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Transmembrane AMPA receptor regulatory protein (TARP) dysregulation in anterior cingulate cortex in schizophrenia

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Abstract

The glutamate hypothesis of schizophrenia proposes that abnormal glutamatergic neurotransmission occurs in this illness, and a major contribution may involve dysregulation of the AMPA subtype of ionotropic glutamate receptor (AMPAR). Transmembrane AMPAR regulatory proteins (TARPs) form direct associations with AMPARs to modulate the trafficking and biophysical functions of these receptors, and their dysregulation may alter the localization and activity of AMPARs, thus having a potential role in the pathophysiology of schizophrenia. We performed comparative quantitative real-time PCR and Western blot analysis to measure transcript (schizophrenia, N = 25; comparison subjects, N = 25) and protein (schizophrenia, N = 36; comparison subjects, N = 33) expression of TARPs (γ subunits 1-8) in the anterior cingulate cortex (ACC) in schizophrenia and a comparison group. TARP expression was also measured in frontal cortex of rats chronically treated with haloperidol decanoate (28.5 mg/kg every three weeks for nine months) to determine the effect of antipsychotic treatment on the expression of these molecules. We found decreased transcript expression of TARP γ-8 in schizophrenia. At the protein level, γ-3 and γ-5 were increased, while γ-4, γ-7 and γ-8 were decreased in schizophrenia. No changes in any of the molecules were noted in the frontal cortex of haloperidol-treated rats. TARPs are abnormally expressed at transcript and protein levels in ACC in schizophrenia, and these changes are likely due to the illness and not antipsychotic treatment. Alterations in the expression of TARPs may contribute to the pathophysiology of schizophrenia, and represent a potential mechanism of glutamatergic dysregulation in this illness.

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5. Author Disclosure
All authors have no disclosures to report.

Conflict of Interest
All authors declare that they have no conflicts of interest.

Contributors
JBD, JT and JHMW designed the study. JBD performed the experiments and statistical analyses, and wrote the first draft of the manuscript. VH provided the human tissue. All authors contributed to and have approved the final manuscript.

Keywords

AMPA receptor auxiliary protein; glutamate receptor; trafficking; postmortem; human

1. Introduction

The glutamate hypothesis of schizophrenia originally proposed that NMDA receptor (NMDAR) hypofunction is associated with the pathophysiology of this illness, and was based in part on the observation that NMDAR antagonists, such as phencyclidine (PCP) and ketamine can induce schizophrenia-like symptoms in normal individuals and exacerbate symptoms in patients with schizophrenia (Allen and Young, 1978; Barbon et al., 2007; Coyle, 1996; Coyle et al., 2003; Ellison, 1995; Lahti et al., 1995; Meador-Woodruff and Healy, 2000). A potential mechanism to explain such hypofunction is abnormal expression and localization of the AMPA subtype of glutamate receptor, whose activation and colocalization with NMDARs at the postsynaptic density (PSD) is required for NMDAR activation and long-term potentiation, and thus critical for glutamatergic neurotransmission (Coyle et al., 2003; Meador-Woodruff and Healy, 2000). Changes in the activity of AMPARs at the PSD, due to either dysregulation of receptor expression or altered insertion stemming from abnormal trafficking, could decrease NMDAR activity and thus contribute to the manifestation of psychotic symptoms. AMPAR modulators, such as ampakines, have been reported to improve cognitive function in schizophrenia (Coyle, 1996), and increased AMPAR binding has been reported in the cortex in this illness (Noga et al., 2001; Zavitsanou et al., 2002). These findings suggest that AMPARs may be abnormally expressed in schizophrenia. Direct examination of AMPAR expression in postmortem brain in schizophrenia, however, has yielded inconsistent results (Breese et al., 1995; Dracheva et al., 2005; Eastwood et al., 1995; Freed et al., 1993; Hammond et al., 2010; Healy et al., 1998; Meador-Woodruff and Healy, 2000; Scarr et al., 2005).

A potential mechanism underlying AMPAR disturbances in schizophrenia is abnormal expression of AMPAR auxiliary proteins that regulate AMPAR function, localization and trafficking (Beneyto and Meador-Woodruff, 2006; Dracheva et al., 2005; Hammond et al., 2010; Malinow and Malenka, 2002; Mirnics et al., 2000; Song and Huganir, 2002; Toyooka et al., 2002; Whiteheart and Matveeva, 2004). Transmembrane AMPAR regulatory protein gamma subunit 2 (TARP γ-2, or stargazin), was the first protein found to interact with AMPARs (Chen et al., 2000; Díaz, 2010; Nakagawa and Sheng, 2000; Tomita et al., 2001; Vandenberghe et al., 2005). γ-2 was initially identified in the naturally occurring mutant stargazer mouse that lacks functional AMPARs at cerebellar granule cell synapses (Chen et al., 1999; Chen et al., 2000; Hashimoto et al., 1999; Letts et al., 1998; Noebels et al., 1990). The gene encoding γ-2 is known as voltage-dependent calcium channel gamma subunit 2 (CACNG2) due to its homology to the skeletal muscle calcium channel subunit γ-1 (Chen et al., 2000; Letts et al., 1998). Currently, eight TARP subunits have been identified, each having varying roles in AMPAR trafficking to the PSD (Chen et al., 2003; Chen et al., 2007; Coombs and Cull-Candy, 2009; Díaz, 2010; Jackson and Nicoll, 2011a; Kato et al., 2007; Klugbauer et al., 2000; Tomita et al., 2003). The TARPs also have biophysical effects on AMPARs, including controlling channel gating, receptor kinetics, glutamate binding affinity, activation and desensitization rates, and receptor stability (Coombs and Cull-Candy, 2009; Kato et al., 2010b; Malinow and Malenka, 2002; Osten and Stern-Bach, 2006; Sager et al., 2009; Tomita, 2010; Ziff, 2007).

In a previous study, we reported increased TARP γ-2 transcript expression in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia (Beneyto and Meador-Woodruff, 2006), but beyond this, little is known about the expression of this family of genes in

schizophrenia. In this study, we hypothesized that TARPs are abnormally expressed in cortex in schizophrenia, which in turn may be associated with AMPAR abnormalities in this illness. We determined both transcript and protein expression of the TARP family using quantitative real-time PCR (qPCR) and Western blot analysis in the anterior cingulate cortex (ACC) from subjects with schizophrenia and a comparison group.

2. Methods and Materials

2.1 Tissue acquisition and preparation

Samples from the full thickness of grey matter from ACC were obtained from the Mount Sinai Medical Center Schizophrenia Brain Collection. Tissue was obtained in compliance with the Mount Sinai School of Medicine Institutional Review Board protocol for postmortem tissue, and was obtained and prepared as previously described (Funk et al., 2012; Hammond et al., 2010). Briefly, patients were diagnosed with schizophrenia using DSM-III-R criteria, and had a documented history of psychiatric symptoms before the age of 40, as well as 10 or more years of hospitalization with a diagnosis of schizophrenia as determined by 2 clinicians. Patients were prospectively recruited and underwent ante mortem clinical assessments, and those with histories of alcoholism, substance abuse, death by suicide, or coma for more than 6 h before death were excluded from study. Neuropathological examinations found no neurodegenerative diseases, including Alzheimer's disease, in any patient. Next of kin consent was obtained for each patient.

Prospective comparison subjects were selected using a formal blinded medical chart review instrument. Subjects were limited to those with no history of alcohol abuse, drug abuse, psychiatric illness, or neurological disease. Assessments included the CERAD battery, the Clinical Dementia Rating Scale and the Positive and Negative Syndrome Scale (Powchik et al., 1998). Additionally, comparison subjects with a diagnosis of dementia or neurodegenerative disease were excluded from study.

Two different sample sets were used for transcript (schizophrenia, N = 25; comparison, N = 25) and protein (schizophrenia, N = 36; comparison, N = 33) studies (Table 1). From these two tissue sets, 11 schizophrenia and 11 comparison samples overlap, and, in several cases, not every subject was available for study in each experiment. A detailed list of subject characteristics is shown in Supplementary Table 1.

2.2 RNA isolation for transcript studies

RNA was isolated from homogenized tissue samples with an AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA, USA), and RNA concentration was determined by UV spectrophotometry. Approximately 1 μg of RNA from each subject was immediately treated following isolation with DNase I (Promega, Madison, WI, USA) for 30 min at 37° C. DNase I was subsequently deactivated by incubating for 15 min at 65° C before reverse transcribing using a High-Capacity cDNA RT Kit (Applied Biosystems, Foster City, CA, USA) containing random primers, dNTPs and transcriptase.

2.3 Comparative quantitative real-time polymerase chain reaction (qPCR)

Taqman® assays (Applied Biosystems, USA) were obtained for all known TARPs, as well as for three housekeeping genes, peptidyl-prolyl isomerase A/cyclophilin A (PPIA), beta-2 microglobulin (B2M) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Supplementary Table 2). qPCR was performed using a Stratagene Mx 3000P qPCR system (Agilent Technologies, Santa Clara, CA, USA) and a FAM-490 detection procedure. Each qPCR reaction contained 0.5 μl Taqman® assay at 1× concentration, 5 μl Jumpstart™ Taq ReadyMix™ (Sigma-Aldrich, St. Louis, MO, USA), 2 μl RNase/DNase-free water

(Molecular Devices, Sunnyvale, CA, USA) and 2.5 μl of cDNA diluted 1:3 for a total volume of 10 μl. Each subject sample was loaded in duplicate into 96-well optical reaction plates (Stratagene, La Jolla, CA, USA), with one gene per plate. In addition, each plate included negative control wells lacking cDNA. To generate a standard curve for the quantification of each gene, a pooled calibrator cDNA sample and a set of serial dilutions ranging from 1:5 to 1:40 were made from aliquots of a pool derived from all subject samples and loaded in triplicate into each plate. Cycling conditions consisted of a 2 min hold at 50°C, followed by one denaturing cycle of 95° C for 10 min, and 50 subsequent denature-anneal cycles of 95° C for 15 s and 60° C for 1 min.

2.4 Quantification and statistical analysis of transcript studies

A standard curve method was used to quantify transcript expression (Larionov et al., 2005). Briefly, cycle thresholds (C_t) were taken during the linear range of the standard curve, averaged, and normalized for each subject to the geometric mean of three housekeeping genes. Multiple regression analyses were performed to determine any significant correlations of dependent measures with sex, age at time of death, tissue pH or postmortem interval (PMI). Differences in gene expression were tested by performing analysis of variance (ANOVA), or covariance (ANCOVA) if a potential covariate was found to be correlated with dependent measures, using Statistica software (StatSoft, Inc., Tulsa, OK, USA). Outliers more than 4 standard deviations from the mean were excluded from statistical analysis. For all tests, $\alpha = 0.05$.

2.5 Western blot analysis

Tissue was prepared for protein analysis as previously described (Funk et al., 2009). Briefly, previously snap-frozen tissue was reconstituted in 5 mM Tris-HCl, pH 7.4, 0.32M sucrose, homogenized using a Power Gen 125 homogenizer (Thermo Fisher Scientific, Rockford, IL, USA) at speed 5 for 60 s, assayed for protein concentration with a BCA protein assay kit, (Thermo Fisher Scientific, USA) and stored at −80° C. Homogenized samples containing 20 μg protein/well were reduced in buffer containing β-mercaptoethanol, and denatured at 70° C for 10 min. Samples were subsequently run in duplicate via electrophoresis on 4–12% SDS-polyacrylamide gels (Invitrogen, Carlsbad, CA, USA) and transferred to polyvinylidene fluoride (PVDF) membranes (Invitrogen, USA) using a semi-dry transblotter (Bio-Rad, Hercules, CA, USA).

Commercially available primary antibodies were used for each of the TARP subunits and valosin-containing protein (VCP), and conditions for each antibody were individually optimized (Table 2). Briefly, blots were blocked with LI-COR blocking buffer (LI-COR Biosciences, Lincoln, NE, USA) or 5% (w/v) bovine serum albumin (BSA) and tris-buffered saline (TBS) for 1 hr at room temperature before incubating with primary antibody in the same buffer overnight at 4° C. Membranes were then washed 2 × 5 min with 0.05% Tween/TBS and subsequently incubated with the corresponding IR-dye labeled rabbit or mouse secondary antibody (LI-COR Biosciences, USA) for 1-4 hrs in the dark at room temperature. Blots were washed 2 × 5 min with 0.05% Tween/TBS and scanned with the LI-COR Odyssey laser-based image detection system (LI-COR Biosciences, USA).

Individual bands from all subjects were measured using Odyssey 3.0 analytical software (LI-COR Biosciences, USA), averaged, and normalized to VCP as a loading control. Changes in protein expression were determined by performing analysis of variance, (ANOVA) or covariance (ANCOVA) if a potential covariate was found to be correlated with dependent measures, using Statistica software (StatSoft, Inc., USA). Outliers more than 4 standard deviations from the mean were excluded from statistical analysis. For all tests, $\alpha = 0.05$.

2.6 Haloperidol-treated rats

Haloperidol decanoate (28.5 mg/kg) or vehicle (sesame oil) injections were injected intramuscularly to house-paired male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) once every three weeks for nine months for a total of 12 injections. This dose was chosen based on previous reports (Harte et al., 2005; Kashihara et al., 1986; Mithani et al., 1987). The animals were sacrificed in compliance with the University of Alabama at Birmingham's IACUC regulations, and the brains immediately harvested, dissected, and stored at −80° C.

Tissue from 10 haloperidol and 10 vehicle treated animals were used for these experiments. For the transcript studies, 1 μg of total rat frontal cortex was stabilized with RNAlater®-ICE (Life Technologies, Carlsbad, CA, USA) and RNA isolated using an RNeasy® Mini RNA isolation kit (Qiagen, USA). Isolated RNA was reverse transcribed using a High-Capacity cDNA RT Kit (Applied Biosystems, USA) containing random primers, dNTPs and transcriptase. Rat-specific Taqman® assays (Applied Biosystems, USA) were obtained for the TARPs, as well as for three housekeeping genes, peptidyl-prolyl isomerase A/cyclophilin A (PPIA), beta-2 microglobulin (B2M) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Supplementary Table 3). Data were analyzed as described above for the human studies.

For protein studies, rat frontal cortex was prepared in homogenization buffer containing 2.5 mM HEPES, pH 7.7, 2 mM EDTA, 2% SDS and a protease inhibitor tablet (Complete Mini, Roche Diagnostics, Mannheim, Germany) using a Model 100 Sonic Dismembrator (Thermo Fisher Scientific, USA) at speed 6 for 3 pulses of 5 s each. The homogenates were assayed for protein concentration with a BCA protein assay kit (Thermo Fisher Scientific, USA) and stored at −80° C. Western blotting procedures and data analyses were performed as described above for the human studies. The same antisera were used in the human and rat studies due to cross-reactivity and antigen homology across both species.

3. Results

3.1 TARP transcript expression in schizophrenia

mRNA levels were measured by qPCR in schizophrenia and comparison subjects (Figure 1). The three housekeeping genes PPIA, B2M and GAPDH were unchanged between diagnostic groups, (average housekeeping C_t = 24.6) and all TARPs except γ-1 and γ-6 were identified and quantifiable (average TARP C_t = 30.1). TARP γ-8 was significantly correlated with PMI ($r = 0.32$, $p = 0.03$); ANCOVA showed a main effect for diagnosis, (N, scz = 24, c = 23; $F(1, 44) = 4.4$, $p = 0.042$) and γ-8 was significantly decreased in schizophrenia. None of the other TARP transcripts were significantly changed in this illness.

3.2 TARP protein expression in schizophrenia

TARP protein expression was determined by Western blot analysis in schizophrenia and comparison subjects (Figure 2). The loading control protein VCP was unchanged between groups. γ-4, (N, scz = 29, c = 27; $F(1, 54) = 8.17$, $p = 0.006$); γ-7, (N, scz = 31, c = 31; $F(1, 60) = 9.69$, $p = 0.003$); and γ-8, (N, scz = 30, c = 26; $F(1, 54) = 5.03$, $p = 0.029$) were all decreased in schizophrenia. None of these proteins were significantly associated or correlated with sex, age, pH or PMI. TARP γ-3 protein expression was significantly correlated with pH, ($r = 0.37$, $p = 0.003$) and ANCOVA showed a main effect for diagnosis, (N, scz = 34, c = 29; $F(1, 60) = 10.9$, $p = 0.002$) and γ-3 was significantly increased in schizophrenia. γ-5 (N, scz = 31, c = 28; $F(1, 57) = 5.35$, $p = 0.024$) was also increased in schizophrenia, and not significantly correlated with any covariant.

3.3 Effects of antipsychotic treatment on TARP expression

To determine whether the changes found for TARP mRNA and protein expression in schizophrenia might be due to the effects of chronic antipsychotic treatment, we conducted parallel transcript and protein studies in the frontal cortex of rats chronically treated with haloperidol. TARP expression in these animals was not significantly different for either transcript (Figure 3) or protein expression (Figure 4).

4. Discussion

In this study, we measured TARP transcript and protein expression in the ACC and found decreased γ-8 mRNA (Figure 1), but unchanged levels of other transcripts. We also found decreased γ-4, γ-7 and γ-8, and increased γ-3 and γ-5 protein levels in schizophrenia (Figure 2). Further, we measured TARP transcript and protein expression in frontal cortex of rats treated chronically with haloperidol, and determined that all TARPs were unchanged (Figures 3 & 4). These data are consistent with AMPAR abnormalities in schizophrenia, and suggest diminished AMPAR trafficking and function in this illness.

A major finding in the current study is that TARP γ-4, γ-7 and γ-8 are all decreased in parallel in schizophrenia. These three subunits have two different yet dynamic functions: first, they specifically increase trafficking of AMPARs to the synapse following AMPAR-TARP coassembly in the endoplasmic reticulum (ER), and second, they modulate biophysical activity of AMPARs by decreasing desensitization and deactivation rates, and increasing resensitization rates, channel conductance and affinity for glutamate, thus effectively increasing the activity of the channel (Díaz, 2010; Jackson and Nicoll, 2011a; Sager et al., 2009; Tomita, 2010). Decreased expression of γ-4, γ-7 and γ-8 suggests fewer TARP-AMPAR complexes within the postsynaptic density, as well as reduced activity of AMPA channels, consistent with the hypothesis of decreased AMPAR activity in schizophrenia.

In contrast, TARP γ-3 and γ-5 were found to be increased. The effect of γ-5 on AMPAR trafficking appears to be minimal; however, this subunit has been shown to increase desensitization and deactivation rates of AMPARs and decrease glutamate affinity, while the other TARP subunits have the opposite effects (Jackson and Nicoll, 2011a; Kato et al., 2008). Further, γ-5 appears to be the only subunit capable of affecting AMPA channel peak open probability (Jackson and Nicoll, 2011a; Soto et al., 2009). Accordingly, in concert with decreased γ-4, γ-7 and γ-8, increased γ-5 in schizophrenia may also be consistent with decreased activity of AMPA channels by increasing desensitization rates, and decreasing open probability and glutamate affinity.

The increased TARP γ-3 protein expression we found is likely to have opposing effects from the convergence of other changes we found on AMPAR regulation in schizophrenia, by increasing the number of AMPARs delivered to the synapse and increasing AMPA channel activity. We suggest that this change in γ-3 is a partial compensatory response to decreased expression of γ-4, γ-7 and γ-8, and increased expression of γ-5. In support, studies performed in TARP knockout mice suggest the ability of these proteins to compensate for loss of function (Jackson and Nicoll, 2011a; Menuz et al., 2008), and increased γ-3 may be a reflection of this mechanism.

Although NMDAR hypofunction has been proposed as a component of the pathophysiology of schizophrenia, this may be associated instead with AMPAR dysregulation. NMDARs and AMPARs are colocalized at the excitatory synaptic membrane, and their sequential coactivation is required for normal glutamatergic neurotransmission. Thus, any abnormality of AMPARs due to alterations of receptor expression, localization or function that resulted

in diminished AMPAR function could result in an apparent alteration of NMDAR activity. These considerations have led to hypotheses of potential AMPAR dysfunction in schizophrenia.

Once AMPARs are coassembled with TARPs in the ER they are trafficked to the extrasynaptic membrane before being laterally translocated to the synaptic cleft for insertion (Coombs and Cull-Candy, 2009; Opazo and Choquet, 2011; Tomita et al., 2003). AMPAR complexes are tethered to the postsynaptic membrane by TARP-PSD95 interaction via PDZ binding domains, but they do not become static: AMPARs remain in constant flux, entering in and out of recycling pathways as needed to regulate synaptic strength and plasticity (Bats et al., 2007; Hanley, 2008; Malinow and Malenka, 2002; Opazo and Choquet, 2011; Sager et al., 2009; Schnell et al., 2002). TARPs are direct modulators of this trafficking pathway, and their dysregulation could contribute to altered AMPAR localization and function at the excitatory synapse. Given the altered expression of TARPs in the ACC, we propose a working model of altered forward trafficking of AMPARs in which abnormal expression of AMPAR auxiliary proteins, such as TARPs, contribute to dysregulated AMPAR trafficking and tethering within intracellular compartments that in turn result in a reduction of functional AMPARs at the synapse. In support of this model are previous reports of alterations in schizophrenia of other AMPAR-associated proteins with known roles in receptor assembly, trafficking, and synaptic localization, including PICK1 (Beneyto and Meador-Woodruff, 2006; Dev et al., 1999; Lu and Ziff, 2005), GRIP1 (Dracheva et al., 2005; Hammond et al., 2010), SAP97 (Hammond et al., 2010; Toyooka et al., 2002), and NSF (Mirnics et al., 2000).

The precise mechanisms underlying TARP-mediated AMPAR trafficking are not yet well understood. TARP mutant mice demonstrate cell-specific and TARP-specific involvement in AMPAR trafficking to synaptic and extrasynaptic membranes (Chen et al., 1999; Chen et al., 2000; Hashimoto et al., 1999; Jackson and Nicoll, 2011a, b; Letts et al., 2005; Menuz et al., 2009; Menuz and Nicoll, 2008; Rouach et al., 2005; Yamazaki et al., 2010). The functions of the TARP subunits are known to significantly overlap, serving to not only increase trafficking and targeting of AMPARs to the synapse, but also to affect biophysical properties of the ion channel (Jackson and Nicoll, 2011a; Sager et al., 2009). TARPs may exhibit binding preferences for specific AMPAR subtypes, (Kato et al., 2008; Kott et al., 2007; Soto et al., 2007; Soto et al., 2009; Suzuki et al., 2008; Zonouzi et al., 2011) or bind in combination with other AMPAR accessory proteins such as the recently described cornichons (Jackson and Nicoll, 2011a; Kato et al., 2010a; Schwenk et al., 2012; Schwenk et al., 2009). AMPARs are not likely to remain bound to the same TARP(s) throughout their lifecycle (Morimoto-Tomita et al., 2009; Tomita et al., 2004), further increasing the complexity of TARP involvement in AMPAR regulation.

A limitation of this study is the potential impact of chronic antipsychotic treatment on transcript and protein expression. To address potential medication effects in this study, we performed parallel transcript and protein studies in frontal cortex from rats chronically treated with haloperidol, and found no significant changes in TARP transcript or protein expression between haloperidol and vehicle-treated control animals. To attempt to address this issue directly in patients in addition to these rodent studies, we performed *post hoc* analyses for each dependent measure grouped by antipsychotic treatment status within the schizophrenia group. Patients were grouped by treatment status, and were either receiving antipsychotic treatment at the time of death, or not treated if they were receiving no antipsychotics for 6 weeks or more prior to death. These analyses revealed no differences in TARP transcript or protein expression in subjects with schizophrenia on or off of these medications. Taken together, these data suggest that the changes in TARP expression we

found in schizophrenia may not be due to chronic antipsychotic treatment but rather the illness itself.

A second limitation of this study is that all of the subjects were elderly and generally in late stages in the progression of this illness with primarily negative and cognitive symptoms. Accordingly, generalization of these findings to younger patients, or those with predominantly positive symptoms, should be made with caution.

In summary, multiple members of the TARP family of AMPAR accessory proteins are abnormally expressed in the ACC in schizophrenia, consistent with our model of abnormal AMPAR trafficking in this illness. Decreased TARP γ-4, γ-7 and γ-8, and increased γ-5 are consistent with abnormal AMPAR localization and decreased function at the synapse in schizophrenia. TARP subunits may work alone or synergistically with other AMPAR auxiliary proteins to modulate the lifecycle and function of AMPARs, potentially affecting normal glutamatergic neurotransmission and contributing to the pathophysiology of schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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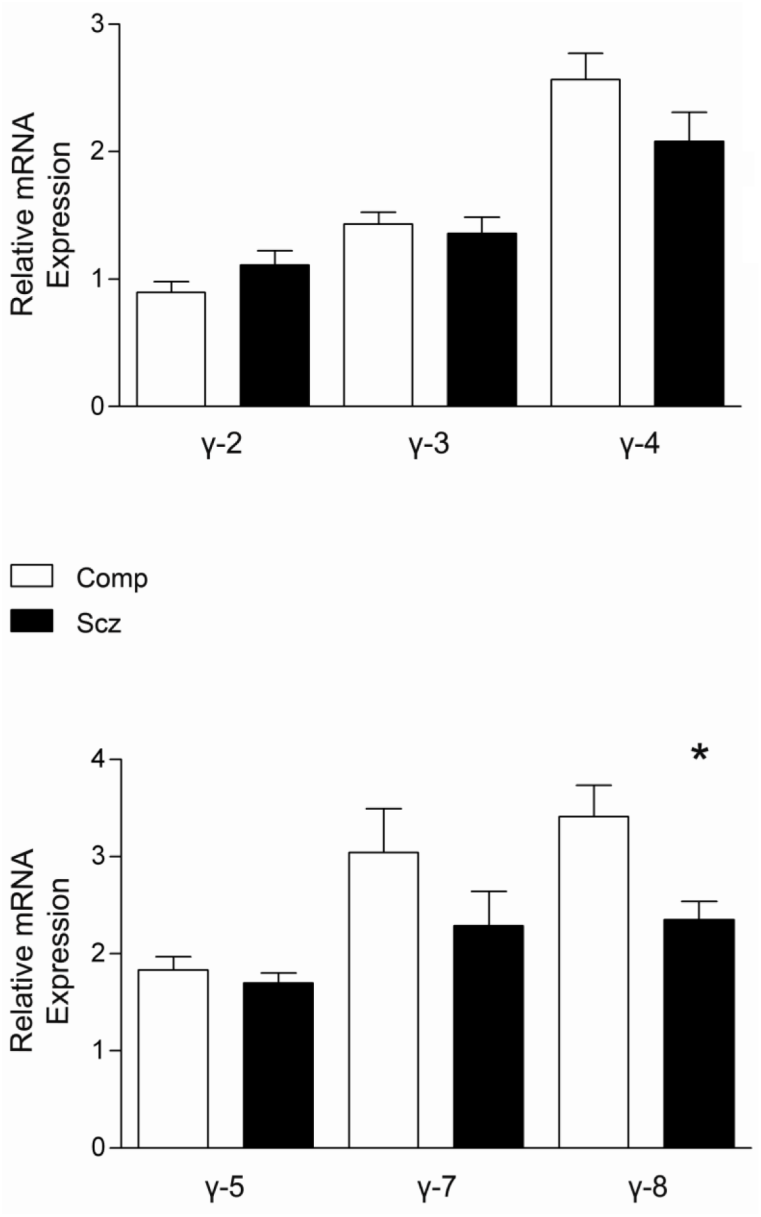

Figure 1.

TARP transcript expression is altered in the ACC in schizophrenia. TARPs were measured by quantitative comparative real-time PCR (qPCR) in schizophrenia (N = 25) and comparison (N = 25) subjects. Data are presented as ratio of gene of interest to the geometric mean of three housekeeping genes. TARP γ-1 and γ-6 mRNAs were not detected. γ-8 transcript expression is significantly decreased in schizophrenia. Data are expressed as means ± SEM. *$p < 0.05$. Comp, comparison subjects; Scz, schizophrenia.

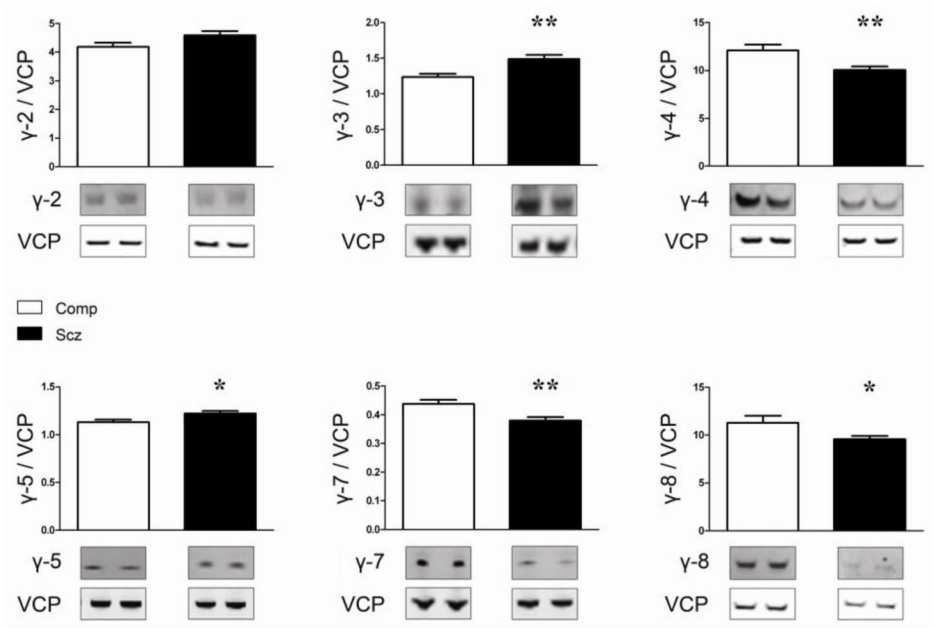

Figure 2.

TARP proteins are abnormally expressed in schizophrenia. TARPs were assayed by Western blot analysis using commercially available antibodies. Samples from schizophrenia (N = 36) and comparison (N = 33) subjects were run in duplicate and normalized to valosin-containing protein (VCP) as a within-lane loading control. Representative blots of each TARP and corresponding VCP blot are shown for both groups. Data are expressed as means ± SEM. *$p < 0.05$, **$p < 0.01$. Comp, comparison subjects; Scz, schizophrenia.

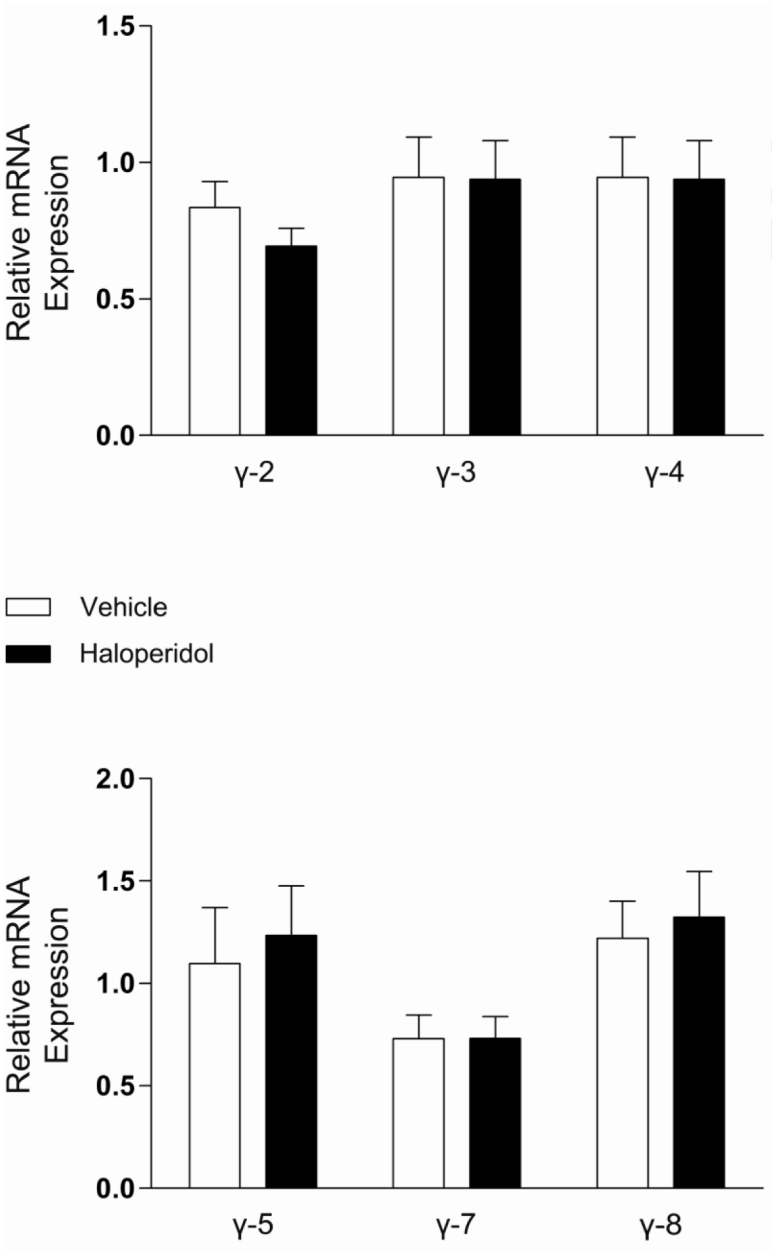

Figure 3.
TARP transcript expression in rats treated for 9 months with haloperidol. TARP transcript expression was assayed by qPCR from frontal cortex of haloperidol (N = 10) and vehicle treated (N = 10) rats. Chronic treatment with haloperidol did not affect TARP transcript expression. Data are expressed as means ± SEM.

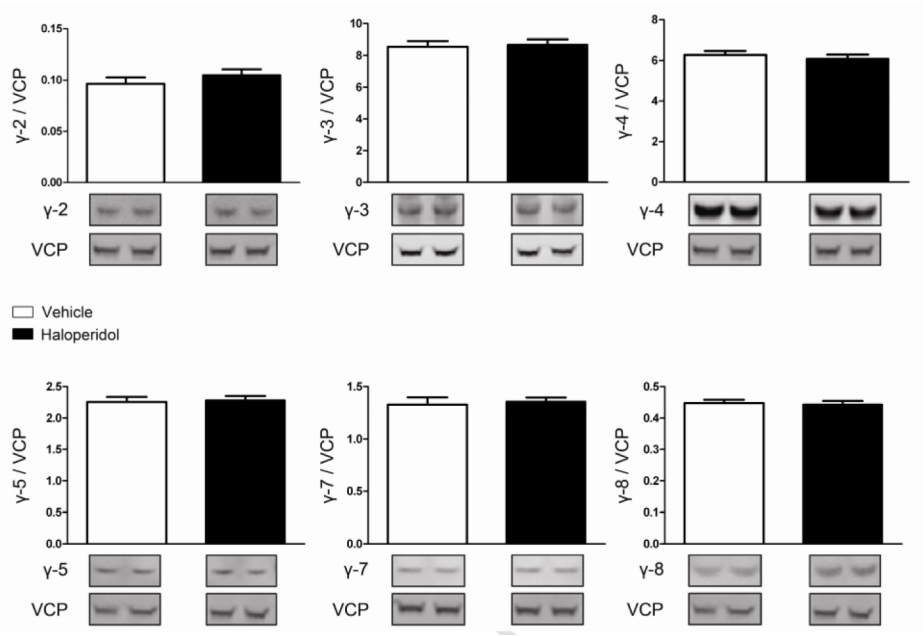

Figure 4.

TARP protein expression in rats treated for 9 months with haloperidol. TARP protein expression was assayed by Western blot analysis from frontal cortex of haloperidol (N = 10) and vehicle treated (N = 10) rats using commercially available antibodies. Chronic treatment with haloperidol did not affect TARP protein expression. Representative blots of each TARP and corresponding valosin-containing protein (VCP) are shown below for both animal groups. Data are expressed as means ± SEM.

Table 1

Subject characteristics.

	Transcript Studies		Protein Studies	
	Schizophrenia	**Comparison**	**Schizophrenia**	**Comparison**
N	**25**	**25**	**36**	**33**
Sex	9 F, 16 M	13 F, 12 M	11 F, 25 M	19 F, 14 M
Age	75.2 ± 12.9	75.8 ± 11.4	74.3 ± 11.7	77.8 ± 14.0
Tissue pH	6.5 ± 0.2	6.6 ± 0.3	6.4 ± 0.3	6.4 ± 0.2
PMI	17.1 ± 10.1	9.6 ± 7.0	13.6 ± 8.2	8.3 ± 6.8
Rx (on/off)	17/8	0/25	25/11	0/33

Data are means ± SD. Abbreviation: PMI, postmortem interval (hours); Rx, treatment with antipsychotic medication (on, receiving these drugs at time of death; off, no antipsychotic treatment for 6 weeks or more prior to death).

Table 2

Antisera and conditions used for Western blot analyses.

Protein	Company	Species/ Molecular weight (kDa)	1°/Buffer[a]	2° Species	2°/Time[b]
TARP γ-2	Cell Signaling	Human/40	1:1000/BSA	Rabbit	1:5000/4hrs
TARP γ-3	Lifespan Biosciences	Human/36	1:100/BSA	Rabbit	1:5000/2hrs
TARP γ-4	Lifespan Biosciences	Human/37	1:1000/LI-COR	Rabbit	1:5000/4hrs
TARP γ-5	Lifespan Biosciences	Human/31	1:500/LI-COR	Rabbit	1:5000/2hrs
TARP γ-7	Lifespan Biosciences	Human/31	1:500/LI-COR	Rabbit	1:5000/3hrs
TARP γ-8	Lifespan Biosciences	Human/43	1:500/LI-COR	Rabbit	1:5000/2hrs
VCP	Abcam	Human/90	1:10000/LI-COR	Mouse	1:10000/1hr

[a]Dilution of primary antisera and corresponding blocking buffer. Bovine serum albumin (BSA) was used at 5% (w/v) in tris-buffered saline (TBS), and all blots were incubated overnight at 4° C.

[b]Dilution of secondary antisera incubated in same buffer as primary antisera, and incubation time in the dark at room temperature.