Brief Communications

Evidence That the BLOC-1 Protein Dysbindin Modulates Dopamine D_2 Receptor Internalization and Signaling But Not D_1 Internalization

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The schizophrenia susceptibility gene dystrobrevin-binding protein 1 (*DTNBP1*) encodes dysbindin, which along with its binding partner Muted is an essential component of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). Dysbindin expression is reduced in schizophrenic brain tissue, but the molecular mechanisms by which this contributes to pathogenesis and symptomatology are unknown. We studied the effects of transfection of DTNBP1 siRNA on cell surface levels of dopamine D_2 receptor (DRD2) in human SH-SY5Y neuroblastoma cells and in rat primary cortical neurons. DTNBP1 siRNA decreased dysbindin protein, increased cell surface DRD2 and blocked dopamine-induced DRD2 internalization. MUTED siRNA produced similar effects. In contrast, decreased dysbindin did not change dopamine D_1 receptor (DRD1) levels, or its basal or dopamine-induced internalization. The DRD2 agonist quinpirole reduced phosphorylation of CREB (cAMP response element-binding protein) in dysbindin downregulated cells, demonstrating enhanced intracellular signaling caused by the upregulation of DRD2. This is the first demonstration of a schizophrenia susceptibility gene exerting a functional effect on DRD2 signaling, a pathway that has long been implicated in the illness. We propose a molecular mechanism for pathogenesis in which risk alleles in *DTNBP1*, or other factors that also downregulate dysbindin, compromise the ability of BLOC-1 to traffic DRD2 toward degradation, but has little effect on DRD1 trafficking. Impaired trafficking of DRD2 decreases dopamine-induced internalization, and with more receptors retained on the cell surface, dopamine stimulation produces excess intracellular signaling. Such an increase in DRD2 signaling relative to DRD1 would contribute to the imbalances in dopaminergic neurotransmission characteristic of schizophrenia.

Key words: **DTNBP1; dysbindin; MUTED; BLOC-1; dopamine D_2 receptor; DRD2; dopamine D_1 receptor; DRD1; internalization; endocytosis; CREB; schizophrenia**

Introduction

Genetic variation in dystrobrevin-binding protein 1 (*DTNBP1*) influences risk for schizophrenia as well as cognition in normal individuals (Straub et al., 2002; Riley and Kendler, 2006; Donohoe et al., 2007). DTNBP1 encodes dysbindin, which is reduced in the prefrontal cortex, midbrain, and hippocampus of brains from patients with schizophrenia (Talbot et al., 2004; Weickert et al., 2004). Dysbindin is an essential component of biogenesis of lysosome-related organelles complex 1 (BLOC-1), a complex that includes proteins from at least seven other genes: *MUTED*, SNARE-associated protein (*SNAPAP*), general control of amino-acid synthesis 5-like 1 (*GCN5L1*), biogenesis of lysosome-related organelles complex 1, subunit 2 (*BLOC1S2*), biogenesis of lysosome-related organelles complex 1, subunit 3 (*BLOC1S3*), pallidin (*PLDN*), and cappuccino (*CNO*) (Wei, 2006). We found previously that *MUTED* is also probably a schizophrenia susceptibility gene (Straub et al., 2005b) as are *BLOC1S2* (Straub et al., 2006) and *BLOC1S3* (Morris et al., 2007).

In primary neurons, downregulation of dysbindin by siRNA decreased levels of SNAP25 and synapsin I, and reduced glutamate release, suggesting that decreased dysbindin may decrease exocytosis of glutamate containing synaptic vesicles (Numakawa et al., 2004). In rat pheochromocytoma cell line (PC12) cells, siRNA induced downregulation of dysbindin increased dopamine (DA) release (Kumamoto et al., 2006). This may be physiologically relevant, because the cortex of the dysbindin mutant mouse (*sandy*, *sdy*) appears to have decreased dopamine levels (Murotani et al., 2007), perhaps from increased dopaminergic transmission and turnover.

Many neuropsychiatric disorders, including Parkinson's disease, mood disorders, and schizophrenia likely involve alterations in dopamine receptor-mediated signaling (Sealfon and Olanow, 2000). Dopaminergic neurotransmission is complex, and mediated via the distinct signaling properties of D_1-like and D_2-like dopamine receptor subfamilies (Missale et al., 1998), and

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dopamine D_2 receptors (DRD2) are a primary target for antipsychotic drugs. It has been proposed that a hyperdopaminergic state in the mesolimbic pathway results in positive symptoms whereas a hypodopaminergic state in the mesocortical pathway results in negative symptoms and cognitive dysfunction (Weinberger, 1987; Davis et al., 1991). In the prefrontal cortex, it appears that over-stimulation of the DRD2 signaling pathway and/or understimulation the DRD1 pathway (i.e., an increased D2/D1 ratio) leads to a reduction of cortical excitation and recurrent inhibition via stimulation of inhibitory GABA interneurons (Seamans et al., 2001; Seamans and Yang, 2004; Winterer and Weinberger, 2004). Such changes would be expected to contribute to the neurophysiological, neuropsychological and clinical problems found in schizophrenia. Last, several studies have shown that DRD2-interacting proteins involved in sorting or trafficking of the receptor, can induce a hyper-sensitized state of DRD2 (Kabbani et al., 2002; Koh et al., 2003; Bartlett et al., 2005).

In this study, we focused on the effect of BLOC-1 on the trafficking of DRD2, as a potential link to the pathogenesis of schizophrenia. We observed that decreasing the BLOC-1 protein dysbindin increased cell surface levels of DRD2, but not DRD1, and increased the strength of the DRD2-mediated G_i signaling pathway. BLOC-1 involvement in DRD2 signaling *in vivo* would be a novel regulatory mechanism, with implications for the understanding of normal dopaminergic signaling and those neuropathologies involving dysregulation of dopamine.

Materials and Methods

Cells. SH-SY5Y human neuroblastoma cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in RPMI-1640 (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), and penicillin-streptomycin-glutamine liquid (Invitrogen). Primary cortical neurons were isolated from embryonic day 18 (E18) rats and maintained in neurobasal media (Invitrogen) supplemented with B-27 (Invitrogen) and GlutaMax-1 (Invitrogen).

siRNA transfection. Transfection of SH-SY5Y cells with Ambion (Austin, TX) DTNBP1 siRNA (ID 33616), MUTED siRNA (ID 148282) or negative control (random) siRNA (ID 4611) was via Lipofectamine2000 (Invitrogen) following the manufacturers protocol, and cells were used 48 h after transfection. Transfection of freshly isolated rat primary neurons with Ambion DTNBP1 siRNA (IDs 282575, 282576, 282577) used Nucleofector (Amaxa, Cologne, Germany) following the manufacturers protocol and cells were used 5–6 d after transfection. Manuals from Invitrogen and Amaxa state that the average siRNA transfection efficiency is ~50%.

Antibodies. A polyclonal antibody against dysbindin was produced as described previously (Numakawa et al., 2004). Polyclonal rabbit anti-DRD2 and anti-DRD1 were obtained from Millipore (Temecula, CA) and monoclonal PE-conjugated anti-cAMP response element-binding protein (CREB) (pS133) was from BD Biosciences (Palo Alto, CA). Secondary antibodies, FITC-conjugated anti-rabbit IgG and AlexaFluor488-conjugated anti-rabbit IgG, were from BD Biosciences and Invitrogen, respectively.

Confocal microscopy. Cells cultured in a four-well chamber slide (Nalge Nunc International, Naperville, IL) were washed twice in PBS and fixed in 4% paraformaldehyde for 30 min at 37°C. After washing, cells were blocked in 5% goat serum, 0.1% Triton X-100 in PBS for 30 min at room temperature. Cells were then incubated with primary antibodies at optimal dilutions in the blocking solution overnight at 4°C. Cells were washed three times with PBS and incubated with secondary antibody for 1 h at room temperature. After PBS washes, slides were mounted using Biomeda Gel/Mount media (Electron Microscopy Sciences, Hatfield, PA). Confocal laser scanning microscopy was performed with a Zeiss (Thornwood, NY) LSM510.

Flow cytometric analysis. After PBS washes, cells were labeled with anti-DRD2 antibody for 1 h on ice. Cells were washed with ice-cold PBS containing 2% BSA and 0.1% NaN_3 and incubated with FITC-conjugated anti-rabbit IgG antibody for 30 min on ice. After PBS washes, cells were fixed with 2% paraformaldehyde in PBS. For the assay to test ligand-induced internalization of DRD2, cells were incubated in serum-free RPMI-1640 medium for 1 h at 37°C and then stimulated with 10 μM dopamine (Sigma, St. Louis, MO) for 30 min. Stimulation was terminated by quickly cooling the cultures on ice. After washing with ice-cold PBS, cells were labeled with anti-DRD2 antibody followed by staining with FITC-conjugated anti-rabbit antibody. After PBS washes, cells were fixed with 2% paraformaldehyde in PBS and analyzed using FACScan (BD Biosciences). To assess DRD2-mediated G_i signaling, CREB phosphorylation was measured using FACScan after addition of the DRD2 agonist quinpirole. Briefly, cells were incubated with serum-free RPMI-1640 for 1 h and treated with phosphodiesterase inhibitors 50 μM forskolin (EMD Biosciences, San Diego, CA) and 20 μM 3-isobutyl-1-methylxanthine (IBMX; EMD Biosciences) for 10 min to increase cAMP and CREB phosphorylation. In the presence of forskolin and IBMX, 10 μM quinpirole (Tocris Biosciences, Ellisville, MO) was added for 20 min at 37°C. After PBS washes, cells were fixed with Phosflow Fix Buffer I (BD Biosciences) for 30 min at 37°C. Cells were permeabilized by Phosflow Perm/Wash Buffer I (BD Biosciences) for 30 min at room temperature. After washing, cells were labeled with PE-conjugated anti-pCREB antibody for 2 h at 4°C. After washing, cells were analyzed using FACScan. CellQuest software (BD Biosciences) was used to acquire and quantify the fluorescence signal intensities.

Statistical analysis. All values for dependent variables are mean ± SD. For comparisons of the effects of various treatments on dopamine receptors and CREB levels, we performed ANOVA followed by *post hoc* Bonferroni's analysis, and all p values presented in the text and figures are *post hoc* unless otherwise noted. Supplemental Tables 1 and 2 (available at www.jneurosci.org as supplemental material) contain all ANOVA and *post hoc* p values, respectively, that we calculated for the experiments presented.

Results

Effect of siRNA for BLOC-1 genes on dysbindin protein and cell surface levels of DRD2 and DRD1

The efficacy of siRNA knockdown of dysbindin protein in SH-SY5Y cells was estimated by immunoblot (supplemental Fig. 1*A*, available at www.jneurosci.org as supplemental material). DTNBP1 siRNA caused a 60% reduction in dysbindin (supplemental Fig. 1*B*, available at www.jneurosci.org as supplemental material) ($p = 0.0048$). Results were similar with either MUTED siRNA alone ($p = 0.002$) or with cotransfection with DTNBP1 siRNA (supplemental Fig. 1*A*,*B*, available at www.jneurosci.org as supplemental material) ($p = 0.0019$). In rat primary cortical neurons transfected with DTNBP1 siRNA, the dysbindin reduction was ~70% (supplemental Fig. 2, available at www.jneurosci.org as supplemental material) ($p = 0.00001$). Using the same transfection protocol, we studied the effects of DTNBP1 siRNA or MUTED siRNA transfection on cell surface DRD2 expression using flow cytometry, and a representative run is shown in Figure 1*A*. Transfected SH-SY5Y cells showed (Fig. 1*B*) increases in cell surface DRD2 of 30% with DTNBP1 siRNA ($p = 0.004$) and of 20% with MUTED siRNA ($p = 0.027$) compared with cells transfected with a control (random sequence) siRNA. In rat primary cortical neurons transfected with DTNBP1 siRNA, cell surface DRD2 was increased 28% (see Fig. 3) ($p = 0.00001$) and DRD1 was unchanged ($p = 0.264$).

Effect of DTNBP1 siRNA on dopamine-induced DRD2 and DRD1 internalization

To check whether the increased cell surface DRD2 was caused by reduced internalization or increased delivery to the plasma membrane, we measured the effect of DTNBP1 siRNA transfection on

dopamine-induced DRD2 internalization in SH-SY5Y cells. In cells transfected with random siRNA, 30 min exposure to dopamine induced an 18% decrease in cell surface DRD2 compared with unstimulated cells (Fig. 2*A*,*B*) ($p = 0.002$). In contrast, surface DRD2 was elevated (13%; $p = 0.022$) in the DTNBP1 siRNA transfected cells, and this was unchanged by dopamine stimulation ($p = 0.922$). Consistent with these flow cytometry results, confocal microscopic analysis of DRD2 also demonstrated dopamine induced reductions in DRD2 immunoreactivity in random siRNA transfected cells (Fig. 2*C*,*D*) whereas little change was apparent after dopamine stimulation of DTNBP1 siRNA transfected cells (Fig. 2*E*,*F*). We then confirmed the effect in rat primary neurons. In cells transfected with a random siRNA, dopamine induced a 12% decrease in cell surface DRD2 compared with unstimulated cells (Fig. 3) ($p = 0.037$). In contrast, in the DTNBP1 siRNA transfected cells, surface DRD2 was unchanged by dopamine stimulation ($p = 0.383$). We also measured cell surface DRD1 and found that both random and DTNBP1 siRNA transfected cells showed decreases in DRD1 caused by dopamine stimulation (13%, $p = 0.013$ and 11%, $p = 0.030$ respectively).

Effect of DTNBP1 siRNA on DRD2 agonist quinpirole inhibition of CREB phosphorylation

To determine whether an increase in cell surface DRD2 increases the strength of the DRD2 stimulated G_i signaling pathway, we designed a flow cytometric assay for phosphorylated CREB (pCREB). DRD2 activates the protein G_i which inhibits adenylate cyclase, reducing cAMP production and decreasing PKA phosphorylation of CREB. Therefore, a reduction in pCREB reflects increased DRD2 signaling. We measured the effect of quinpirole on pCREB levels in rat primary neurons (Fig. 4). Before the addition of quinpirole, we incubated with forskolin and IBMX to increase cAMP, which increased pCREB. We found that basal and forskolin/IBMX induced pCREB levels were the same in random siRNA and DTNBP1 siRNA transfected cells ($p = 0.978$ and $p = 0.161$ respectively). In contrast, pCREB after stimulation with quinpirole was significantly lower (~20%; $p = 0.0018$) in DTNBP1 siRNA transfected cells (Fig. 4).

We also measured pCREB in SH-SY5Y cells (supplemental Fig. 3, available at www.jneurosci.org as supplemental material) and found that in contrast to a lack of a basal effect in primary cortical neurons, DTNBP1 siRNA reduced the basal level of pCREB by 33% ($p = 0.0034$). In cells stimulated by forskolin/IBMX, pCREB was increased, and DTNBP1 siRNA caused a 49% decrease (supplemental Fig. 3, available at www.jneurosci.org as supplemental material) ($p = 0.0001$) compared with random siRNA. Finally, pCREB was decreased by addition of quinpirole, and further lowered by DTNBP1 siRNA transfection (supplemental Fig. 3, available at www.jneurosci.org as supplemental material) (28% decrease; $p = 0.023$).

To determine whether the basal effect of DTNBP1 siRNA in SH-SY5Y cells on pCREB required extracellular dopamine stimulation of DRD2, we used the DRD2 antagonist haloperidol (supplemental Fig. 4, available at www.jneurosci.org as supplemental material). We found that with random siRNA, basal pCREB was unchanged by haloperidol, whereas the basal reduction (6% decrease; $p = 0.013$) of pCREB because of DTNBP1 siRNA was prevented (4% increase; $p = 0.083$).

Discussion

Dysbindin is a component of both the BLOC-1 complex and the dystrophin-associated protein complex, as demonstrated by co-immunoprecipitation and other methods (Li et al., 2003; Wei, 2006). However, there is little evidence that the two complexes interact in the brain (Nazarian et al., 2006).

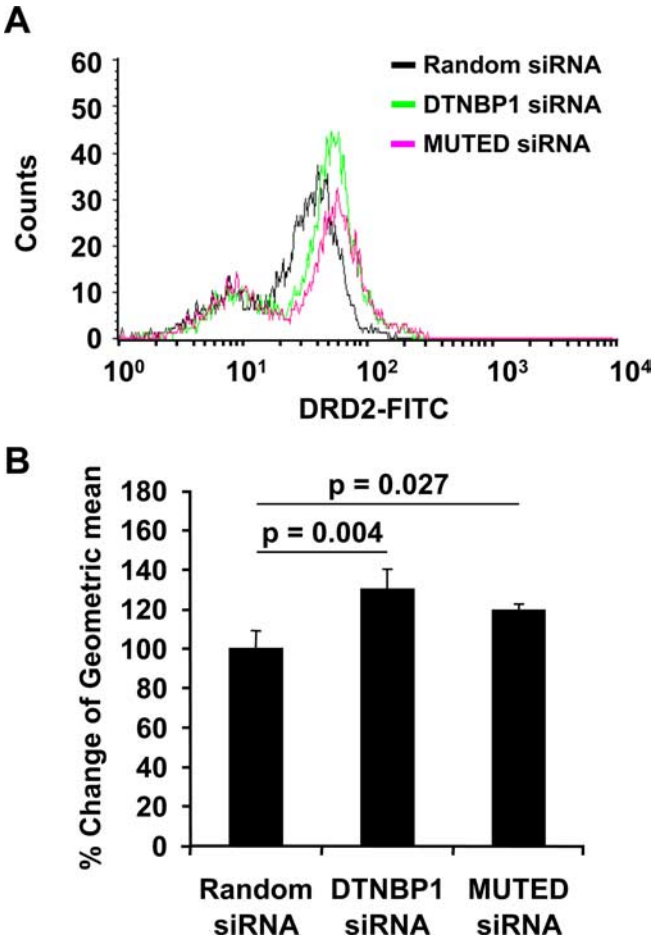

Figure 1. Effects of siRNA on cell surface levels of DRD2 in SH-SY5Y cells. ***A***, Representative data from flow cytometric analysis of cell surface DRD2 expression. The overlaid histogram shows relative fluorescence intensities of DRD2 from 10,000 cells transfected with either random siRNA (black), DTNBP1 siRNA (green), or MUTED siRNA (pink). ***B***, Changes in cell surface DRD2 expression by DTNBP1 siRNA or MUTED siRNA transfection. The geometric mean of the DRD2 fluorescence signal was obtained by flow cytometric analysis. Values are expressed as a percentage of random siRNA transfected cells. Error bars represent means ± SD ($n = 3$).

The BLOC-1 complex is involved in vesicular trafficking in melanocytes and fibroblasts (Li et al., 2003; Nguyen and Wei, 2004), but little is known about it's role in the developing or adult nervous system. BLOC-1 components pallidin and snapin can bind members of the SNARE family, syntaxin 13 and SNAP25 respectively (Huang et al., 1999; Ilardi et al., 1999). These proteins have been localized to early-, sorting-, and recycling-endosomes (Hong, 2005), further supporting a role for BLOC-1 in membrane trafficking. Destabilization of dysbindin protein in the mutant muted mouse (*mu*) and conversely destabilization of muted in the *sandy* mouse have been reported (Li et al., 2003). We too observed a reduction of dysbindin protein in MUTED siRNA transfected SH-SY5Y cells.

It has been hypothesized that schizophrenia is due in part to overstimulation of DRD2, understimulation of DRD1, or both, particularly in the cortex (Seamans et al., 2001; Winterer and Weinberger, 2004). From the perspective of potential BLOC-1 dysfunction caused by dysbindin deficiency and the unexplained imbalances in dopamine neurotransmission in schizophrenia, we

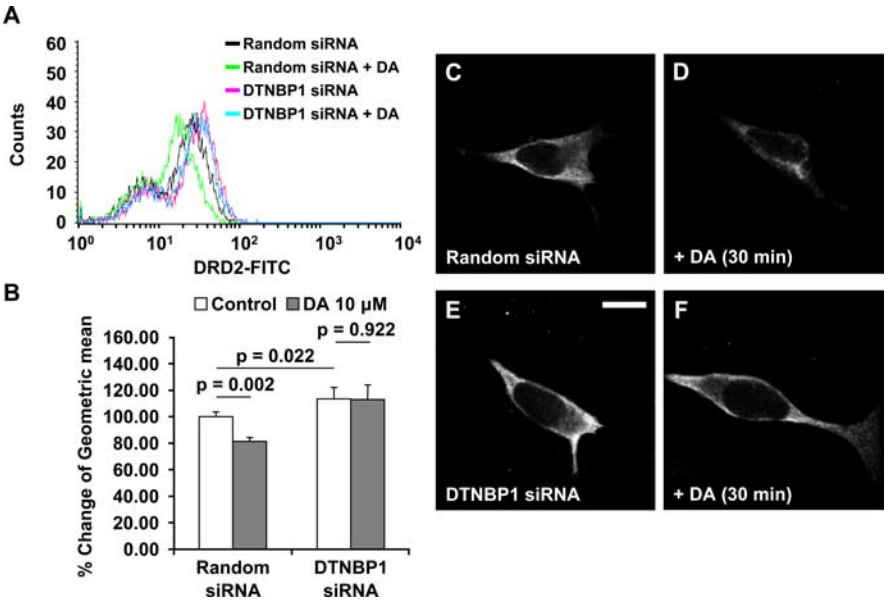

Figure 2. Effects of dopamine stimulation on cell surface levels of DRD2 in DTNBP1 siRNA transfected SH-SY5Y cells. ***A***, Representative data from flow cytometric analysis of cell surface DRD2. The overlaid histogram shows relative fluorescence intensities of DRD2 from cells treated with random siRNA (black), random siRNA plus dopamine (DA; green), DTNBP1 siRNA (pink), or DTNBP1 siRNA plus DA (blue). ***B***, Dopamine-induced changes in cell surface DRD2 expression. The geometric mean of cell surface of DRD2 fluorescence was obtained by flow cytometric analysis. Values are expressed as a percentage of random siRNA transfected cells. Error bars represent means ± SD ($n = 4$). ***C–E***, Representative confocal images of DRD2 expression in SH-SY5Y cells transfected with random siRNA (***C***, ***D***) or DTNBP1 siRNA (***E***, ***F***). ***C***, ***E***, Not treated. ***D***, ***F***, Treated with 10 μM DA for 30 min. Scale bar, 10 μm.

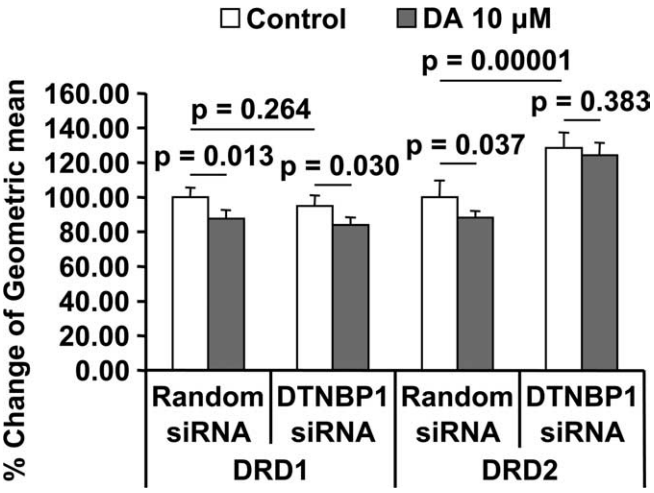

Figure 3. Effects of dopamine stimulation on cell surface DRD1 or DRD2 levels in siRNA transfected rat primary neurons. The geometric mean of cell surface of DRD1 or DRD2 fluorescence was obtained by flow cytometric analysis. Values are expressed as a percentage of random siRNA transfected cells. Error bars represent means ± SD ($n = 4$).

asked whether BLOC-1 proteins were involved in trafficking of DRD2 or DRD1. We demonstrated that downregulated dysbindin, as is found in the schizophrenic brain, increased cell surface DRD2 in human SH-SY5Y neuroblastoma cells as well as in rat primary cortical neurons. Importantly, ligand-induced DRD2 internalization was completely blocked, whereas DRD1 internalization was unaffected. The specific mechanism by which a deficiency in BLOC-1 proteins might modulate DRD2 is unclear, but is likely to be attributable to the fact that internalized DRD2 is subject to degradation via an endosomal-lysosomal pathway, whereas most DRD1 is recycled to the plasma membrane (Bartlett et al., 2005; Martin-Negrier et al., 2006).

This role in differential trafficking would also be consistent with recent work showing that BLOC-1 interacts with two other complexes, AP-3 and BLOC-2, and traffics lysosome-associated membrane proteins from the early endosome to the late endosome, to lysosomes, and to the *trans*-Golgi network (for review, see Newell-Litwa et al., 2007; Setty et al., 2007).

In primary neurons with decreased dysbindin, we observed a decrease of pCREB after quinpirole treatment. This shows that in addition to elevated cell surface DRD2, after agonist stimulation, downstream signaling was also abnormal. We blocked the DTNBP1 siRNA induced decrease in pCREB with haloperidol, which indicates that the effect of siRNA on pCREB required agonist activation, and therefore is unlikely to be independent of DRD2.

Accurate measurement of DRD2 binding potential *in vivo* is difficult and complicated by multiple factors such as neuroleptic treatment and endogenous dopamine, and this is particularly so in the cortex where the density of DRD2 is very low (Seeman and Kapur, 2000; Seeman, 2006). Nevertheless, several PET or SPECT studies have shown that striatal DRD2 was elevated in schizophrenic patients (Wong et al., 1986; Abi-Dargham et al., 2000; Hirvonen et al., 2005) and we suggest that a deficiency in dysbindin may contribute to this. Compared with the number of studies of striatum, there have been very few of cortex. Although a decrease was observed in temporal cortex (Tuppurainen et al., 2003) and anterior cingulate cortex (Suhara et al., 2002), two other studies found no difference in frontal cortex, temporal cortex, or thalamus (Talvik et al., 2003; Glenthoj et al., 2006). Therefore it appears that the issue of DRD2 levels in the cortex of schizophrenics is still unresolved. Furthermore, it should be emphasized that only some families will have a dysfunctional BLOC-1 pathway based on inheritance of risk alleles in BLOC-1 genes, and so studies of relatively small samples may fail to detect the upregulation described here. It should also be noted that radiolabeled DRD2 antagonists can bind to unoccupied cell surface and internalized receptors (Seeman and Kapur, 2000), but may be relatively insensitive to occupied receptors.

Even if, because of compensatory mechanisms for example, *DTNBP1* does not turn out to upregulate DRD2 in the adult cortex, it still may increase risk via a developmental upregulation that affects adult cortical function. Transient over-expression of DRD2 in the striatum of a transgenic mouse elicited impairments in working memory and behavioral flexibility, and also increased dopamine levels, decreased dopamine turnover, and increased DRD1 activity in the medial prefrontal cortex (Kellendonk et al., 2006). These phenotypes were not rescued when DRD2 overexpression was reversed in the adult. These mice also exhibited motivational and interval timing deficits, which were proposed to be related to prefrontal cortical function deficits in patients (Drew et al., 2007). In addition, previous work has shown that

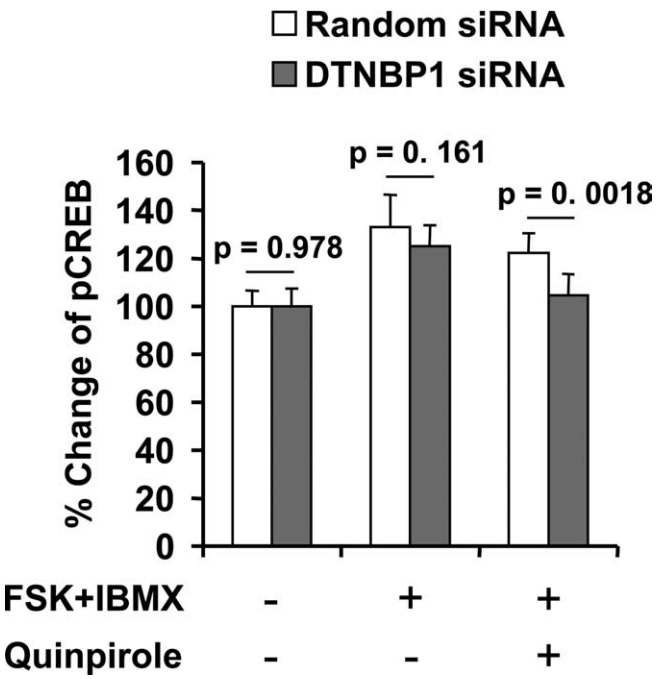

Figure 4. Effects of DTNBP1 siRNA on CREB phosphorylation in rat primary neurons. Bars represent the percentage change in geometric mean of phospho-CREB fluorescence, assayed by flow cytometry with anti-pCREB antibody. Pretreatment with 50 μM forskolin (FSK) and 20 μM IBMX for 30 min was followed by the addition of 10 μM quinpirole for 20 min at 37°C. Error bars represent means ± SD ($n = 6$).

dopamine can modulate GABA neuron migration from the basal forebrain to the cerebral cortex in embryonic mice and that altering the physiological balance between DRD1 and DRD2 activation can impair GABA neuronal migration (Crandall et al., 2007).

The effects of reduced dysbindin on dopaminergic transmission may be complex, involving multiple, perhaps opposing mechanisms, and we do not know whether a dysbindin-related DRD2 upregulation is presynaptic, postsynaptic, or both. The putative increase in dopamine turnover in the *sandy* mouse (Murotani et al., 2007) presumably caused by increased dopamine transmission, would be consistent with DTNBP1 siRNA experiments on PC12 cells (Kumamoto et al., 2006). Presynaptic DRD2 can function as an autoreceptor, which allows an inhibitory feedback mechanism by altering dopamine synthesis, release, and reuptake in response to increased synaptic dopamine. Even greater regulatory complexity may occur via direct protein–protein interactions, whereby DRD2 facilitates the recruitment of the dopamine transporter to the plasma membrane and enhanced dopamine reuptake (Lee et al., 2007).

We have observed a statistical interaction between single nucleotide polymorphisms in *DTNBP1* and the Val^{158}Met polymorphism in COMT (Straub et al., 2005a). *DTNBP1* is associated with both schizophrenia and cognitive phenotypes only in families where the affected individual has the Met/Met genotype. Because Met/Met individuals tend to have higher cortical dopamine levels, they should suffer the greatest imbalance in signaling if decreased dysbindin increases the availability of DRD2. This imbalance and COMT interaction might be exacerbated if decreased dysbindin does indeed cause greater dopamine release *in vivo*.

The novel result reported here is that dysbindin deficiency can increase the level of cell surface DRD2 and enhance the strength of DRD2 signaling although leaving DRD1 levels unchanged. This may be one of the mechanisms underlying some of the dopaminergic disturbances implicated in schizophrenia that are benefited by drugs that antagonize DRD2. Thus, it is important to determine if any of the numerous schizophrenia susceptibility genes (Straub and Weinberger, 2006) impact BLOC-1 function or otherwise disturb DRD2 signaling. More generally, investigation into the effects of BLOC-1 dysfunction on the normal balance and integration of dopaminergic, glutamatergic, and GABAergic signaling during development and adulthood is warranted.

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