Fyn Is Required for Haloperidol-induced Catalepsy in Mice

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MATERIALS AND METHODS

Animals—Fyn tyrosine kinase-deficient mice were generated by inserting the β-galactosidase gene (lacZ) into the reading frame of the fyn gene as described previously (20). Because the lacZ introduced is expressed in both heterozygous (+/fyn) and homozygous (fyn/fyn) mice, heterozygous mice were mainly used as the controls instead of wild-type mice to compensate for the possible effect of lacZ expression as a foreign gene. The background of this mutant’s strain is C57BL/6J. Genotypes were analyzed by the PCR. All animals were maintained under standard laboratory conditions as described previously (15). All experimental procedures were in accordance with the 1996 National Institutes of Health guidelines and were approved by the Animal Care Committee of the Chiba University Graduate School of Medicine.

Typical antipsychotic agents, such as haloperidol and chlorpromazine, have extrapyramidal side effects (EPS) that resemble Parkinson disease. Drug-induced catalepsy, the impairment of movement initiation, in rodents is an animal model of EPS and is mainly caused by blockade of the dopamine D2 receptor (D2-R) (1, 2). Haloperidol-induced responses are also dependent on N-methyl-D-aspartate receptor (NMDA-R) activity, because prior administration of the NMDA-R antagonist MK-801 attenuates haloperidol-induced catalepsy (3, 4). D2-R and NMDA-R are co-expressed in close proximity along the dendrites of medium spiny neurons in the striatum, and they are functionally coupled in terms of controlling extrapyramidal functions (5).

The NMDA-Rs are hetero-oligomeric ligand-gated ion channels composed of a single NR1 subunit and one type of NR2 (A–D) subunit (6). The most abundant receptor subunits in the striatum are NR1, NR2A, and NR2B (7, 8). These three subunits are involved in extrapyramidal functions (5), and we have found that an NR2B-selective antagonist attenuates haloperidol-induced catalepsy (9).

Phosphorylation of tyrosine residues on the NMDA-R has been reported to modulate its channel characteristics (10, 11). Depriving the striatum of dopaminergic input increases the tyrosine phosphorylation of the striatal NMDA-R and the motor response (12, 13), but infusing the striatum with a tyrosine kinase inhibitor, genistein, attenuates both the tyrosine phosphorylation and the motor response induced by dopaminergic deprivation (13).

Fyn is a member of the Src family kinases (SFKs) and is associated with the NMDA-R at postsynaptic densities. Fyn phosphorylates NMDA-R subunits and modifies their channel activity (14). One of the NMDA-R subunits, NR2B, is preferentially phosphorylated by Fyn, and its phosphorylation has been implicated in several brain functions, including ethanol tolerance, long term potentiation, and seizure susceptibility (15–18). The Tyr-1472 of NR2B is a particularly key site for Fyn activation and the subsequent tyrosine phosphorylation of NR2B alters striatal neuronal activity, thereby inducing the behavioral changes that are manifested as a cataleptic response.

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The abbreviations used are: EPS, extrapyramidal side effects; BSS, balanced salt solution; D2-R, dopamine D2-receptor; HAL, Haloperidol; NMDA, N-methyl-d-aspartate; NMDA-R, N-methyl-d-aspartate receptor; PKA, protein kinase A; PKC, protein kinase C; SFKs, Src family kinases; TBS, Tris-buffered saline; TH, tyrosine hydroxylase.
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Osaka University, and the National Institute of Neuroscience, National Center of Neurology and Psychiatry.

Pharmacological Agents—Haloperidol, the dopamine D2-R-selective antagonist L-741,626 (21), and the D2-R-selective agonist (−)-quinpirole were purchased from Sigma. The drugs were administered by intraperitoneal injection in a volume of 10 µl/g body weight. All solutions were prepared immediately prior to the experiments. To exclude the effect of drug tolerance, no animals were used more than once in the pharmacological experiments.

Antibodies—Goat polyclonal anti-D2-R antibody (N19) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal anti-tyrosine hydroxylase (TH) antibody was obtained from Chemicon (Temecula, CA). A mouse monoclonal anti-phenylalanine hydroxylase (TYR) antibody (1:100) was purchased from Cell Signaling Technology (Beverly, MA). Phosphorylation site-specific rabbit polyclonal antibody against p-Src (Y418) and p-Src (Y529) was obtained from BIO-SOURCE (Camarillo, CA) and against p-NR2B (Y1472) was from Sigma. Rabbit polyclonal anti-NR2B antibody was a gift from Dr. Masa- hiko Watanabe (22). Anti-Src mouse monoclonal antibody (GD11) was purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). The anti-Fyn rat monoclonal antibody (γC3) was raised by Dr. Masahiro Yasuda (23). The anti-βIII tubulin mouse monoclonal antibody was purchased from Promega (Madison, WI).

Assessment of Catalepsy—Catalepsy was measured by a bar test (24). The test was carried out 1 h after intraperitoneal injection of haloperidol (0–1.0 mg/kg) or L-741,626 (0–10 mg/kg). A 3-mm-diameter wooden bar was fixed horizontally 4 cm above the floor of a Plexiglas cage. The animals were placed inside the test cage and allowed to acclimatize for 5 min prior to performing the bar test. Both forepaws were then gently placed on the bar, and the length of time during which each mouse maintained the initial position was measured (maximum cut-off time, 180 s).

Analysis of Rigidity—Muscle rigidity after haloperidol administration was assessed by a mechanographic technique using a modified device designed for rat experiments (25). The mouse was placed in a narrow, well ventilated plastic tube to restrict body movement, and one hind leg was bound to a force sensor (AD4937-5N, A & D Co. Ltd., Tokyo, Japan) that recorded linear reciprocating motion (15-mm distance, 15 cycles/min) via a crank and motor. The raw data from the force sensor were analyzed on a Macintosh computer connected to an A/D converter and software (PowerLab 4s, chart version 3.6 ADInstruments, Mountain View, CA), and the resistance of the flexor and extensor muscles to forced extension and flexion of the knee and ankle joint was measured. The mice were attached to the above device, and the difference in muscle resistance before and after the administration of vehicle or haloperidol (1.0 mg/kg) was recorded. The mean amplitude of 10 consecutive waves at each time point was calculated. Spikes that indicated spontaneous movements of the mice were excluded from the count.

In Situ Hybridization Histochemistry—The distribution of D2-R gene expression in the striatum of the control and Fyn-deficient mice was compared. The probe was prepared as follows. A cDNA fragment encoding the sequence of mouse D2-R (1.3 kbp, a gift from Dr. T. Kaneko, Kyoto University) was cloned into the pBluescript II/ KS− vector, and the clone was digested and used as a DNA template to synthesize an antisense or sense digoxigenin-labeled cRNA probe. The probe was prepared with T7 or T3 RNA polymerase and a digoxigenin RNA labeling kit (Roche Applied Science). Staining was performed as reported previously (26). The sense cRNA probe was employed as the control, and no signals in the brain were detected with it.

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Immunoblot Analysis—One hour after administration of the vehicle, haloperidol (1.0 mg/kg), or L-741,626 (5 mg/kg), the striatum was immediately dissected and frozen in liquid nitrogen. The striatum was placed in buffer containing 10% sucrose, 3% SDS, 10 mM Tris-HCl, pH 6.8, 1 mM sodium orthovanadate, and 1 µM phenylmethylsulfonyl fluoride and homogenized with a Polytron homogenizer (Kinematica AG, Lucerne, Switzerland). Samples were spun down (20,000 × g, 15 min) to remove insoluble material, and the protein concentration was determined with the BCA protein assay reagent (Pierce). After the addition of 40 mM dithiothreitol, the samples were boiled for 5 min, and an equal amount of protein (40 µg per lane) from each sample was separated by electrophoresis on 10% polyacrylamide gels. The gels were transferred onto Immobilon membranes (Millipore, Bedford, MA). The membranes were blocked with 1% bovine serum albumin in Tris-buffered saline (TBS; pH 7.5) containing 0.1% Tween 20 (TBS-T) or 10% skim milk in TBS-T for 1 h and probed with primary antibodies (1.750 dilution for TH, 1:4000 for anti-βIII tubulin, and 1:1000 for other antibodies). After washing three times with TBS-T, the membranes were incubated with horseradish peroxidase-labeled secondary antibodies (anti-goat, anti-rabbit, anti-rat, or anti-mouse IgG, 1:20,000 dilution, all purchased from The Jackson Laboratories, West Grove, PA). After washing three times, the signals were detected with ECL Plus (Amer- sham Biosciences) and ATTO Cool Saver (ATTO Corp., Tokyo, Japan). The membranes were then incubated with stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl, pH 6.7) at 50 °C for 30 min, washed, blocked, and reprobed with other antibodies.

Immunoprecipitation and Western Blotting—The procedures for immunoprecipitation were as described previously (15). Striatum obtained 1 h after vehicle or haloperidol (1.0 mg/kg) administration was placed in lysis buffer (10 mM Tris-HCl, pH 7.4, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 0.15 mM NaCl, 1 mM EDTA, and 1 mM sodium orthovanadate) and homogenized with a Polytron homogenizer. Samples were spun down to remove insoluble material, and the protein concentration was determined. Equal amounts of protein (500 µg) were then used for immunoprecipitation. Samples were preclaried with protein G-Sepharose (Amersham Biosciences), incubated for 1 h at 4 °C with 1 µg of the anti-Fyn or anti-Src antibody, and then incubated for 1 h at 4 °C with 10 µl of protein G-Sepharose. After three washes with lysis buffer, the pelleted protein G-Sepharose was boiled for 5 min in 30 µl of SDS sample buffer, and 15 µl of the supernatant was subjected to SDS-PAGE. The separated proteins were subsequently blotted onto Immobilon, probed with each antibody, and visualized as described above.

Primary Cultures of Striatal Neurons—Primary cultures of striatal neurons were prepared from the fetal striata of wild-type and Fyn-deficient mice at embryonic day 17. Striata from 6 to 8 fetal brains were dissected and placed in Hanks’ balanced salt solution (Invitrogen) and then were transferred into a dissociation medium containing Hanks’ balanced salt solution, 0.05% DNase I, and 1% trypsin/EDTA and incubated at 37 °C for 7 min. After sedimentation, the supernatant was removed, and the pellet was washed three times with Hanks’ balanced salt solution containing 1% penicillin/streptomycin. The tissue was gently placed in Hanks’ balanced salt solution containing 0.05% DNase I and triturated with a plastic pipette until a homogeneous suspension was obtained. After centrifugation at 130 × g for 8 min, the cell pellet was resuspended in Neurobasal/B27 medium (Invitrogen) containing 0.5 mM l-glutamine and penicillin/streptomycin (100 units/ml). The cell cultures were seeded at a density of 3 × 10^5 cells/cm² on 0.1% polyethyleneimine-coated cover glasses in 1.9 cm² well dishes (Nunc, Nunc). Cells were maintained at 37 °C under a humidified 5% CO₂.
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ATMOSPHERE. The cultured striatal neurons were identified immunochemically with anti-GAD65 antibody (Chemicon) and anti-MAP2 antibody (Sigma). More than 95% of both the wild-type and Fyn-deficient neurons were double-labeled by anti-GAD65 and anti-MAP2 (supplemental Fig. S1).

Calcium Imaging—Calcium imaging was carried out as described previously (27). Briefly, striatal primary cells were incubated with 10 μM fura-2/AM (Dojindo) for 1 h at 30 °C in balanced salt solution (BSS) consisting of (in mM) NaCl 130, KCl 5.4, glucose 5.5, HEPES 10, and CaCl₂ 2, and adjusted to pH 7.4 with NaOH. After washing, the cover glasses that contained cultured neurons were mounted on the stage of an inverted fluorescence microscope (IX50; Olympus) and perfused with BSS at a flow rate of 1.8 ml/min. The perfusion medium was prewarmed and maintained at 32.6 ± 1.1 °C in the measurement dish. Fluorescence images obtained by alternate excitation with 340 and 380 nm light through the ×20 objective lens and CCD camera (C2400-8; Hamamatsu Photonics, Hamamatsu, Japan) were fed into an image processor (Argus 50, Hamamatsu) for ratiometric analysis. The effect of the D₂-R antagonist L-741,626 on the channel activity of NMDA receptors was investigated in the presence of the selective D₂-R agonist quinpirole in the perfusion medium. As shown in supplemental Fig. S2, quinpirole alone had a dose-dependent inhibitory effect on the channel activity of the NMDA receptors of the striatal primary neurons of the control mice consistent with its inhibitory effect reported in the striatal slice culture (28). Quinpirole was observed to have almost the same degree of the inhibitory effect on Fyn-deficient neurons (49% decrease at 50 μM).

Preparation of Protein Samples from the Cultured Neurons—Striatal neurons were cultured in 1.9 cm²/well dishes (Nunclon, Nunc) as described above. Each solution used in the following experiments was prewarmed to 37 °C in a water bath. Culture dishes were warmed on a heat block to 37 °C. The culture medium was removed, and the cultured cells were incubated with BSS for at least 5 min. The cells were then incubated with the following: 1) BSS for 7 min followed by incubation in quinpirole (50 μM) in BSS for 7 min, or 2) in quinpirole (50 μM) in BSS for 7 min followed by a mixture of quinpirole (50 μM) and L-741,626 (10 μM) in BSS for 7 min. The solution was removed, and the cells were immediately lysed in 150 μL of SDS sample buffer.

Statistical Analyses—The results of the catalepsy assessment and calcium imaging were evaluated by the Kruskal-Wallis test followed by the Mann-Whitney U test. The results of the muscle rigidity analysis were evaluated by a two-way repeated measure ANOVA. The results of Western blotting were evaluated by one-way ANOVA followed by Bartlett’s test. All data are expressed as the mean ± S.E.

RESULTS

Haloperidol induced catalepsy in the +/fyn² mice, and the duration of the catalepsy increased in a dose-dependent manner (Fig. 1). By contrast, the duration of the catalepsy in the fyn²/fyn² mice was significantly shorter (Fig. 1). At the 1.0 mg/kg dose, there was no difference in the cataleptic response between the +/fyn² mice and the wild-type mice (154.5 ± 47.1 s). The D₂-R-selective antagonist L-741,626 was confirmed to induce catalepsy in the control mice (supplemental Fig. S3), as reported previously in rats (29), but the duration of the catalepsy was significantly reduced in fyn²/fyn² mice (supplemental Fig. S3). Because there was no significant difference in the temporal patterns of the locomotor activity between +/fyn² mice and fyn²/fyn² mice (30), the altered cataleptic response in the fyn²/fyn² mice was concluded not to be due to a locomotion defect.

Because Fyn-deficient mice are more fearful than control mice (31), we suspected that they might avoid a procedure like the “bar test” and that the duration of catalepsy would be misleadingly short as a result. We therefore also measured muscular rigidity to minimize any such emotional influence on the response to haloperidol. Haloperidol induced a marked increase in hind limb muscle rigidity in the +/fyn² mice that was detectable as early as 45 min after administration (1.0 mg/kg) and persisted for more than 2 h, but no increase in muscle rigidity was detected in the fyn²/fyn² mice (Fig. 2 and supplemental Fig. S4).

To exclude the possibility that the failure to respond to haloperidol was because of a difference in D₂-R expression, in situ hybridization of D₂-R mRNA and Western blotting of D₂-R protein were performed on the striatum of +/fyn² and fyn²/fyn² mice. As shown in Fig. 3, no clear difference was observed in either striatal D₂-R gene expression (Fig. 3A) or the protein level (Fig. 3B). Western blotting analysis of striatal TH was also performed to determine whether there was any difference between the two genotypes in the abundance of the rate-limiting enzyme in dopamine biosynthesis, but little difference in the amount of TH protein was found (Fig. 3C).

The effect of haloperidol on protein tyrosine phosphorylation in the
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The striatum of the +fynZ + and fynZ/fynZ mice was compared by Western blotting. One hour after haloperidol administration (1.0 mg/kg), a marked increase in tyrosine phosphorylation of several proteins, including 60-, 110-, and 180-kDa proteins, was observed in the striatum of the +fynZ mice but not of the fynZ/fynZ mice (Fig. 4A).

Because the 60-kDa protein corresponds in size to SFKs, we measured tyrosine phosphorylation of the activation-related Tyr-418 residue. As shown in Fig. 4B, phospho-Tyr-418 increased after haloperidol injection in the +fynZ mice, while no such effect was observed in the fynZ/fynZ mice. Basal Tyr(P)-418 immunoreactivity was much lower in the fynZ/fynZ mice. Because the anti-pY418 antibody recognizes both Fyn and Src, we immunoprecipitated Fyn and Src, and we examined the phosphorylation of the activation-related residue, Tyr-418, and of the inhibition-related residue, Tyr(P)-529, by Western blotting in the +fynZ mice. As shown in Fig. 4C, Fyn but not Src was activated at Tyr-418 by haloperidol, and no change was observed in the phosphorylation at Tyr-529. Because the 180-kDa protein corresponds in size to the NR2B subunit, we also measured the phosphorylation of the Tyr-1472 of NR2B, the key phosphorylation site, by Fyn. The results showed that phospho-Tyr-1472 increased in the +fynZ mice but not in the fynZ/fynZ mice (Fig. 4D). The basal level of Tyr(P)-1472 in the fynZ/fynZ mice was not significantly different from the basal level in the +fynZ mice. Marked increases in tyrosine phosphorylation of the 60-, 110-, and 180-kDa proteins and up-regulation of Tyr(P)-418 and Tyr(P)-1472 were also observed following L-741,626 administration to the control mice, but no such effects were observed in the fynZ/fynZ mice (supplemental Fig. S5).

There were no sex differences in the results of either the behavioral or biochemical studies (data not shown). The same findings in regard to the haloperidol-induced enhancement of tyrosine phosphorylation were also observed in the wild-type mice, and no clear difference was detected between +fynZ mice and wild-type mice (data not shown). To investigate whether the Fyn-mediated increase in NMDA receptor phosphorylation by D2-R blockade affects NMDA receptor activity, we prepared striatal primary cultures and assessed the channel activity of NMDA receptors by the calcium imaging method.

After 4–7 days of culture, we loaded 10 μM fura-2/AM into the primary cells and measured the increase in intracellular calcium concentration ([Ca2+]i) by calcium fluorideimetry. Exposure to 3 μM NMDA/10 μM glycine for 30 s induced a robust response in more than 95% of the cells analyzed, and repeated applications of NMDA/glycine at 5-min intervals evoked reproducible responses (data not shown), indicating little desensitization of the NMDA receptors under our experimental conditions.

We first examined the effect of a D2-R-selective antagonist,
We then examined the effect of L-741,626 on Fyn activation and NMDA receptor phosphorylation in the presence of quinpirole (50 μM) by Western blot analysis, as shown in Fig. 6. Primary cells were exposed or not exposed (control) to 10 μM L-741,626 for 7 min prior to sample preparation. In the wild-type cells, immunoreactivity for anti-pY1472 antibody and anti-pY418 antibody in the L-741,626-treated cell extracts was stronger than in the control cell extracts. By contrast, when Fyn-deficient cells were used, there were no significant differences in immunoreactivity for anti-pY1472 antibody and anti-pY418 antibody between the L-741,626-exposed cell extracts and the control cell extracts.

**DISCUSSION**

The results of this study show that Fyn is required for haloperidol-induced catalepsy. We also found that haloperidol induces Fyn activation and a Fyn-dependent increase in NR2B phosphorylation in mouse striatum. We used striatal primary neurons to verify that D2-R blockade induced Fyn activation, enhancement of NR2B phosphorylation, and potentiation of the channel activity of NMDA receptor at the cellular level, and the latter two effects were significantly reduced in Fyn-deficient neurons. On the basis of these findings, we propose a new molecular mechanism that underlies haloperidol-induced catalepsy in which the D2-R antagonist induces Fyn activation in the striatum, and the subsequent phosphorylation of the NR2B subunit by the activated Fyn increases the channel activity of NMDA receptors, which leads to changes in neural transmission and results in the cataleptic response.

Because haloperidol-induced catalepsy and muscular rigidity are mainly caused by blockade of dopamine D2-Rs in the striatum (1, 2), sensitivity to haloperidol should be altered by changes in dopaminergic transmission. However, there were no clear differences between Fyn-deficient mice and control mice in the expression pattern of the D2-R gene or the amounts of D2-R protein and tyrosine hydroxylase, and measurements by microdialysis showed no significant difference in striatal basal dopamine levels (32). Thus, it is rather unlikely that the
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reduced sensitivity to haloperidol in the Fyn-deficient mice is because of defective dopaminergic transmission.

We found that haloperidol increased phosphorylation of the Tyr-418 residues of Fyn. The catalytic activity of SFKs is controlled through autokatylic phosphorylation and dephosphorylation, particularly at amino acid residues Tyr-418 and Tyr-529 (14), and the phosphorylated Tyr-529 intramolecularly interacts with an Src homology 2 domain to form a loop, thereby suppressing kinase function. Intermonomorphic auto-phosphorylation at Tyr-418, on the other hand, activates SFKs by displacing Tyr-418 from the substrate-binding site, thus allowing the kinase to gain access to substrates (33). We found that the haloperidol-induced increase in Tyr-418 phosphorylation occurred specifically in Fyn and did not occur in Src, whereas phosphorylation of Tyr-529 was the same in both Src and Fyn. Thus, Fyn is specifically activated by haloperidol in vivo.

Haloperidol also increased the phosphorylation of Tyr-1472 in the NR2B subunit, and because no increase was observed in Fyn-deficient mice, the haloperidol-induced phosphorylation of NR2B subunit must be dependent on Fyn. Moreover, we confirmed this D2-R antagonist induced Fyn-mediated enhancement of NR2B phosphorylation at the cellular level in primary cultures of striatal neurons.

Fyn-mediated phosphorylation of NR2B and potentiation of NMDA-R channel activity are involved in several brain functions, including ethanol tolerance (15), seizure susceptibility (23), and long term potentiation (17). Activation of Eph receptors has also been reported to result in increased phosphorylation of NR2B and an increase in NMDA-R channel activity measured by Ca2+ imaging in hippocampal primary cultures (34). The use of HEK293T cells transfected with a mutant NR2B construct in this study also showed that Fyn-mediated tyrosine phosphorylation of NR2B is required for the increase in NMDA-R channel activity. In our study, NMDA-R channel activity in most wild-type striatal neurons was increased by the blockade of D2-R, and the proportion of such neurons was significantly reduced in Fyn-deficiency. Thus, the increased NMDA-R activity after D2-R blockade in most of the striatal neurons was Fyn-dependent. However, NMDA-R may also be activated by a Fyn-independent pathway, because a certain proportion of the neurons in the Fyn-deficient striatal culture exhibited increased NMDA-R activity.

It has been repeatedly observed that the NMDA-R antagonist MK-801 attenuates haloperidol-induced catalepsy (9, 35–37), and we recently reported that prior exposure to the NR2B-selective antagonist CP-101,606 significantly reduces haloperidol-induced catalepsy (9). Thus, haloperidol-induced catalepsy is specifically dependent on NR2B function, and activation of NR2B function by Fyn-mediated phosphorylation is likely to be required for catalepsy to occur.

NMDA-R dysfunction is hypothesized to be the pathogenetic mechanism responsible for schizophrenia, because NMDA-R antagonists cause psychotic states resembling schizophrenia (38–40), and mice with reduced NMDA-R expression have been reported to display schizophrenia-related behaviors (41). Because unmedicated schizophrenic patients exhibit attenuated EPS shortly after haloperidol administration compared with healthy controls (42), the lower responsiveness to haloperidol in Fyn-deficient mice may mimic a feature of schizophrenia.

Several mutant mice, including mice deficient in the D2-R (24), A3A-adenosine receptor (43), retinoic X receptor y1 (44), and protein kinase A (PKA) (45), show reduced cataleptic responses to haloperidol. The reduced cataleptic response in one of them, the PKA-deficient mutant, is likely to be caused by a molecular mechanism similar to that in Fyn deficiency, because an increase in PKA-mediated serine phosphorylation of striatal NR1 subunits increases following haloperidol adminis-

tration (46). The scaffolding protein RACK1 binds to both Fyn and NR2B, and the three molecules form a complex in rat hippocampus (16, 47). Dissociation of RACK1 from this RACK1-Fyn-NR2B complex facilitates Fyn-mediated phosphorylation of NR2B (47). Because PKA activation has been demonstrated to dissociate RACK1 from this complex (16), the above PKA-RACK1-Fyn pathway may also exist downstream of D2-R in the striatum.

Another molecule that may act between D2-R and Fyn is PKC. Activation of G-protein-coupled receptors, such as muscarinic and metabotropic glutamate receptors, in the hippocampus increases NMDA-evoked currents via protein kinase C (PKC) (48). The increase in NMDA-R function is mediated by the activation of SFKs, because the PKC-induced NMDA-R up-regulation is blocked by an inhibitor of Src and Fyn and does not occur in Src-deficient cells (48). D2-R is another G-protein-coupled receptor, and because haloperidol administration acutely increases PKC activity in the rat striatum (49), Fyn activation after D2-R blockade may be mediated by the PKC pathway.

Other molecules, including receptor tyrosine kinases (34, 50) and a cytokine receptor (51), have also been reported to be involved in SFK-mediated phosphorylation and activation of NMDA-R, and they may be involved in the striatal activation of Fyn after D2-R inhibition.

In this study we found that blockade of D2-R causes Fyn activation, Fyn-mediated NMDA-R phosphorylation, and potentiation of its channel activity in the striatal neurons that may be responsible for haloperidol-induced catalepsy. Further investigation should focus on the above-postulated Fyn-activation mechanisms initiated by D2-R blockade, and these transduction steps should be drug targets for controlling not only motor function but higher cognitive brain function.

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