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Research Report

Altered hippocampal circuit function in C3H α7 null mutant heterozygous mice

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ABSTRACT

The α7 subtype of nicotinic receptor is highly expressed in the hippocampus where it is purported to modulate release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). The α7 receptor-mediated release of GABA is thought to contribute to hippocampal inhibition (gating) of response to repetitive auditory stimulation. This hypothesis is supported by observations of hippocampal auditory gating deficits in mouse strains with low levels of hippocampal α7 receptors compared to strains with high levels of hippocampal α7 receptors. The difficulty with comparisons between mouse strains, however, is that different strains have different genetic backgrounds. Thus, the observed interstrain differences in hippocampal auditory gating might result from factors other than interstrain variations in the density of hippocampal α7 receptors. To address this issue, hippocampal binding of the α7 receptor-selective antagonist α-bungarotoxin as well as hippocampal auditory gating characteristics were compared in C3H wild type and C3H α7 receptor null mutant heterozygous mice. The C3H α7 heterozygous mice exhibited significant reductions in hippocampal α7 receptor levels and abnormal hippocampal auditory gating compared to the C3H wild type mice. In addition, a general increase in CA3 pyramidal neuron responsivity was observed in the heterozygous mice compared to the wild type mice. These data suggest that decreasing hippocampal α7 receptor density results in a profound alteration in hippocampal circuit function.

1. Introduction

The α7 subtype of nicotinic receptor is a rapidly desensitizing, ligand-gated ion channel (Zhang et al., 1994) that directly fluxes cations, particularly calcium (Seguela et al., 1993), resulting in increased intracellular calcium levels. The α7 receptor is expressed in many brain regions, but is especially dense in the hippocampus (Breese et al., 1997; Seguela et al., 1993; Whiteaker et al., 1999). This receptor has been localized to hippocampal neurons containing the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Breese et al., 1997; Fabian-Fine et al., 2001; Frazier et al., 1998a; Freedman et al., 1993; Khiroug et al., 2003) and activation of the receptor leads to the release of GABA in hippocampal cultures and slices (Alkondon and Albuquerque, 2001; Alkondon et al., 1999; Buhler and Dunwiddie, 2002; Radcliffe et al., 1999).

The α7 receptor-mediated release of GABA appears to be important for hippocampal sensory processing. Luntz-Leybman

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et al. (1992) examined cholinergic regulation of sensory processing in Sprague–Dawley rat hippocampus using an auditory conditioning–testing paradigm. In this paradigm, two identical tones were presented with an intertone interval of 500 ms. Both the first (conditioning) and second (test) tones elicited an evoked potential response in hippocampal area CA3, i.e. increased the activity of CA3 pyramidal neurons. The pyramidal neuron response to the test tone was significantly less than that to the conditioning tone, suggesting that pyramidal neuron activation was inhibited or "gated" during presentation of the test tone. The decrease in pyramidal neuron response to the second tone appeared to be due to a persistent increase in CA3 hippocampal interneuron discharge that was initiated by the conditioning tone (Miller and Freedman, 1995). Intracerebroventricular infusion of the α7-selective antagonist α-bungarotoxin (α-BTX) resulted in similar pyramidal neuron responses to both the conditioning and test tones, i.e. a disruption of auditory gating, while infusion of a high affinity nicotinic receptor channel blocker or a muscarinic receptor antagonist did not (Luntz-Leybman et al., 1992). These data suggest that the α7 receptor influences hippocampal sensory processing by modulating hippocampal inhibitory circuit function.

Individuals with schizophrenia exhibit a deficit in auditory gating relative to control subjects. Schizophrenics respond to the presentation of tone pairs with conditioning and test responses of similar amplitude, indicative of abnormal auditory gating. The schizophrenia-associated deficit in auditory gating has been genetically linked to a dinucleotide polymorphism at the chromosome 15q13–14 site of the α7 receptor (Freedman et al., 1997). In addition, a reduction in α-BTX binding has been observed in postmortem hippocampus of schizophrenics, including those that have never smoked or have been free of neuroleptic treatment for at least a year (Freedman et al., 1995; Pomper et al., 1999). The reduction in α-BTX binding observed in postmortem schizophrenic hippocampus may be secondary to alterations in the α7 receptor gene in schizophrenic brain. Point mutations in the promoter region for the α7 gene reduce its expression *in vitro* and are significantly associated with schizophrenia (Leonard et al., 2002), although they are also found in control hippocampus (Gault et al., 2003). Together, these studies suggest that decreased hippocampal α7 receptor density is associated with a disruption of normal hippocampal sensory processing.

Different strains of inbred mice vary considerably in their hippocampal α7 receptor density (Marks et al., 1989; Stevens et al., 1996). Two of these strains, C3H and DBA/2, have been used in a number of previous studies to examine how differences in hippocampal α7 receptor levels correlate with differences in hippocampal morphology and function. C3H mice have higher (mean 69.2 fmol/mg protein) levels of hippocampal α-BTX binding while DBA/2 mice have lower (mean 45.6 fmol/mg protein) levels of hippocampal α-BTX binding, a 35% reduction in density compared to the C3H mice (Marks et al., 1989). The differences in hippocampal α-BTX binding in C3H and DBA/2 mice appear to result from an interstrain variation in the α7 receptor gene (*Acra7*) locus on mouse chromosome 7 (Stitzel et al., 1996). Functionally, C3H mice exhibit robust hippocampal auditory gating while DBA/2 mice are deficient in hippocampal auditory gating (Stevens et al., 1996). The disruption of auditory gating in the mouse strain with lower hippocampal α7 receptor levels again suggests that the α7 receptor influences hippocampal sensory processing via an influence on hippocampal inhibitory circuit function. Further support for this hypothesis was provided by studies demonstrating improvement in auditory gating in DBA/2 mice with administration of agonists which bind the α7 nicotinic receptor (Stevens et al., 1998; Stevens and Wear, 1997). Acute nicotine administration was also found to influence auditory gating in awake C57BL/6J and DBA/2Hsd mice (Metzger et al., 2007). In this study, however, the P20 and N40 components of the auditory evoked potentials were differentially affected, as nicotine increased the amplitude and gating of the P20 component but decreased the amplitude and gating of the P40 component in each strain.

The difficulty with this two-strain animal model, however, is that the strains have different genetic backgrounds. Bowers et al. (1999) found that the impact of a null mutation of the γ-protein kinase C gene on initial sensitivity to ethanol was

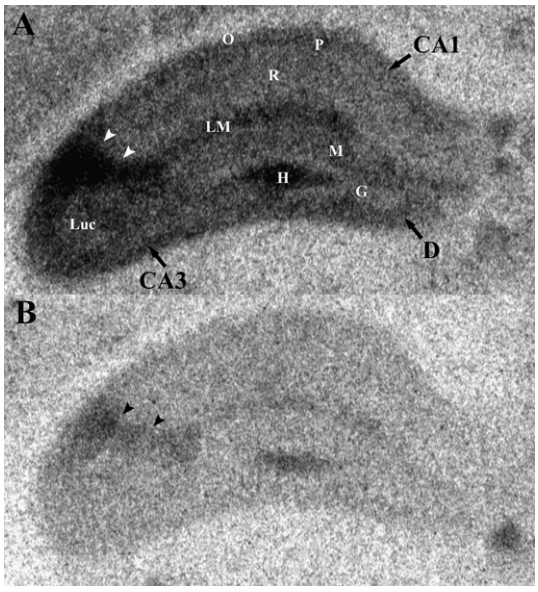

Fig. 1 – Film images of transverse sections through the hippocampus of C3H wild type (A) and C3H α7 receptor heterozygous (B) mice processed for ^{125I}α-BTX autoradiography. Note the significantly decreased level of ^{125I}α-BTX binding in the section from the C3H α7 receptor heterozygous mouse compared to the section from the wild type mouse. In contrast, the pattern of ^{125I}α-BTX binding is comparable between the two groups of mice. The dense band of ^{125I}α-BTX binding across all layers at the CA3/CA1 border zone is still clearly observable (arrow heads), as is the greater density of labeling within CA1 stratum lacunosum/moleculare and within the hilus of the dentate gyrus. Abbreviations: D — dentate gyrus, CA3 — hippocampal area CA3, CA1 — hippocampal area CA1, O — stratum oriens, P — stratum pyramidale, R — stratum radiatum, LM — stratum lacunosum/moleculare, Luc — stratum lucidum, M — molecular layer of the dentate gyrus, G — granule cell layer of the dentate gyrus, H — hilus of the dentate gyrus.