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Synergistic interactions between PDE4B and GSK-3: DISC1 mutant mice

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ABSTRACT

Disrupted-In-Schizophrenia-1 (DISC1) is a strong genetic risk factor associated with psychiatric disorders. Two distinct mutations in the second exon of the DISC1 gene (Q31L and L100P) lead to either depression- or schizophrenia-like behavior in mice. Both phosphodiesterase-4B (PDE4B) and glycogen synthase kinase-3 (GSK-3) have common binding sites on N-terminal region of DISC1 and are implicated into etiology of schizophrenia and depression. It is not known if PDE4B and GSK-3 could converge signals in the cell via DISC1 at the same time. The purpose of the present study was to assess whether rolipram (PDE4 inhibitor) might synergize with TDZD-8 (GSK-3 blocker) to produce antipsychotic effects at low doses on the DISC1-L100P genetic model. Indeed, combined treatment of DISC1-L100P mice with rolipram (0.1 mg/kg) and TDZD-8 (2.5 mg/kg) in sub-threshold doses corrected their Pre-Pulse Inhibition (PPI) deficit and hyperactivity, without any side effects at these doses. We have suggested that rolipram-induced increase of cAMP level might influence GSK-3 function and, hence the efficacy of TDZD-8. Our second goal was to estimate how DISC1-Q31L with reduced PDE4B activity, and therefore mimicking rolipram-induced conditions, could alter pharmacological response to TDZD-8, GSK-3 activity and its interaction with DISC1. DISC1-Q31L mutants showed increased sensitivity to GSK-3 inhibitor compare to DISC1-L100P mice. TDZD-8 (2.5 mg/kg) was able to correct PPI deficit, reduce immobility in the forced swim test (FST) and increased social motivation/novelty. In parallel, biochemical analysis revealed significantly reduced binding of GSK-3 to the mutated DISC1-Q31L and increased enzymatic activity of GSK-3. Taken together, genetic variations in DISC1 influence formation of biochemical complex with PDE4 and GSK-3 and strength the possibility of synergistic interactions between these proteins.

This article is part of a Special Issue entitled 'Schizophrenia'.

1. Introduction

DISC1 (*Disrupted-in-Schizophrenia-1)* was identified as a gene at the site of the chromosome 1 breakpoint in a large Scottish family with multiple cases of major mental illness, including schizophrenia, bipolar disorder, and major depression (Millar et al., 2000). Several independent genetic studies have confirmed DISC1 as a one of the most substantiated risk factors for schizophrenia (reviewed in Harrison and Weinberger, 2005; Chubb et al., 2008; Brandon et al., 2009; Jaaro-Peled et al., 2009). Studies of the DISC1 and its signaling pathways have developed a system for how to dissect and understand the molecular basis of neuropsychiatric disorders. Much of the insights have been gained from studies of the DISC1 complex. Indeed, DISC1 is a multifunctional scaffold protein, which integrates signaling through a number of distinct pathways to regulate different aspects of neurodevelopment (e.g. cytoskeletal organization, cell cycle, or cell proliferation), signal transduction and intracellular transport/exocytosis (Chubb et al., 2008; Brandon et al., 2009; Jaaro-Peled et al., 2009).

The idea that two or more signals converge to elicit specific biological action is a common theme in cellular regulation. This could be exemplified by the synergistic interactions of different second messengers such as cAMP and Ca^{2+} in the regulation of cardiac contractility and insulin secretion from pancreatic β islets (Saltiel and Kahn, 2001; Bers, 2008). DISC1 is required for the parallel processing of signals from GSK-3 and PDE4 signaling pathways, important for normal neurodevelopment and brain function. DISC1 affects progenitor proliferation through modulation of GSK-3 activity as elucidated in details (Mao et al., 2009). GSK-3 is widely expressed in the brain (Perez-Costas et al., 2010), and is implicated in fundamental neuronal functions, such as neurodevelopment (Kim et al., 2009),

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neurotransmitter function (Li et al., 2007; Beaulieu et al., 2004), neuroinflammation (Beurel and Jope, 2009) and synaptic plasticity (Peineau et al., 2007). There is accumulating evidence that implicates deregulation of GSK-3 in neuropsychiatric disorders, including bipolar disorder and schizophrenia. GSK-3 inhibitors have been beneficially used to correct neurological conditions in animals such as bipolar disorder and depression (Gould et al., 2004; Kaidanovich-Beilin et al., 2004; Rosa et al., 2008; Beaulieu et al., 2008), Alzheimer's Disease (Muñoz-Montaño et al., 1997; Noble et al., 2005; Engel et al., 2006; Huang and Klein, 2006) and schizophrenia (Beaulieu et al., 2004; Lipina et al., 2010).

DISC1 also has been implicated in the regulation of cAMP signaling via its interaction with cAMP-specific PDE4B (Millar et al., 2005; Murdoch et al., 2007). High level of cAMP increase PDE4s activity following protein kinase A (PKA)-mediated phosphorylation. As a result cAMP is metabolized by PDE4s and PKA activity is decreased. Distinct PDE4 isoforms, interacting with specific sites/signaling complexes, regulate cAMP responses in the cell (reviewed in Houslay et al., 2005; Boswell-Smith et al., 2006), and, hence, many aspects of neuronal function. PDE4 has been reported as risk factor for schizophrenia (Millar et al., 2005; Pickard et al., 2007; Numata et al., 2008; Fatemi et al., 2008; Tomppo et al., 2009), and it's inhibitor, rolipram, acts as a cognitive enhancer (Barad, 1998; Zhang et al., 2004; Davis and Gould, 2005), antidepressant (O'Donnell and Zhang, 2004; Zhang et al., 2006) and antipsychotic (Maxwell et al., 2004; Kanes et al., 2007; Lipina and Roder, 2010).

It is possible that DISC1 may provide the cellular environment to integrate GSK-3 and PDE4 signals, which could occur at the level of GSK-3, an enzyme that is inactivated upon PKA phosphorylation (Fang et al., 2000). Therefore, DISC1 may represent an example of a scaffold protein with the capacity to integrate distinct and independent upstream signaling pathways in a cell. However, whether all of DISC1 binding partners are present in a single complex at the same time is not yet known, as well as how alterations in the stability of the DISC1 complex contributes to the onset of schizophrenia or depression. Notably, missense mutations in the mouse DISC1, which differently alter binding of PDE4s and GSK-3 to the DISC1, lead to the expression of schizophrenia or depressive behavioral endophenotypes (Clapcote et al., 2007; Lipina et al., 2010). Hence, we suggested that the formation of the DISC1-PDE4B-GSK-3 enzymatic complex can contribute to the psychiatric disorders.

The mouse missense mutation, DISC1-L100P reduces binding of both PDE4B (Clapcote et al., 2007) and GSK-3 (Lipina et al., 2010) to DISC1 without alteration of enzymatic activities and results in schizophrenia-like behavioral endophenotypes in mice. Another DISC1-Q31L mutation only slightly decreased interaction with PDE4B (Clapcote et al., 2007; Murdoch et al., 2007), but leads to a reduction of PDE4B activity of 50% and depressive endophenotypes in mutant mice (Clapcote et al., 2007). Pharmacological inhibition of either PDE4 by rolipram or GSK-3 by TDZD-8 was able to correct schizophrenia-like behavior in DISC1-L100P mutant mice (Clapcote et al., 2007; Lipina et al., 2010). The DISC1-Q31L showed resistance to rolipram treatment (Clapcote et al., 2007) in agreement with their low PDE4B enzymatic activity and their pharmaco-behavioral response to GSK-3 inhibitor was not studied yet. Therefore, we sought here to probe if DISC1 could provide a place of convergence for PDE4B and GSK-3 signals, influencing schizophrenia- or depression-like behavior in mouse models. Our results suggest synergy between PDE4 and GSK-3 signaling in a DISC1-dependent manner.

2. Materials and methods

2.1. Animals

DISC1-Q31L and DISC1-L100P homozygous mutants and their wild type littermates were generated as previously described (Clapcote et al., 2007). Both DISC1 mutant lines were backcrossed for 12–16 generations to C57BL/6 mice. Experiments were performed with 12–16 week old male mice. Male mice were tested in the open field, PrePulse Inhibition (PPI) of Acoustic Startle Response, social affiliation/novelty, and immobility in the forced swim test (FST). Groups of three to five same-gender littermates were housed in filtered polycarbonate cages under controlled temperature (21 ± 1 °C), lighting (lights on: 7am–7pm) and humidity (50–60%). The animals were given *ad libitum* sterile food (Purina mouse chow) and water. All animal procedures were approved by the Animal Care and Use Committee of Toronto Centre of Phenogenomics (TCP) and were conducted in accordance with the requirements of the Province of Ontario Animals for Research Act 1971 and the Canadian Council on Animal Care.

2.2. Behavioral studies

Behavioral tests were done between 9am and 4pm. All pharmacological experiments were performed on drug-naïve animals. Prior to all experiments mice were acclimatized to the experimental room for 30 min. The behavioral equipment was cleaned with 70% ethanol between mice.

2.2.1. Locomotor activity

Each mouse was placed in a 41 cm × 41 cm × 33 cm^3 activity monitoring chamber (AccuScan Instruments Inc, Ohio, USA), and activity was measured for 30 min by an automated recording system connected to infrared beams (VersaMax Animal Activity Monitoring System, Ohio, USA). Data were analyzed by two-way ANOVAs followed by Fisher least significance difference (LSD) post-hoc test.

2.2.2. Prepulse inhibition of acoustic startle response

PPI procedure was conducted using four sound-attenuated chambers (ENV-022s; MED Associates, St. Albans, VT, USA), as previously described (Lipina et al., 2005). Five types of trials were used. Pulse alone trials consisted of a single white noise burst (120 dB, 40 ms). Prepulse + pulse trials (PP69P, PP73P, PP81P) consisting of a prepulse of noise (20 ms at 69, 73, or 81 dB respectively) followed 100 ms after prepulse onset by a startling pulse (120 dB, 40 ms). No-stimulus trials consisted of background noise only (65 dB). Sessions were structured as follows: 1) 15 min acclimation at background noise level; 2) five Pulse trials; 3) ten blocks each containing all five trials (Pulse, PP69P, PP73P, PP81P, No-stimulus) in pseudorandom order; 4) five Pulse trials. The force intensity for each trial was recorded as the startle level. The percentage PPI induced by each prepulse intensity was calculated as [1-(startle amplitude on prepulse trial)/(startle amplitude on pulse alone)]*100%. PPI data were analyzed by two-way ANOVA with repeated-measures followed by Fisher least significance difference (LSD) post-hoc test.

2.2.3. Social affiliation and novelty

The social behavior was estimated as previously described (Clapcote et al., 2007). The apparatus for this test consisted of a clear Plexiglas box (53 cm length × 25.6 cm width × 23 cm height) divided into three interconnected chambers. The outer chambers were identical to each other and divided from the central chamber by clear Plexiglas partitions (7.3 cm width × 23 cm height) that each had a centrally placed opening (11 cm width × 23 cm height) and a retractable chamber divider. Two identical wired cages with a cylindrical shape (8 cm diameter, 13 cm height) were used inside the apparatus, one in each side chamber. Throughout the experimental sessions, the wired cages were located at the center of each outer chamber and permitted auditory, visual, and olfactory exploration.

At the beginning of each experimental session, the test mouse was placed in the central chamber and was allowed to freely explore for 5 min. Next, an unfamiliar mouse (male C57BL/6J, "stranger 1") was placed inside a cylinder in one of the outer chambers. The cage and outer chamber containing the stranger mouse was alternated across subjects. Containing the stranger mouse in the cylinder ensures that social approaches were initiated by the subject mouse and was investigatory only, without direct physical contact. Time spent by the test mouse in each outer chamber was recorded over a 10-min period, to estimate social motivation (session I). Another unfamiliar mouse (male C57BL/6J, "stranger 2") was then placed inside an identical cylinder in the opposite outer chamber, and the activity of the test mouse was likewise recorded for a further 10 min, to evaluate social novelty (session II). The test mouse was considered to be in the chamber when its head and two front paws entered the chamber. The amount of time spent exploring each chamber and the number of entries into each chamber were scored using event-recording software (The Observer 5.0, Noldus Information Technology, Netherland). The apparatus was cleaned with 70% ethanol after the session II between mice.

2.2.4. Forced swim test

The protocol was performed as described (Clapcote et al., 2007). The mice were released individually into a transparent plastic cylinder (25 cm height, 18 cm diameter), which contained water at 25 °C to the depth of 18 cm. The experiment lasted 6 min, and an observer scored the floating (no limb movement and making only minimal movements to keep the head above the water) in the last 4 min of the trial using The Observer 5.0 (Noldus Information Technology, Netherland). Each mouse was allowed to dry after the test, and the water was changed between cages.