Impaired coupling of glycolytic enzymes and characterization of the EAAT2 interactome in schizophrenia

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Introduction

Excitatory amino acid transporter 2 (EAAT2) belongs to a family of sodium-dependent glutamate transporters that maintain low synaptic concentration of glutamate by removing glutamate from the synaptic cleft into astroglia and neurons. Efficient reuptake of glutamate by EAAT2 relies on sodium and potassium gradients generated principally by Na+/K+ ATPase and energy intermediates (ATP) that drive glial glutamate reuptake. Hexokinase 1 (HK1), an initial enzyme of glycolysis, binds to mitochondrial outer membrane where it couples cytosolic glycolysis to mitochondrial oxidative phosphorylation, producing ATP utilized by the EAAT2/Na+/K+ ATPase complex to facilitate glutamate reuptake. In this study, we hypothesized that EAAT2 doesn’t work independently but works cooperatively with Na+/K+ ATPase and HK1 in a large multiprotein complex; breakdown of this complex may lead to abnormal glutamate transmission, contributing to the pathophysiology of schizophrenia.

Materials and Methods

Tissue and Subjects: Tissue from subjects with schizophrenia and a comparison group in dorsolateral prefrontal cortex (DLPFC) was provided by the Bronx VA/Mount Sinai Medical Center brain bank (SZC, n = 35; CTL, n = 26).

Subcellular fractionation: The fractionation was performed as described previously (Hammond et al., PLoS One. 2012;7(6):e39190). 18 schizophrenia subjects and 18 comparisons were matched for sex, age, pH, and PMI.

Immunoprecipitation (IP): Pierce Crosslink IP Kit (cat# 26147, Thermo Scientific). Rabbit EAAT2 antibody (Santa Cruz) was used for IP, guinea pig EAAT2 antibody (gMillipore) was used for Western Blot.

Mass Spectrometry: Eluted proteins were resolved using 4–12% Bis-Tris gradient gels (Invitrogen). The gel bands on either IgG or IP were excised above 50KD. Proteins were identified using nano liquid chromatography-tandem mass spectrometry.

Immunofluorescence: Frozen sections were fixed with fresh 4% formaldehyde in PBS for 10 min at 4°C, incubated with the primary antibodies or IgG controls. After incubated with 2nd antibodies, the sections were examined using a Leica confocal microscope.

Statistical Analyses: Factorial ANOVA or paired 2 sample t- test, *P < 0.05.

Results

Hypothesis 1: EAAT2, Na+/K+ ATPase and mitochondria are cocompartmentalized in postmortem prefrontal cortex.

Colocalization was confirmed by mass spectrometry and immunofluorescence.

Hypothesis 2: Expression of total protein may not be changed in SCZ.

Expression of proteins was examined in DLPFC tissue homogenate by Western Blots.

Hypothesis 3: Protein expression may be changed in subcellular fractions.

Expression of proteins was examined in subcellular fraction in DLPFC subjects with schizophrenia (SZC, n = 18; CTL, n = 18).

Summary

1. EAAT2 is associated with Na+/K+ ATPase α1, HK1 and aconitate in a large multiprotein complex in postmortem prefrontal cortex.
2. Total protein expression of EAAT2, EAAT2B, EAAT2 exon 9 skipping, HK1, Na+/K+ ATPase α1, J1, UQCRCC2, aconitase1 and 2 was not changed in the subjects with schizophrenia in the DLPFC.
3. EAAT2B isoform of EAAT2 and aconitase1 were increased in L/C or mitochondrial fraction respectively in the DLPFC in schizophrenia.
4. HK1 detachment from the mitochondrial outer membrane suggests impaired energy metabolism in this illness.

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