Subcortical dopaminergic deficits in a DISC1 mutant model: a study in direct reference to human molecular brain imaging

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Imaging of the human brain has been an invaluable aid in understanding neuropsychopharmacology and, in particular, the role of dopamine in the striatum in mental illness. Here, we report a study in a genetic mouse model for major mental illness guided by results from human brain imaging: a systematic study using small animal positron emission tomography (PET), autoradiography, microdialysis and molecular biology in a putative dominant-negative mutant DISC1 transgenic model. This mouse model showed augmented binding of radioligands to the dopamine D2 receptor (D2R) in the striatum as well as neurochemical and behavioral changes to methamphetamine administration. Previously we reported that this model displayed deficits in the forced swim test, a representative indicator of antidepressant efficacy. By combining the results of our two studies, we propose a working hypothesis for future studies that this model might represent a mixed condition of depression and psychosis. We hope that this study will also help bridge a major gap in translational psychiatry between basic characterization of animal models and clinico-pharmacological assessment of patients mainly through PET imaging.

INTRODUCTION

A central role for dopamine in the pathology of psychosis has been proposed for many years. The initial proposal by Van Rossum (1) is supported by the classic discovery that antipsychotics block the dopamine D2 receptor (D2R). On the basis of the action of antipsychotics, many human brain imaging studies have been conducted to validate this theory further. A meta-analysis of studies that employ positron emission tomography (PET) comparing D2 receptor parameters in patients with schizophrenia (SZ) and controls revealed a small but significant elevation of striatal D2 receptors in unmedicated SZ patients (2,3). Amphetamine-induced decrease in [11C]raclopride or [123I]IBZM binding, a measure of dopamine release, is augmented in the striatum of unmedicated SZ patients compared with well-matched controls (4–6).

Although human PET studies are crucial in addressing the neurochemical basis of mental disorders, long-term medication has been a major confounding factor. Because it is considerably more difficult to design PET studies with sufficient numbers of non-medicated patients, the use of animal models may be an alternative approach to overcome this limitation. Some of the animal models generated to model SZ and related disorders have been studied in conjunction with dopaminergic neurotransmission (7–12). Nevertheless, as far as we are aware, these animal models have not been examined in

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reference to human brain imaging, especially PET studies in a systematic manner. We believe that this is currently a major gap in ‘translational psychiatry.’

The DISC1 gene is specifically disrupted by a hereditary chromosomal abnormality in a large Scottish pedigree, which leads to several major mental illnesses, including SZ, with very high penetrance (13–15). Many follow-up genetic studies have suggested that DISC1 is not a strong and specific risk factor for SZ per se. However, modulating this gene in rodents leads to behavioral changes including deficits relevant to SZ (12,16–21). Consistent with these behavioral observations, changes in dopaminergic neurotransmission have been suggested in multiple genetic models for DISC1 (10–12).

We recently reported that a transgenic model in which a putative dominant-negative DISC1 is expressed under the αCaMKII promoter (DN-DISC1) almost exclusively in the cortex and hippocampus showed a significant reduction of parvalbumin immunostaining in the prefrontal cortex (16), a cortex and hippocampus showed a significant reduction of parvalbumin immunostaining in the prefrontal cortex (16), a pathological hallmark in brains from patients with SZ (22). However, previous studies with this model have not addressed potential dysfunction of the dopaminergic system (16,23,24). Here, we systematically assessed possible changes in striatal dopaminergic transmission in this model by using small animal PET, autoradiography, microdialysis and molecular approaches in direct reference to human PET studies.

RESULTS

Augmentation of striatal dopamine D2 receptor in DN-DISC1 mice: PET, autoradiography and molecular analyses

We performed small animal PET after administering the D2R antagonist, [11C]raclopride, to pairs of DN-DISC1 and wild-type (WT) littermates and imaging with the GE eXplore VISTA scanner. We used a brain-centered dynamic protocol covering 0–60 min post-injection. We compared the striatum/cerebellum ratio for each pair to control for specific versus non-specific uptake of [11C]raclopride. The striatum/cerebellum ratio was consistently higher for the DN-DISC1 mouse in each of the three pairs (Fig. 1A), suggesting that the DN-DISC1 mice have more striatal D2R available for binding. It may also indicate a lower baseline level of dopamine available to compete with the injected [11C]raclopride on binding to the D2 receptors in the striatum of the DN-DISC1 mice.

To examine the levels of D2R more directly, we performed autoradiography with the D2R antagonist, [3H]spiperone. Densitometric analysis was done after 6 to 7 weeks of exposure. We detected a small but consistent increase in the level of D2R in the striatum of DN-DISC1 mice compared with the level in WT mice. This increase reached significance for the right medial part of the rostral striatum (Fig. 1B). Real-time PCR with primers specific to the long, postsynaptic isoform of dopamine D2R also detected increased D2R messenger RNA levels in the striatum (Fig. 1C). These results are consistent with a small but significant elevation of striatal D2 receptors in untreated SZ patients (2,3,25). We also observed upregulation of dopamine D1 receptor messenger RNA (Supplementary Material, Fig. S1).

Hypersensitivity to methamphetamine in DN-DISC1 mice

In the striatum, dopamine is normally cleared rapidly from the synaptic cleft by the dopamine transporter (DAT), which retrieves dopamine to the presynaptic terminal. The psychomimetic drug methamphetamine (a derivative of amphetamine) increases extracellular dopamine by releasing it from presynaptic nerve terminals, inhibiting its reuptake, redistributing dopamine from synaptic vesicles to the cytosol and promoting reverse transport (26). Amphetamine use can induce psychosis (27,28), and SZ patients are hypersensitive to the effects of amphetamine (29). Furthermore, amphetamine challenge has been reported to elevate dopamine release in untreated SZ patients compared with matched controls (6).

Therefore, we tested the effects of a methamphetamine challenge (1 mg/kg, i.p.) in the DN-DISC1 model. We found a higher percentage increase in extracellular dopamine in the ventral striatum using in vivo microdialysis (Fig. 2A) and significantly greater augmentation in the locomotion of the DN-DISC1 mice in comparison to WT littermates (Fig. 2B). These results are also consistent with those from clinico-pharmacological and PET studies in humans.

Changes in baseline dopamine and dopamine transporter (DAT) in DN-DISC1 mice

Next, we compared the baseline levels of extracellular dopamine in the ventral striatum of DN-DISC1 mice with those in WT mice. In vivo microdialysis results indicated that the baseline extracellular dopamine, as measured by averaging three dopamine readings before saline injection, was significantly decreased in the DN-DISC1 mice (Fig. 3A). To determine whether the differences were due to altered levels of DAT, we examined these and observed that DAT was increased in the striatum of DISC1-DN mice compared with that of WT controls in a DN-DISC1 dose-dependent manner (Fig. 3B). We also compared the present data from DN-DISC1 mice with those from published transgenic mice over-expressing DAT and found substantial qualitative similarity between these two models (Table 1) (30,31). Imaging studies employing a radiotracer that targets the DAT will ultimately be needed to address the functional significance of DAT in these findings. However in the present study, we could not access the ligand.

DISCUSSION

One of the most fruitful areas of research in neuropsychopharmacology and studies on major mental illnesses is human brain imaging, especially that on dopamine in the striatum. Recently, biological psychiatry has advanced by generating many genetic animal models for major mental illnesses. Nonetheless, few studies have tried to link these two research areas systematically. In the present study, we brought the power of neuroimaging, which is routinely applied to humans, to characterize a DISC1 genetic mouse model (DN-DISC1). The results from small animal PET, autoradiography and real-time PCR using the mouse model are consistent with human PET data on D2 receptors from untreated SZ patients, i.e. subjects with psychosis. The behavioral and neurochemical responses
to methamphetamine challenge in this model are also compatible with data from patients with SZ or psychosis.

We previously reported deficits in the forced swim test, a paradigm to test the pharmacological efficacy of antidepressants, in this model (16). Furthermore, in an independent study to address the frontal function of this DN-DISC1 model, DN-DISC1 mice also showed impoverished motivational functions in tests of progressive ratio and

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**Figure 1.** Increased D2Rs in the DN-DISC1 mouse model. (A) In vivo PET scan with [11C]raclopride on three pairs of heterozygous DN-DISC1 and WT mice. Representative example of the PET-MRI images. DN-DISC1 mice showed increased binding in the striatum (normalized to binding to the cerebellum). (B) Autoradiography with [3H]spiperone. Regions of interest: lateral and medial parts of the rostral, medial and caudal striatum. DN-DISC1 mice had consistently higher binding in the striatum, which reached significance (\( P < 0.05 \)) in the medial part of the right rostral striatum (\( N = 5 \) WT, \( N = 6 \) homozygous DN-DISC1). (C) Real-time PCR to quantify post-synaptic dopamine D2R mRNA in the striatum (\( N = 9 \) WT, \( N = 7 \) homozygous DN-DISC1). DN-DISC1 mice had a significantly higher (\( P < 0.05 \)) D2R/GAPDH ratio.
of the dynamic modulation of reward value by effortful action, which may be possibly linked to an endophenotype of depression (A. Johnson, H. Jaaro-Peled, A. Sawa and M. Gallagher, manuscript in preparation). In the Scottish pedigree in which the disruption of DISC1 was originally identified, the majority of subjects with this mutation were diagnosed as having major depression rather than SZ (14,15). It is very interesting to further validate a DISC1 animal model based on the possibility that it may represent a condition of concurrent mood disturbance and psychosis. Given that increased DAT availability has been reproducibly reported in patients with major depression (32–36), application of PET with a DAT tracer to the DN-DISC1 model will be an important experiment to address this question in future studies.

A challenging question that remains is why preferential modulation of DISC1 in the cortex, especially in the pyramidal neurons as shown in our previous publication (16), leads to these subcortical pathologies. Although we cannot exclude the possibility that a very low level of DN-DISC1 protein in the subcortical areas might underlie these pathologies, it seems most likely that dysfunction of pyramidal neurons in the cortex due to expression of DN-DISC1 protein under the aCaMKII promoter leads to the subcortical pathologies. A rescue experiment with supplement of WT DISC1 protein in the pyramidal neurons by a viral expression vector may enable us to address this question.

Furthermore, a classic hypothesis that was originally proposed mainly to explain the pathophysiology of SZ or psychosis may assist this conceptual framework indirectly: primary pathological changes in the frontal cortex, especially those in which interneuron dysfunction leads to dysregulation of pyramidal neurons, might decrease the baseline ‘tonic’ striatal dopamine, leading to a compensatory potentiation of ‘phasic’ responsivity of dopamine upon stimuli (37). Less extra-synaptic dopamine in the ventral striatum of the DN-DISC1 model, as determined by microdialysis under baseline conditions, fits with this theory of decreased tonic dopamine in psychosis and SZ. Increased levels of D2 receptors, as determined by real-time PCR and autoradiography, are likely compensations for decreased tonic dopamine. Methamphetamine challenge elicits a larger increase in dopamine in the DN-DISC1 mice, which is possibly due to elevated DAT levels.
A comparison with the recent publications on DAT transgenic mice shows remarkable qualitative similarity (Table 1) (30,31), suggesting that an increase in DAT protein could reduce baseline dopamine levels and elicit a compensatory increase in D2R. Future study on developmental trajectory of the dopaminergic abnormalities may help elucidate the sequence of events and provide us a clearer clue of the causal relationship. Interestingly, the levels of DAT and dopamine receptors were also increased in a mouse model expressing dominant-negative ErbB4, another promising risk factor for major mental illnesses (38). Involvement of presynaptic mechanisms, such as DAT, will be an important subject for studying the pathophysiology of major mental illnesses (39). If we use genetic animal models that are fully characterized in direct reference to human PET studies, we will be able to address mechanistic questions of how genetic risk factors for mental illnesses affect subcortical dopaminergic signaling in a clinically relevant manner.

Unlike other medical fields, psychiatry is still dependent on subjective interviews for diagnosis. More objective means to evaluate pathophysiology and determine diagnoses are required. Exploration of physiological assessments that are translatable between humans and animal models may facilitate basic understanding and bring about more objective measures of psychiatric illness that can be employed in diagnosis, prognosis and therapeutic monitoring. Further studies of models for mental illness with PET, magnetic resonance imaging (MRI) and other molecular and functional modalities may be an important first step towards this goal.

**MATERIALS AND METHODS**

The procedures used were in accordance with the guidelines published in the Institute of Laboratory Animals Resources Commission on Life Sciences’ 1996 *Guide for the Care and Use of Laboratory Animals*.

**Mice**

We have two lines of DN-DISC1 transgenic mice in the pure C57BL/6N background. Line 37 has higher expression of the transgene than line 10 (16). By systematic breeding, we have also developed a homozygous line from line 37, validated by western blotting (Supplementary Material, Fig. S2). Heterozygous DN-DISC1 mice were used for some experiments in this study, because of administrative difficulty of shipping homozygotes among institutions. Unless specifically noted, DN-DISC1 indicates homozygous line 37 mice. All experiments were performed on young adult male mice.

**PET scan with [11C]raclopride**

Three pairs of DN-DISC1 heterozygotes and littermate WT mice were scanned by high-resolution MRI (9.4T, 2D sequence) followed by [11C]raclopride PET. Radiopharmaceutical in the range of 7.4–22.2 MBq (200–600 μCi) per mouse was injected intravenously and doses were matched in each pair. The average specific radioactivity was 15 016 Ci/mmol end of synthesis. DN-DISC1 and WT mice were scanned side by side. Mice were imaged using a GE eXplore VISTA small animal PET scanner, with a brain-centered dynamic protocol covering 0–90 min post-injection. The PET and MRI data were then co-registered manually via the AMIDE program (http://amide.sourceforge.net) using harderian gland anatomical structures delineated by MRI. Regions of interest (ROIs) were then drawn through the striatum and cerebellum. Radioactivity and area were then calculated for each set of drawn ROIs following entry of the injected dose and the experimentally determined correction factor into the AMIDE program. Striatum: cerebellum ratios were calculated to normalize specific versus non-specific [11C]raclopride uptake.

**Autoradiography of dopamine D2 receptors with [3H]-Spiperone**

Three-month old DN-DISC1 mice (N = 6) and C57BL/6 controls (N = 5) were used for the experiment. Brain slices were pre-washed in 50 mm Tris buffer (pH 7.1) containing 120 mm NaCl, 5 mm KCl, 2 mm CaCl₂ and 1 mm MgCl₂ for 5 min at 36°C. Total binding was determined by incubating slides at 36°C for 30 min in the same buffer containing 40 nm ketanserin to block the binding of the tracer to serotonergic receptors and 1.4 nM [3H]spiperone (specific activity: 15 Ci/mmol, Perkin Elmer Life and Analytical Sciences, Boston, MA). Non-specific binding was determined in adjacent brain slices incubated with the addition of 1 μM (+)-butaclamol (Sigma, St Louis, MO, USA). After the 30 min incubation period, slides were rapidly washed three times in buffer at 0°C, followed by a rinse in ice-cold H₂O₂. Slides were then dried at
37°C for 1 h, left to dry at room temperature overnight and then apposed to Kodak Bio-Max MR films for 6 to 7 weeks at 22–24°C. Reference [³H] microscale standards (Amersham, Arlington Heights, IL, USA) were included with each film to ensure linearity of optical density and to allow the quantitative analysis of the images. Images from autoradiograms were captured using an image analysis system (Loats Associates Inc., Westminster, MD, USA) and densitometric analysis was performed using NIH Image v1.63.

Real-time PCR of dopamine D2 receptors

Three-month old DN-DISC1 mice (N = 7) and C57BL/6 controls (N = 9) were used for the experiment. Real-time quantitative PCR was performed using the SYBR green-based assay on ABI 7900HT by normalizing each sample to GAPDH and using genomic DNA dilutions as standards. The program included 40–50 cycles of 50°C 2 min, 95°C 10 min, 95°C 15 s, 60°C 1 min and a dissociation step.

The following primers were used: D2R-sense: 5′-GTCTCTG TCCCTACACATCCTTG-3′, D2R-antisense: 5′-CGAGAC GATGGAGGAGTAGACC-3′.

Methamphetamine effect on DN-DISC1 mice

Open field

DN-DISC1 heterozygous line 37 (N = 5), line 10 (N = 5) and WT littermates (N = 6) were used for this experiment. To measure locomotor activity, each mouse was placed in a transparent acrylic cage. Locomotion and rearing were measured in 5 min blocks for 120 min using digital counters with infrared sensors (Scanet SV-10; Merquest) as described previously. Mice received one injection of saline or methamphetamine (1 mg/kg, i.p.) after 30 min of pre-habituation in a transparent acrylic cage and were placed into the cage for an additional 90 min to measure the methamphetamine-induced hyperactivity. Data were analyzed by an ANOVA with repeated measures followed by post-hoc testing.

In vivo microdialysis

Mice were anesthetized with sodium pentobarbital, and a guide cannula (AG-8, Eicom) was implanted into the ventral striatum (anteroposterior: +1.7 mm, mediolateral: −0.75 mm from bregma, dorsoventral: −4.0 mm from the dura) according to the Atlas of Franklin and Paxinos (40) and secured to the skull with stainless steel screws and dental acrylic cement. A dialysis probe (AI-8-1; 1 mm membrane length, Eicom) was inserted through the guide cannula and perfused continuously with Ringer’s solution (147 mM NaCl, 4 mM KCl and 2.3 mM CaCl₂) at a flow rate of 1.0 µl/min. The dialysates were collected every 10 min and analyzed by an HPLC system with electrochemical detection (Eicom). Three samples were used to establish baseline levels of dopamine before the administration of saline and again before the administration of methamphetamine (1 mg/kg, i.p.).

Immunoblot for dopamine transporter

The level of DAT in total striatal lysates was assessed by immunoblotting with rat monoclonal antibody (Millipore MAB369 at 1:500). WT (N = 15), heterozygous (N = 8) and homozygous (N = 15) DN-DISC1 mice were compared.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.

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Conflict of Interest statement. None declared.

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