Archival Report

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Regulation of Brain-Derived Neurotrophic Factor Exocytosis and Gamma-Aminobutyric Acidergic Interneuron Synapse by the Schizophrenia Susceptibility Gene Dysbindin-1

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ABSTRACT

BACKGROUND: Genetic variations in dystrobrevin binding protein 1 (*DTNBP1* or dysbindin-1) have been implicated as risk factors in the pathogenesis of schizophrenia. The encoded protein dysbindin-1 functions in the regulation of synaptic activity and synapse development. Intriguingly, a loss of function mutation in *Dtnbp1* in mice disrupted both glutamatergic and gamma-aminobutyric acidergic transmission in the cerebral cortex; pyramidal neurons displayed enhanced excitability due to reductions in inhibitory synaptic inputs. However, the mechanism by which reduced dysbindin-1 activity causes inhibitory synaptic deficits remains unknown.

METHODS: We investigated the role of dysbindin-1 in the exocytosis of brain-derived neurotrophic factor (BDNF) from cortical excitatory neurons, organotypic brain slices, and acute slices from dysbindin-1 mutant mice and determined how this change in BDNF exocytosis transsynaptically affected the number of inhibitory synapses formed on excitatory neurons via whole-cell recordings, immunohistochemistry, and live-cell imaging using total internal reflection fluorescence microscopy.

RESULTS: A decrease in dysbindin-1 reduces the exocytosis of BDNF from cortical excitatory neurons, and this reduction in BDNF exocytosis transsynaptically resulted in reduced inhibitory synapse numbers formed on excitatory neurons. Furthermore, application of exogenous BDNF rescued the inhibitory synaptic deficits caused by the reduced dysbindin-1 level in both cultured cortical neurons and slice cultures.

CONCLUSIONS: Taken together, our results demonstrate that these two genes linked to risk for schizophrenia (BDNF and dysbindin-1) function together to regulate interneuron development and cortical network activity. This evidence supports the investigation of the association between dysbindin-1 and BDNF in humans with schizophrenia.

Keywords: Brain-derived neurotrophic factor (BDNF), DTNBP1, Dysbindin-1, Exocytosis, Gamma-aminobutyric acid (GABA), Interneuron synapse, Schizophrenia

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In the mammalian central nervous system, neurons receive inhibitory synaptic inputs from gamma-aminobutyric acid (GABA)ergic interneurons and excitatory inputs from glutamatergic neurons (1,2). Converging experimental and clinical evidence suggests that the dysfunction of appropriate GABAergic inhibition and the consequent imbalance between excitation and inhibition in the cerebral cortex underlie the pathophysiology of schizophrenia, a complex psychiatric disorder with lifetime morbid risk close to 1% of the general population (3–5). For example, in the neocortex of schizophrenia patients, the activity of the rate-limiting synthetic enzyme glutamic acid decarboxylase (GAD) is reduced (6). This finding has been repeatedly confirmed and extended in subsequent studies that showed alterations in several presynaptic and postsynaptic components of the GABAergic system (7).

Dystrobrevin binding protein 1 (*DTNBP1* or dysbindin-1), which encodes dysbindin-1, has been implicated in several studies as a potential susceptibility gene for schizophrenia (8–11). In schizophrenia, dysbindin-1 expression has been reported to be reduced in cortical and limbic neurons, suggesting that reduced dysbindin-1 protein levels may be a disease-related trait (11–15). In mice, a loss-of-function mutation in *Dtnbp1* (dys-MT) disrupts both glutamatergic and GABAergic transmission in the prefrontal cortex, and this disruption is associated with working memory deficits (16–21). Most importantly, in dys-MT mice, cortical pyramidal neurons in the prefrontal cortex display enhanced excitability, presumably due to a reduction in GABAergic signaling (19–21). The mechanism underlying the loss of inhibitory activity in dys-MT mice remains unclear because dysbindin-1 expression is

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largely restricted to excitatory neurons rather than interneurons (13,22).

In this study, we discovered an unexpected role of dysbindin-1 in the exocytosis of brain-derived neurotrophic factor (BDNF), an important trophic factor for the differentiation and survival of inhibitory neurons from excitatory neurons (23). We further determined that this reduction in BDNF secretion reduces the number of functional GABAergic synapses that are transneuronally formed on excitatory neurons (24). Given that interneurons cannot synthesize BDNF by themselves but require BDNF for the formation and maintenance of their synapses (25–27), our results demonstrate that these two well-known schizophrenia susceptibility gene products (BDNF and dysbindin-1) function together to regulate interneuron synapse development. Furthermore, our results provide considerable insight into the cellular and molecular mechanisms that regulate the development of the neural circuitry in the brain and link these abnormalities in BDNF secretion to cognitive disease.

METHODS AND MATERIALS

A detailed description of the experimental procedures can be found in the Supplement. All animal experiments in this study were approved by the Institutional Animal Care and Use Committee of the Duke-National University of Singapore Graduate Medical School and the Lieber Institute for Brain Development.

Cell Culture

Cortical neurons were prepared from embryonic day 17 rat embryos and cultured in Neurobasal medium with B27 supplement (Invitrogen, Carlsbad, California). At day-in-vitro (DIV) 7, cultures were transfected with a calcium phosphate transfection kit (Clontech, Madison, Wisconsin). Human embryonic kidney 293 cells and PC12 cells were maintained using standard protocols and were transfected with Lipofectamine 2000 (Invitrogen).

Molecular Biology

Dysbindin-1 and scrambled short hairpin RNAs (shRNAs) were generated using pLentilox 3.7 vector. Dysbindin-1 shRNA target sequences were conserved in both mouse and rat dysbindin-1A and C isoforms. pCAG-MCS2-myc-dysbindin-1 was generated as reported previously (28), and the shRNA-resistant mutant of dysbindin-1 was generated by polymerase chain reaction-based mutagenesis.

Fluorescent Immunocytochemistry

Cultured rat neurons and mouse brain slices (postnatal day [P] 28) were stained with various primary antibodies, followed by incubation with appropriate fluorophore-conjugated secondary antibodies and imaged. Detailed antibody information can be found in the Supplement.

Imaging

Images were acquired with an LSM 710 (Zeiss, Singapore) confocal microscope using either 40× or 63× oil objectives and total internal reflection fluorescence microscopy imaging was conducted as previously reported (29).

Electrophysiology

Cortical neurons were transfected with various constructs as previously described (29). Whole-cell voltage clamp recordings were performed on DIV14 to DIV15 neurons. For acute slice recording, wild-type and dys-MT male mice at P15 to P35 were used as previously described (21).

Statistical Analysis

Unless otherwise stated, error bars represent the standard error of the mean. All statistical analyses are listed in the Supplement.

RESULTS

Increased Excitatory Synaptic Transmission in Layer 2/3 Pyramidal Neurons in the Prefrontal Cortex of dys-MT Mice

The prefrontal cortex (PFC) is a brain structure that is known to play a critical role in executive function and is prominently implicated in the pathophysiology of schizophrenia (21). In the PFC, top-down information is transmitted via pathways from layer (L) 2/3 pyramidal neurons to pyramidal neurons in L5, which is a major corticofugal output layer of the PFC network. We characterized the synaptic properties of L2/3 pyramidal neurons within the prelimbic area (PrL), homologous to the dorsolateral prefrontal cortex in primates, by measuring spontaneous excitatory postsynaptic currents (sEPSCs) using whole-cell patch-clamp recordings (Figure 1A, B) (20). Intriguingly, we found that dys-MT mice exhibited a dramatic increase in the frequency of the sEPSCs that were recorded from L2/3 pyramidal cells, without changing the amplitude (WT: 2.69 ± .62 Hz, 10.50 ± .29 pA; dys-MT: 6.65 ± 1.50 Hz, 10.99 ± 1.25 pA; $p < .05$) (Figure 1C, D). These results indicate an increase in excitatory synaptic transmission in the PFC of dys-MT mice.

Previous reports suggested that the decreased excitability of interneurons and decreased inhibitory transmission onto pyramidal neurons partially account for the increased frequency of sEPSCs in the PFC of dys-MT mice (20). To test this further, we monitored spontaneous miniature inhibitory postsynaptic currents (mIPSCs) in L2/3 pyramidal neurons within PrL. In the presence of glutamatergic antagonists and sodium channel blockers, we observed significant reduction in the frequency of mIPSCs (WT: 5.23 ± .30 Hz; dys-MT: 4.29 ± .27 Hz; $p < .05$) (Figure 1E, F). However, no change in mIPSC amplitude was detected in PrL of dys-MT mice (WT: 22.19 ± 1.98 pA; dys-MT: 18.31 ± 1.32 pA) (Figure 1E, G). Intriguingly, immunocytochemistry revealed that the density (WT: 1.52 ± .08 per 10 μm^2; dys-MT: 1.24 ± .04 per 10 μm^2; $p < .01$) and size (WT: 1.46 ± .05 μm^2; dys-MT: 1.33 ± .04 μm^2; $p < .05$) of vesicular GABA transporter (VGAT) (an inhibitory presynaptic marker)-expressing boutons, which represent GABAergic inhibitory synaptic terminals, in the L2/3 within the PrL of dys-MT mice were reduced significantly, indicating the reduced inhibitory inputs onto L2/3 pyramidal neurons (Supplemental Figure S1).