was also detected in C3H *Chrna7* KO mice with no sex difference. In contrast, an increase in δ subunit protein was observed in C3H *Chrna7* Het mice while a decrease in this subunit was observed in C3H *Chrna7* KO mice, with δ subunit protein levels being greater in males than in females. Finally, an increase in γ2 subunit protein was found in C3H *Chrna7* KO mice with the levels of this subunit again being greater in males than in females. The increases in hippocampal parvalbumin and GAD67 observed in C3H *Chrna7* mice are contrary to reports of reductions in these proteins in the postmortem hippocampus from schizophrenic individuals. We hypothesize that the disparate results may occur because of the influence of factors other than *CHRNA7* that have been found to be abnormal in schizophrenia. © 2014 Published by Elsevier Ltd. on behalf of IBRO.

Key words: *Chrna7*, λ-aminobutyric acid (GABA), $GABA_A$ receptors, parvalbumin, glutamate decarboxylase 67 (GAD67), schizophrenia.

INTRODUCTION

Cognitive dysfunction is a core feature of schizophrenia (Barch and Ceaser, 2012; Minzenberg and Carter, 2012). Deficits have been observed in a variety of cognitive domains, including sensory filtering (gating), attention, spatial working memory, executive function, context processing and episodic memory (Piskulic et al., 2007; Barch and Ceaser, 2012; Minzenberg and Carter, 2012). Cognitive abnormalities are detected at the first presentation of the disease, but are also present prior to illness onset (Kalkstein et al., 2010).

Normal cognitive function depends upon a number of brain regions, including the hippocampus (Kroes and Fernandez, 2012). Schizophrenia-associated cognitive deficits may occur, at least in part, because of abnormalities found in the hippocampus of schizophrenic individuals (Todtenkopf and Benes, 1998; Tregellas et al., 2004, 2007; Heckers and Konradi, 2010; Konradi et al., 2011; Williams et al., 2011). The factors contributing to schizophrenia-associated hippocampal abnormalities are currently ill-defined.

Schizophrenia is thought to arise from a complex interaction between genetic and environmental risk factors (Pickard, 2011; Hosak, 2013). A large number of genes have been proposed as potential risk factors for schizophrenia, including the gene encoding the α7* subtype of nicotinic receptor, C*HRNA7*(human)/*Chrna7* (mouse) (Pickard, 2011; Hosak, 2013; Mowry and Gratten, 2013). *CHRNA7* is found in the q13–q14 region of chromosome 15 in humans (Leonard and Freedman,

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Abbreviations: 5-HT_3, serotonin 3 receptor; BDNF, brain-derived neurotrophic factor; *CHRNA7*/*Chrna7*, gene (human/mouse) encoding the α7 nicotinic acetylcholine receptor; DLPFC, dorsal lateral prefrontal cortex; EDTA, ethylenediaminetetraacetic acid; ERK1/2, extracellular signal-regulated kinase 1/2; GABA, γ-aminobutyric acid; GAD-65, L-glutamic acid decarboxylase-65; GAD-67, L-glutamic acid decarboxylase-67; Het, heterozygous; KO, knockout ; mRNA, messenger RNA; NMDA, *N*-methyl-D-aspartate; PKA, protein kinase A; TBS, Tris-buffered saline; TBST, TBS containing 0.05% Tween-20; TrkB, tropomyosin-related kinase-B; WT, wildtype.

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2006). The 15q13–q14 region has been linked to a P50 auditory gating deficit observed in schizophrenic patients as well as to schizophrenia in a number of studies, although not all (Stephens et al., 2009). A recurrent microdeletion at 15q13.3, which includes *CHRNA7*, has been shown to significantly associate with schizophrenia (Stefansson et al., 2008). In addition, the *CHRNA7* gene has been associated with schizophrenia and with the memory and P50 auditory sensory gating deficits observed in schizophrenic individuals (Leonard and Freedman, 2006; Stephens et al., 2012).

The density of $\alpha7^*$ receptors is significantly reduced in postmortem hippocampus from schizophrenic individuals (Heckers and Konradi, 2010). The reduction in hippocampal $\alpha7^*$ receptor levels may contribute to schizophrenia-associated cognitive dysfunction as hippocampal $\alpha7^*$ receptors have been implicated in several cognitive domains impaired in schizophrenia, including sensory gating, spatial working memory, context processing and episodic memory (Thomsen et al., 2010; Graef et al., 2011; Wallace and Porter, 2011). Treatment with $\alpha7^*$ receptor agonists has been shown to improve cognitive abnormalities in both animals and humans. The selective $\alpha7^*$ receptor agonist TC-5619 corrected abnormalities in social behavior as well as deficits in paired-pulse inhibition (PPI), a measure of sensory gating, in transgenic mice and Sprague–Dawley rats (Hauser et al., 2009). Improved performance on a social recognition task was observed in Wistar rats following treatment with the selective $\alpha7^*$ receptor agonist AR-R 17779 (Van Kampen et al., 2004). Another selective $\alpha7^*$ receptor agonist, ABT-107, improved auditory gating abnormalities in DBA/2 mice (Radek et al., 2012). The P50 gating deficit observed in schizophrenic individuals was significantly improved by administration of tropisetron, a partial agonist at $\alpha7^*$ receptors and an antagonist at serotonin 3 receptor (5-HT_3) receptors (Koike et al., 2005). DMXB-A, a partial agonist at the $\alpha7^*$ receptor and an antagonist at $\alpha4\beta2^*$ receptors, improved attention, working memory, and negative symptoms in nonsmoking patients with schizophrenia (Olincy and Freedman, 2012). Improvement in both cognitive deficits and negative symptoms was also reported in smoking and nonsmoking schizophrenic patients following treatment with EVP-6124, an $\alpha7$ agonist and 5-HT_3 antagonist (Wallace and Bertrand, 2013). Finally, the full $\alpha7$ agonist TC-5619 improved cognitive dysfunction as well as negative symptoms in a 12-week study of both smoking and nonsmoking schizophrenic individuals (Wallace and Bertrand, 2013). These data suggest that treatment with $\alpha7^*$ receptor agonists may be a viable approach for improving schizophrenia-associated cognitive deficits.

Hippocampal $\alpha7^*$ receptors are expressed by neurons containing the inhibitory neurotransmitter λ-aminobutyric acid (GABA) as well as by neurons containing the excitatory neurotransmitter glutamate (Thomsen et al., 2010; Wallace and Porter, 2011). Activation of the receptor modulates the release of both hippocampal GABA and glutamate (Thomsen et al., 2010; Wallace and Porter, 2011). Therefore, the $\alpha7^*$ receptor can influence the inhibitory/excitatory balance in the hippocampus, an important factor underlying normal cognition (Leiser et al., 2009; Morellini et al., 2010). A decrease in $\alpha7^*$ receptor density could disrupt hippocampal inhibitory/excitatory homeostasis, thereby altering hippocampal-mediated cognitive function. Q4

We hypothesize that reduced *CHRNA7* expression is one of many factors contributing to schizophrenia-associated hippocampal abnormalities. To test this hypothesis, we are using a mouse model of reduced *Chrna7* expression (C3H *Chrna7* mice) to examine to what extent a decrease in $\alpha7^*$ receptor density may, or may not, be associated with specific hippocampal abnormalities observed in schizophrenia. Compared to wild-type (C3H *Chrna7* WT) mice, C3H mice heterozygous for *Chrna7* (C3H *Chrna7* Het mice) have significant reductions in hippocampal $\alpha7^*$ receptor density, deficits in hippocampal auditory gating, increased hippocampal activity (Adams et al., 2008) and significant decreases in hippocampal glutamate decarboxylase-65 (GAD65) and $GABA_A$ receptor levels (Adams et al., 2012). With the exception of the decreased $GABA_A$ receptor levels, the hippocampal alterations observed in the C3H *Chrna7* Het mice are comparable to those reported in the hippocampus of schizophrenic individuals (Todtenkopf and Benes, 1998; Tregellas et al., 2004, 2007; Heckers and Konradi, 2010; Konradi et al., 2011; Williams et al., 2011). These data suggest that decreased *CHRNA7* expression may be a primary factor underlying some, but not all, of the hippocampal abnormalities observed in schizophrenia.

The current study expanded our investigation by examining protein levels of parvalbumin, GAD67 and $GABA_A$ receptor subunits in the hippocampus from male and female C3H *Chrna7* WT, C3H *Chrna7* Het and C3H *Chrna7* KO (knockout) mice. We investigated parvalbumin and GAD67 because the $\alpha7^*$ receptor is expressed by many subtypes of GABAergic neurons (Freedman et al., 1993; Thomsen et al., 2010; Wallace and Porter, 2011), including a subpopulation which contains parvalbumin (Arevalo-Serrano et al., 2008) and each of these proteins has been reported to be decreased in the postmortem hippocampus from schizophrenic patients (Zhang and Reynolds, 2002; Benes et al., 2007; Heckers and Konradi, 2010; Konradi et al., 2011; Thompson Ray et al., 2011). Parvalbumin is a calcium-binding protein that plays an important role in regulating pyramidal neuron activity (Freund, 2003), while GAD67 is one of two enzymes that synthesize GABA (Martin and Tobin, 2000). We examined the levels of $GABA_A$ receptor subunit proteins to determine whether decreases in these subunits could account for the reduction in hippocampal $GABA_A$ receptor binding observed in male C3H *Chrna7* Het mice (Adams et al., 2012).

EXPERIMENTAL PROCEDURES

Animals

Mice were originally obtained from the Institute for Behavioral Genetics breeding colony at the University of Colorado, Boulder, CO, USA. The C3H *Chrna7* KO mice were derived by mating $\alpha7$ mixed background (129 × C57BL/6) mice with C3H mice (both sexes

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