Schizophrenia Research 146 (2013) 254–263

Contents lists available at SciVerse ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Potential molecular mechanisms for decreased synaptic glutamate release in dysbindin-1 mutant mice ☆

Shalini Saggu [a,1], Tyrone D. Cannon [b,c], J. David Jentsch [d], Antonieta Lavin [a,*]

[a] Dept of Neuroscience, Medical University of South Carolina, Charleston, SC, USA
[b] Dept of Psychology, University of California, Los Angeles, CA, USA
[c] Dept of Psychiatry & Biobehavioral Sciences, University of California, Los Angeles, CA, USA
[d] Dept of Human Genetics, University of California, Los Angeles, CA, USA

ARTICLE INFO

Article history:
Received 20 November 2012
Received in revised form 28 January 2013
Accepted 30 January 2013
Available online 6 March 2013

Keywords:
Prefrontal cortex
Calcium
Dysbindin
Synapsin
Synaptotagmin
Synaptic vesicles

ABSTRACT

Behavioral genetic studies of humans have associated variation in the DTNBP1 gene with schizophrenia and its cognitive deficit phenotypes. The protein encoded by DTNBP1, dysbindin-1, is expressed in forebrain neurons where it interacts with proteins mediating vesicular trafficking and exocytosis. It has been shown that loss of dysbindin-1 results in a decrease in glutamate release in the prefrontal cortex; however the mechanisms underlying this decrease are not fully understood. In order to investigate this question, we evaluated dysbindin-1 null mutant mice, using electrophysiological recordings of prefrontal cortical neurons, imaging studies of vesicles, calcium dynamics and Western blot measures of synaptic proteins and Ca^{2+} channels. Dysbindin-1 null mice showed a decrease in the ready releasable pool of synaptic vesicles, decreases in quantal size, decreases in the probability of release and deficits in the rate of endo- and exocytosis compared with wild-type controls. Moreover, the dysbindin-1 null mice show decreases in the $[Ca^{2+}]_i$,expression of L- and N-type Ca^{2+}channels and several proteins involved in synaptic vesicle trafficking and priming. Our results provide new insights into the mechanisms of action of dysbindin-1.

1. Introduction

Schizophrenia is a relatively common neuropsychiatric disorder that often involves debilitating and treatment-refractory cognitive deficits that can significantly limit the psychosocial function of affected persons (Green et al., 2000). The disorder is highly heritable, and a number of candidate susceptibility genes have emerged recently (Gejman et al., 2010; Ayalew et al., 2012).

Of these putative risk genes, the gene encoding dystrobrevin-binding-protein-1 (i.e., dysbindin-1) — DTNBP1 is of particular interest. DTNBP1 lies within the chromosome 6p24-22 susceptibility locus (Straub et al., 1995), and multiple associations have been reported between variants of DTNBP1 and schizophrenia (Talbot et al., 2009; Maher et al., 2010). Beyond association between sequence variants and the disorder, a large proportion of schizophrenia patients exhibit lower dysbindin-1C protein in tissue from the PFC (Tang et al., 2009). There are three isoforms of dysbindin: dysbindin 1A, 1B and 1C. Dysbindin 1C is mainly found in postsynaptic sites in human tissue; however it has also been reported in presynaptic sites (Talbot et al., 2011). In mice, dysbindin 1C is the only form of protein found presynaptically (Talbot et al., 2011). Amongst its functions, dysbindin-1 is involved in the control of presynaptic release of glutamate. Recent studies (Chen et al., 2008; Jentsch et al., 2009) have reported that reduced expression of dysbindin-1 in mice dampened glutamate release in the PFC and hippocampus.

Dysbindin-1 is part of the Biogenesis of Lysosome-related Organelle Complex 1 (BLOC-1 complex) (Starcevic and Dell'Angelica, 2004) which is compromised by 8 proteins (dysbindin, snapin, muted, pallidin, cappuccino and BLOS 1–3). This complex has been related to multiple cellular functions including synaptic vesicle dynamics and stabilization of the t-SNARE complex (Larimore et al., 2011; Mullin et al., 2011; Newell-Litwa et al., 2009, 2010). Interestingly, decreases in dysbindin-1 reduce the level of snapin (Feng et al., 2008) which, in turn, affects its association with SNAP25 and the interactions between SNAP25 and the calcium sensor synaptotagmin-1, thus impairing priming of vesicles. Moreover, changes in dysbindin-1 produce changes in synapsin 1 (Numakawa et al., 2004), which controls the movement of synaptic vesicles from the reserve pool to the ready-releasable pool (RRP) (Cesca et al., 2010), consequently facilitating synaptic vesicle trafficking following high frequency stimulation.

Here, we provide evidence that mice with loss dysbindin-1 expression exhibit a decrease in glutamate release that may be underlain by decreases in the expression of L- and N-type Ca^{2+} channels, resulting

☆ This research was funded by PHS grants MH-83269 (TC, JDJ, AL).
* Corresponding author at: Dept. of Neuroscience, Medical University of South Carolina, Charleston, SC 29425, USA. Tel.: +1 843 792 6799.
E-mail address: lavina@musc.edu (A. Lavin).
[1] Actual address: Department of Biology, Faculty of Sciences, University of Tabuk, Tabuk, Saudi Arabia.

0920-9964/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.schres.2013.01.037

in deficits in $[Ca^{2+}]_i$, abnormalities in synaptic vesicle priming and deficits in the replenishment of the ready releasable pool.

2. Methods

2.1. Animals

Studies were performed on mice carrying a large genomic deletion (exons 6–7; introns 5–7, Li et al., 2003) contained wholly within the DTNBP1 gene. We used mice that had been backcrossed to the C57Bl/6J background (Jackson Laboratories, Bar Harbor, Maine). All animals were genotyped as previously described (Jentsch et al., 2009). All the WT mice were littermates of the dys −/− mice. Male mice were used in the electrophysiological and molecular experiments described here; with the exception of the studies using FM1-43 (for which the subjects were 20–30 days of age), all subjects were 45–60 days of age at the time of study. All experimental protocols were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee.

2.2. Electrophysiology

Brain slices (300 μm) were prepared from 10 dysbindin-1 wild-type (WT) and 13 null mutant (dys −/−) mice. Subjects were anesthetized with isoflurane (Abbott Laboratories). The brain was removed, and coronal slices containing the infralimbic and prelimbic PFC were cut at 300 μm thickness in ice-cold high-sucrose solution containing (in mM): sucrose, 200; KCl, 1.9; Na_2HPO_4, 1.2; $NaHCO_3$, 33; $MgCl_2$, 6; $CaCl_2$, 0.5; D-glucose, 10; ascorbic acid, 0.4. Slices were incubated at 33 °C for at least 1 h before recordings; the incubation medium contained (in mM): NaCl, 125; KCl, 2.5; NaH_2PO_4,1.25; $NaHCO_3$, 25; MgCl, 4, CaCl, 1, D-glucose, 10; sucrose, 15; ascorbic acid, 0.4, aerated with 5%CO_2/95%O_2. After incubation, slices were transferred to a submerged chamber and superfused with oxygenated artificial cerebrospinal fluid (aCSF) (in mM): 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 2.0 $CaCl_2$, 1.3 $MgCl_2$, 10 D-glucose and 0.4 ascorbic acid at room temperature. Recordings were made using a Multiclamp 700B amplifier (Axon Instruments, CA), connected to a computer running Windows XP and Axograph X software. All recordings were obtained from pyramidal neurons in layers V or VI of the prelimbic or infralimbic cortex, identified using infrared-differential interference contrast optics and video-microscopy.

Voltage clamp: picrotoxin (50 μM) was included in the perfusion solution to block $GABA_A$ receptors. For voltage-clamp recordings, electrodes (3–7 MΩ resistance in situ) were filled with a solution containing (in mM): 135 CsCl, 10 HEPES, 2 $MgCl_2$, 1 EGTA, 4 NaCl, 2 Na-ATP, 0.3 tris-GTP, 1 QX-314, 10 phosphocreatine; and 285 mOsmols. Series resistances (10–20 MΩ), and input resistances were continually monitored throughout the experiment via a −1 mV (100 ms) hyperpolarizing pulse. Pyramidal neurons were clamped at −80 mV. Electric stimulation was delivered via a bipolar concentric electrode positioned in layer II of the PFC. Evoked EPSCs (eEPSCs) were elicited via the stimulation electrode, and the amplitude of the eEPSCs was adjusted to 75% of the maximum amplitude. In order to deplete the RRP, we use a protocol consisting of 20 pulses (1 ms duration) at 40 Hz (delivered 30 times). Miniature EPSCs (mEPSCs) were measured after adding 1 μM TTX to the buffer solution. In order to control for a possible role for endocannabinoid release, depletion experiments were repeated using a minimal stimulation protocol. Briefly, the amplitude of the eEPSCs was adjusted to the minimum amount of current that elicited a constant amplitude eEPSC across 5 trials, then 20 pulses (1 ms duration) at 40 Hz (delivered 30 times) were applied.

2.3. Preparation of synaptosomes

Infralimbic and prelimbic PFC (referred as PFC) tissue from three animals/genotype (unless otherwise indicated) was pooled together to make N = 1 in each group. The tissue was homogenized with 10 strokes in a Potter homogenizer holding 5 ml of ice-cold isolation buffer containing 320 nM sucrose, 1 mM Na-EDTA, 10 mM Tris-HEPES (pH 7.4) and a protease inhibitor cocktail (Sigma, catalog # P8340). The homogenates were centrifuged at 600 *g* for 10 min to obtain a pellet fraction (P1) enriched in cell debris, intact cells and nuclei. The post-nuclear fraction (S1) was collected and centrifuged for 15 min at 9200 *g*. The pellet was collected and washed by resuspension in Krebs buffer containing 125 mM NaCl, 5 mM KCl, 0.1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM D-glucose and 10 mM HEPES-NAOH, pH 7.4. After washing, the P2 pellet was resuspended in Krebs buffer, and protein concentration was determined by a Bradford assay (Bio Rad). The pellet was a crude synaptosomal fraction.

2.4. Loading of synaptosomes with FM1-43

Synaptosomes were loaded with dye according to Meffert et al. (1994) with modifications. In brief aliquots of synaptosomes (0.4–0.5 mg of protein/ml) were resuspended in Krebs buffer and loaded with 5 μM of FM1-43 for 10 min at 30 °C, followed by the addition of 40 mM KCl for 1 min. Following loading, synaptosomes were pelleted by brief centrifugation followed by washing, repelleting, and resuspension in Krebs buffer containing 1 mM $CaCl_2$. Fluorescence measurements were carried out as described by Meffert et al. (1994).

2.5. Determination of synaptosomal $[Ca^{2+}]_i$

Synaptosomal fractions from PFC were incubated with 5 μM Fluo-3 and Fluo-4 and 4 μM Fura-2 (Molecular Probes) for 30 min at 37 °C (Yamaguchi et al., 1998). Samples were centrifuged at 10,000 *g* for 3 min, and pellets were resuspended with 200 μl of pre-warmed Krebs buffer. Fluo-3, Fluo-4 and Fura-2 loaded synaptosomes were placed in 96-well microplates (0.3 mg per well), and plates inserted in a fluorometer (Fluoroskan Ascent-Thermo Labsystems; Waltham, MA). $[Ca^{2+}]_i$ was measured by determining the changes in the ratio (R) of fluorescence at 340 (F1) and 380 (F2) nm of excitation for Fura-2 and for Fluo-3 and Fluo-4 measured at 488 nm of excitation, with an emission cut-off of 510 nm. Synaptosomal $[Ca^{2+}]_i$ was calculated according to the formula $[Ca^{2+}]_i = Kd \cdot B(R - R_{min})/(R_{max} - R)$, using 0.4% Triton X-100 and 7.5 mM EG pH 8.0 to calculate R_{max} and R_{min} values, respectively.

2.6. FM1-43 staining and destaining

PFC slices (250 μm) from 7 WT and 9 dys −/− mice were labeled with FM1-43 (8 μM; Invitrogen) for 5 min in aCSF containing CNQX (10 μM). They were transferred to CNQX + FM1-43 in high $[K^+]_0$ (45 mM) and 2 mM Ca^{2+} for 15 min to stimulate the uptake of FM1-43 via endocytosis of vesicles. All labeling and washing protocols were performed in the presence of CNQX to prevent synaptically-driven action potentials from accelerating dye release. After loading, slices were washed for 10 min prior to imaging. After taking basal images, slices were washed for 15 min in dye-free aCSF containing 100 μM sulforhodamin-101 and scanned. Depolarization-dependent de-staining was obtained by application of 90 mM K^+. All FM1-43 experiments were performed using a Zeiss LSM 510 confocal laser-scanning microscope with a 40× objective. Images were captured after FM1-43 uptake, after the application of the sulforhodamine (S-Rhd) quenching and again after 15 min of high K^+ depolarization. The filtering strategy used an emission filter with a narrow band pass at 540 ± 20 nm, a range of wavelengths over which FM1-43 emits but S-Rhd does not. Each image was 1024 × 1024 pixels. To monitor exocytosis, the brightness of single cells (containing clusters of synaptic vesicles) was quantified during all destaining or at specific time intervals.