Increased Dopamine Receptor Signaling and Dopamine Receptor-G Protein Coupling in Denervated Striatum

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ABSTRACT

Chronic interruption of the nigrostriatal dopaminergic pathway leads to sensitized dopaminergic responses in striatum. We attempted to explore the mechanism(s) underlying this dopaminergic supersensitivity by assessing dopamine receptor signaling and receptor-G protein coupling in unilateral 6-hydroxydopamine-lesioned rats. Dopamine-stimulated adenylyl cyclase activity as well as dopamine-activated guanosine 5'-O-(3-thiotriphosphate) ([γS]GTPγS) binding and [3H]palmitate incorporation by Gα proteins were enhanced in tissues obtained from denervated striata without apparent changes in Gα protein levels. Moreover, high-affinity binding sites of the D2 dopamine receptor increased in lesioned compared with control striata without altering the expression level of the receptor. These denervation-mediated changes appear to correlate with the increase in D1 dopamine receptor binding sites that co-immunoprecipitated with Gαs(olf)/q(11) proteins. In contrast, the total number of D2 receptor binding sites was increased, yielding an increase in absolute number of high-affinity sites without significant changes in the proportion of high-affinity sites. Stimulation of the D2 dopamine receptor enhanced coupling to Gαi protein; this was increased in the striata lesioned. The results provide an important molecular mechanism by which dopamine receptor-regulated signaling is enhanced following denervation of dopaminergic input to striatum. Although D1 dopamine receptor supersensitivity appears to be mediated by enhanced coupling of the receptor to its G proteins, sensitization in the D2 dopamine receptor system is mediated by increased D2 receptor density and enhanced D2 receptor-Gi protein coupling.

In the brain, dopamine transmits synaptic information by binding to specific cell surface receptors. Five subtypes of dopamine receptors have been identified and cloned to date (Dearry et al., 1990; Sokoloff et al., 1990; Zhou et al., 1990; Sunahara et al., 1991; van Tol et al., 1991). Stimulation of the D1-like dopamine receptors D1 and D5 activates adenylyl cyclase via Gi/o proteins (Dearry et al., 1990; Sokoloff et al., 1990; Zhou et al., 1990; Sunahara et al., 1991; Teberi et al., 1991). A D1-like dopamine site, which couples to Gq/11 protein, has been linked to the modulation of phosphatidylinositol hydrolysis (Wang et al., 1995; Jin et al., 2001). On the other hand, D2-like dopamine receptors D2, D3, and D4 are negatively coupled to adenylyl cyclase via Gi/o proteins (Onali et al., 1985; Potenza et al., 1994; Tang et al., 1994; McAllister et al., 1995). In addition, both D1 and D2 dopamine receptors have been shown to regulate calcium signaling (Missale et al., 1998) and to stimulate mitogen-activated protein kinase pathways (Zhen et al., 1998; Cai et al., 2000).

The nigrostriatal dopaminergic pathway is a major dopaminergic projection in the brain, which originates in substantia nigra and innervates striatal medium-sized spiny neurons. Dopamine released from dopaminergic nerve terminals interacts with dopamine receptors and thus regulates a wide range of neuronal functions including locomotor activity. Chronic interruption of this neuronal pathway increases the sensitivity of striatal D1 and D2 dopamine receptors in response to receptor stimulation (Arnt and Hyttel, 1985). Although the enhanced responsiveness of striatal D2 dopamine receptors has been associated with increased D2 dopamine receptor density (Qin et al., 1994; Chalon et al., 1999; Araki et al., 2000), the adaptive mechanism(s) underlying D2 dopaminergic supersensitivity are not well defined. Previous studies have demonstrated no alteration or even a decrease in D1 dopamine receptor expression (Hamdi and Kostrzewa, 1991; Qin et al., 1994), suggesting that sensitization of the D1 dopamine receptor system is mediated by change(s) at a site distal to the receptor. In our previous experiments in which dopaminergic supersensitivity was elicited by reserpine-in-
duced depletion of neuronal dopamine, sensitization of striatal D₁ dopamine receptor responses was associated with an increase in receptor-stimulated GTPγS binding to Gα protein (Butkerait et al., 1994) and increased expression of striatal GαS messenger RNA (Butkerait and Friedman, 1993), suggesting that alteration at the Gα protein level may lead to enhanced signaling via the D₁ dopamine receptor/Gα protein system and contribute to the development of D₁ dopamine receptor supersensitivity.

The present study utilizing the unilateral 6-hydroxydopamine (6-OHDA)-lesioned rat model provides direct evidence that sensitization of striatal D₁ dopamine receptors is associated with increased D₁ dopamine receptor-Gα protein coupling, whereas development of striatal D₂ dopamine receptor supersensitivity is dependent on increased D₂ dopamine receptor expression and enhanced D₂ dopamine receptor-Gα protein coupling.

Materials and Methods

Chemicals. [3H]SCH23390 (71.3 Ci/mmol), [3H]raclopride (69.5 Ci/mmol), [35S]GTPγS (1311 Ci/mmol), 9,10-[3H]palmitic acid (47 Ci/mmol), and [α-32P]ATP (800 Ci/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA). West Pico supersignal chemiluminescence reagents were purchased from Pierce Chemical (Rockford, IL). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Unilateral Lesion of Nigrostriatal Dopaminergic Pathway.

The animal protocol employed in the present study was approved by the Institutional Animal Care and Use Committee of the City University of New York, and all procedures conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (publication no. 85-23, revised 1996). Male Sprague-Dawley rats (200–250 g) were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and injected with 6-OHDA (8 μg/ml saline containing 0.05% ascorbic acid) into the left mid-limbic bundle at coordinates AP -2.5 mm, LAT +1.5 mm, DV -8.0 mm using bregma as a marker. All rats were pretreated with desipramine-HCl (25 mg/kg, i.p.) to prevent damage of noradrenergic neurons. Lesioned rats were screened 3 weeks after surgery by monitoringrotational locomotor activity. Rotational behavior was assessed by placing rats in 50-cm bowls where the bowls are truly hemispheric, and rotations were counted between 10 to 15 min after apomorphine administration (0.2 mg/kg, s.c.). Rats showing fewer than 20 full rotations per 5 min were eliminated from further experiments. Successful lesions were further evaluated by measuring striatal dopamine content. Striatal tissues from both control and lesioned sides were collected and homogenized by sonication in 10 volumes of 0.1 M perchloric acid with 1 μM 2,3-dihydroxybenzoic acid. The homogenate was centrifuged at 27,000 g for 20 min. Dopamine content in 10 μl of supernatant was determined by high-performance liquid chromatography. Rats showing more than 20 rotations per 5 min were found to have a minimum of 85% reduction in striatal dopamine content (0.9 ± 0.1 versus 6.9 ± 0.3 ng/mg b.wt.).

Adenylyl Cyclase Assay. Striatal tissues were homogenized by Teflon glass homogenizer in chilled buffer containing 10 mM imidazole, 2 mM EGTA, and 10% sucrose, pH 7.3. The homogenates were centrifuged at 1,000g for 10 min, and supernatants were centrifuged at 27,000g for 20 min. The pellets obtained were washed twice and suspended in 10 mM imidazole, pH 7.3. Membrane protein was determined by Bradford’s method using bovine serum albumin as standard. Adenylyl cyclase activity was measured by calculating the conversion rate of [32P]ATP to [32P]cAMP (Salomon et al., 1979). The assays were performed in 250 μl of solution containing 10 mM imidazole, pH 7.3, 2 mM MgCl₂, 0.1 mM papaverine, 0.2 mM EGTA, 1 mM dithiothreitol, 1 μM GTP, 0.1 mM ATP; 2 mM phosphocreatine, 5 units of creatine phosphokinase, and 1 μCi of [α-32P]ATP. The reaction mixture was preincubated at 30°C for 5 min, and the reaction was initiated by adding 50 to 60 μg of membrane proteins and incubated for an additional 10 min. The reaction was terminated by the addition of 300 μl of stopping solution (2% SDS, 25 mM ATP, and 1.3 mM cAMP). Formed [32P]cAMP was separated from [32P]ATP by chromatography through Dowex and alumina columns. Radioactivity in each sample was determined by liquid scintillation spectroscopy. [3H]cAMP was added to each reaction for estimation of column recovery.

GTPγS Binding to Gα Proteins. The striata were homogenized in 10 volumes of ice-cold 25 mM HEPES buffer, pH 7.4, which contained 1 mM EDTA, 0.1 M sucrose, 50 μg/ml leupeptin, 0.04 mM phenylmethylsulfonyl fluoride (PMSF), 2 μg/ml soybean trypsin inhibitor, and 0.2% 2-mercaptoethanol. The homogenates were centrifuged at 800g for 5 min, and the supernatants were centrifuged at 49,000g for 20 min. The pellets were suspended in 10 volumes of reaction buffer, which contained 25 mM HEPES, pH 7.4, 100 mM NaCl, 50 μg/ml leupeptin, 2 μg/ml soybean trypsin inhibitor, 0.04 mM PMSF, and 0.2% 2-mercaptoethanol. The resulting striatal membranes (200 μg) were incubated at 30°C for 2 min in reaction buffer that contained in addition 1 mM MgCl₂ and 5 mM GTP. After the addition of 50 nM [35S]GTPγS (10 μCi/assay), the incubation, in a total volume of 250 μl, continued for 3 min in the absence or presence of agonists. The reaction was terminated by dilution with 750 μl of ice-cold reaction buffer that also contained 1 mM EGTA and 20 mM MgCl₂ and immediately centrifuged at 16,000g for 5 min. The resulting pellet was solubilized in 0.5 ml of buffer, which contained 100 mM Tris-HCl, pH 7.4, 200 mM NaCl, 20 mM MgCl₂, 10 mM EDTA, 1.25% (v/v) Nonidet P-40, 0.04 mM PMSF, and 0.2% SDS. The solubilization of tissues was facilitated by using sonication for 10 s. Normal rabbit serum (100 μg/ml) was added to 1 ml of lysate and incubated at 25°C for 30 min. Nonspecific immune complex was removed by incubation with 100 μl of standardized protein A (10% Pansorbin; Calbiochem, San Diego, CA) at 25°C for 30 min followed by centrifugation at 5000g at 4°C for 5 min. The supernatant was incubated at 25°C for 30 min with antisera raised against specific Gα proteins (1:1000 dilution). The immunocomplex was collected by incubation at 25°C for 30 min with 100 μl of 10% Pansorbin and centrifugation at 5000g at 4°C for 5 min. The pellet was washed and suspended in buffer containing 50 mM Tris-HCl, pH 8.0, and 1% Nonidet P-40. The radioactivity in the suspension was determined by liquid scintillation spectrometry.

Palmitoylation of Gα Proteins. Striatal membranes were prepared as in the GTPγS binding assay. The final reaction volume was 250 μl containing 200 μg of membrane proteins, 800 μCi/ml 9,10-[3H]palmitate, 1 mM MgCl₂, and 50 mM Gpp(NH)p. The reaction was carried out at 30°C for 15 min in the absence or presence of agonists, terminated by adding 750 μl of ice-cold Krebs-Ringer buffer containing 1 mM EGTA, and immediately centrifuged at 16,000g for 5 min. The pellets were solubilized in 1 ml of immunoprecipitation buffer containing 100 mM Tris-HCl, pH 7.4, 1.25% (v/v) Nonidet P-40, 200 mM NaCl, 20 mM MgCl₂, 10 mM EDTA, and 0.2% SDS by brief sonication. The specific Gα proteins in solubilized membrane were immunoprecipitated by incubation with antisera raised against specific Gα proteins. The immunoprecipitates were washed and suspended in immunoprecipitation buffer. The radioactivity in the suspension was measured by liquid scintillation spectrometry. The radioactivity precipitated with normal rabbit serum was considered background and subtracted from all agonist-stimulated values.

Radioligand Binding Studies. The ligand binding assays for D₁ and D₂ dopamine receptors were performed as described previously (Billard et al., 1984; Kohler et al., 1985). Briefly, striatal tissue was homogenized with Teflon glass homogenizer in 20 volumes of ice-cold buffer containing 50 mM Tris-HCl, pH 7.4, 2 mM EGTA, and 10% sucrose. The homogenate was centrifuged for 5 min at 800g, and the supernatant was centrifuged for 20 min at 49,000g. The pellet was washed twice and suspended in 50 mM Tris-HCl buffer, pH 7.4.
Immunoblot Analysis. Striatal membranes were prepared, and 20–μg aliquots were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were washed with PBS and blocked overnight at 4°C with 10% milk followed by washing with PBS with 0.1% Tween 20 (PBST). Brains were washed with PBST and incubated for 1 h with a 1:2,000 dilution of anti-rabbit IgG-horse-radish peroxidase and washed with 0.3% PBST followed by washing with 0.1% PBST. Immunoreactivity was visualized by reacting with enhanced chemiluminescence reagent for exactly 5 min and immediately exposing to X-ray film. Specific bands were quantitated by soft laser densitometry.

Commmunoprecipitation. Aliquots of striatal membrane preparations were solubilized by gentle end-over-end shaking for 60 min in PBS containing 1.5% digitonin, 0.5 mM phenylmethylsulfonyl fluoride, 25 μg/ml leupeptin, 20 μg/ml aprotonin, 25 μg/ml pepstatin, and 0.01 unit/ml soybean trypsin inhibitor. The sample was centrifuged at 49,000 g for 30 min, and 200 μg of supernatant was incubated for 3 h with antibodies directed against specific Ga proteins (PerkinElmer Life Sciences; 1:1000 dilution) followed by a 60-min incubation with 100 μl of 10% suspension of protein A bearing Staphylococcus aureus cells (Pansorbin cells). Dopamine receptors that coprecipitated with Ga proteins were determined by dopamine receptor binding assay of the Ga protein immunoprecipitates. Receptor binding assay was assayed in washed pellets that were suspended in 500 μl of binding buffer containing 50 mM Tris-HCl, pH 7.5, and 5 mM MgCl2. The suspension was incubated at 37°C for 30 min with 2 nM [3H]SCH23390 or 10 nM [3H]raclopride and the appropriate cold ligands. The reactions were terminated by filtering through 10-kDa molecular mass cutoff filters (Cole-Parmer Instrument Co., Vernon Hills, IL). The amount of radioactivity trapped on the filters was determined by liquid scintillation spectrometer.

Statistical Analysis. All data are presented as mean ± S.E.M. The dose-response curves were evaluated by two-way ANOVA followed by Newman-Keuls test for multiple comparisons. Two-tailed Student’s t test was used to compare particular responses between two groups. The threshold for significance was p < 0.05.

Results
Dopamine Receptor-Mediated Locomotor Activity. Administration of the nonselective dopamine receptor agonist apomorphine (0.2 mg/kg, s.c.) induced contralateral rotations in unilateral 6-OHDA-lesioned rats. The apomorphine-induced locomotor response appeared 3 min after drug injection, reached maximal response between 5 to 10 min, and persisted for up to 40 min after drug administration. The selective D1 dopamine receptor agonist SKF38393 (5 mg/kg, s.c.) or the D2 dopamine receptor agonist l-quinpirole (1 mg/kg, s.c.) also induced contralateral rotations in lesioned rats as previously reported (Cai et al., 2000).

Dopamine-Stimulated Adenylyl Cyclase Activity. Dopamine dose-dependently stimulated adenylyl cyclase activity in striatal membranes. This dopamine-mediated effect was blocked by the selective D1 dopamine receptor antagonist SCH23390 but not by the D2 dopamine receptor antagonist raclopride. The maximal response to dopamine was increased in membranes obtained from lesioned striata without significant change in EC50 (Fig. 1A). Similarly, activation of G protein with the nonhydrolyzable GTP analog Gpp(NH)p also stimulated adenylyl cyclase, and the Gpp(NH)p-mediated stimulation of adenylyl cyclase was higher in denervated striata (Fig. 1B). However, basal and forskolin-stimulated cyclase activities were comparable in striatal membranes prepared from control and denervated striata (174 ± 10 versus 187 ± 18 and 1002 ± 80 versus 1092 ± 92 pmol/min/mg, respectively).

Receptor-Stimulated GTPγS Binding to Ga Proteins. There were no differences in basal [35S]GTPγS binding to membrane Gαi (504 ± 62 versus 556 ± 59 cpm), Gαo (379 ± 42 versus 378 ± 43 cpm), Gαq/11 (461 ± 59 versus 448 ± 55 cpm), Gai (504 ± 62 versus 556 ± 59 cpm), and Gαo (379 ± 42 versus 378 ± 43 cpm).

Fig. 1. Effect of 6-OHDA lesion on dopamine (A)- and Gpp(NH)p (B)-stimulated adenylyl cyclase activities in striatal membranes. Striatal membranes were prepared from control and lesioned striata, and adenylyl cyclase activity was measured by conversion of [32P]cAMP to [32P]cAMP in the presence of increasing concentration of dopamine (0.1–1000 μM) or Gpp(NH)p (0.01–100 μM). [32P]cAMP formed was separated by Dowex and alumina columns and quantitated by liquid scintillation spectrometer. The data are depicted as the mean specific adenylyl cyclase activities in picomoles of [32P]cAMP formed per minute per milligram of tissue derived from six individual experiments each in duplicate. Vertical bars represent S.E.M. Dopamine- and Gpp(NH)p-stimulated adenylyl cyclase activities were increased in lesioned compared with control striata (*, p < 0.01, Newman-Keuls test for multiple comparison).

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71 cpm), or Goo (584 ± 64 versus 599 ± 77 cpm) proteins between control and lesioned striata. As shown in Fig. 2, activation of dopamine receptor by dopamine increased GTP\(_g\) binding to Gs/oIf, Gaq/11, and Gai proteins but not to Gao protein in a concentration-dependent manner. Furthermore, dopamine-stimulated increases in [\(^{35}\)S]GTP\(_g\) binding to Gs/oIf, Gaq/11, and Gai proteins were enhanced by 28 to 66% in the denervated compared with the control striata.

To test whether the observed increase in dopamine receptor signaling is a specific consequence of dopaminergic pathway denervation, striatal muscarinic cholinergic receptor-mediated stimulation of GTP\(_g\) binding was examined. Carbachol (1 \(\mu\)M), a muscarinic receptor agonist, stimulated [\(^{35}\)S]GTP\(_g\) binding to Gaq/11, Gai, and Gao but not Gs/oIf proteins. More importantly, carbachol-stimulated GTP\(_g\) binding to the G\(_o\) protein was not affected by the lesion (Fig 3).

Receptor Stimulation-Induced Incorporation of Palmitate into G\(_o\) Proteins. As shown in Fig 4, dopamine receptor stimulations increased palmitate incorporation by Gs/oIf, Gaq/11, and Gai but not Gao protein. Dopamine-induced palmitoylation of Gao, Gaq/11, and Gai proteins was increased by 115, 108, and 110%, respectively, in lesioned striata when compared with control striata. However, basal palmitoylation of the G\(_o\) proteins was not different in membranes obtained from lesioned and control striata.

Expression Levels of Dopamine Receptors and G\(_o\) Proteins. To determine whether alteration in dopamine receptor expression level is responsible for the change in dopaminergic response, the densities of D\(_1\) and D\(_2\) dopamine receptors were determined by saturation receptor binding assays using the selective D\(_1\) dopamine receptor ligand [\(^{3}\)H]SCH23390 and the selective D\(_2\) dopamine receptor ligand [\(^{3}\)H]raclopride. The data were subjected to Scatchard analysis, and the results summarized in Tables 1 and 2 indicate that no significant difference in D\(_1\) dopamine receptor expression was noted, although the density of D\(_2\) dopamine receptors was increased by 37% in the denervated striata compared with the control tissue.

G protein levels were assessed by immunoblot analyses using specific G protein antibodies. Although Gs/oIf, Gai, Gaq/11, and Gao were detectable in membranes obtained from control and lesioned striata, no significant changes in the expression levels of these G\(_o\) proteins were observed between control and lesioned sides (Fig 5).

Coupling of Dopamine Receptors to G\(_o\) Proteins. G protein-coupled receptors exist in high- and/or low-agonist binding states that depend on whether they are coupled to G proteins. Assessment of agonist binding affinity, therefore, helps in evaluating the coupling of the receptor to its associated G protein(s). The high/low-binding affinity states of the D\(_1\) and D\(_2\) dopamine receptors were determined by analyses of dopamine competition curves of the selective D\(_1\) receptor ligand [\(^{3}\)H]SCH23390 or of the selective D\(_2\) receptor ligand
TABLE 1
The effects of unilateral 6-OHDA lesion on the expression and binding characteristics of D<sub>1</sub> dopamine receptor in striatal membranes

D<sub>1</sub> dopamine receptor binding was assessed in membranes prepared from control and lesioned striata using the selective D<sub>1</sub> dopamine receptor antagonist [3H]SCH23390 as ligand. For saturation binding, striatal membranes were incubated at 37°C for 30 min with increasing concentrations of [3H]SCH23390 (0.1–6.4 nM) and terminated by vacuum filtration through Whatman GF/B filters. Nonspecific binding was defined as binding in the presence of 10 μM homolergine. To prevent nonspecific association of [3H]SCH23390 with serotonin receptors, 10 μM mesulergine was added to the assay mixture. Maximal binding (B<sub>max</sub>) and binding affinity (K<sub>d</sub>) were determined as described above. Data are presented as mean ± S.E.M. derived from four individual experiments each performed in duplicate.

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<th></th>
<th>B&lt;sub&gt;max&lt;/sub&gt; (fmol/mg)</th>
<th>K&lt;sub&gt;d&lt;/sub&gt; (nM)</th>
<th>B&lt;sub&gt;high&lt;/sub&gt; (%)</th>
<th>B&lt;sub&gt;low&lt;/sub&gt; (%)</th>
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<tr>
<td>Control</td>
<td>643 ± 64</td>
<td>1.5 ± 0.2</td>
<td>33 ± 4</td>
<td>67 ± 4</td>
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<tr>
<td>Lesioned</td>
<td>851 ± 43</td>
<td>1.6 ± 0.2</td>
<td>49 ± 3*</td>
<td>51 ± 3*</td>
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* p < 0.05 compared with respective values of control group by two-tailed Student’s t test.

TABLE 2
The effects of unilateral 6-OHDA lesion on the expression and binding characteristics of D<sub>2</sub> dopamine receptor in striatal membranes

D<sub>2</sub> dopamine receptor binding was assessed in membranes prepared from control and lesioned striata using the selective D<sub>2</sub> dopamine receptor antagonist [3H]raclopride as ligand. For saturation binding, striatal membranes were incubated at 37°C for 30 min with increased concentrations of [3H]raclopride (0.25–16 nM) and terminated by vacuum filtration through Whatman GF/B filters. Nonspecific binding was defined as binding in the presence of 1 μM haloperidol. Maximal binding (B<sub>max</sub>) and binding affinity (K<sub>d</sub>) were calculated by Scatchard analysis of specific [3H]raclopride binding. For competition experiment, [3H]raclopride (4 nM) binding was carried out in the presence of 10<sup>-10</sup> to 10<sup>-4</sup> M dopamine (eight concentrations in 10-fold increments). The dopamine-mediated competition curve was fitted and the percentage of high- (B<sub>high</sub>) and low- (B<sub>low</sub>) affinity binding sites were calculated according to a two-site binding model (Munson and Rodbard, 1980). Data are presented as mean ± S.E.M. derived from four individual experiments each performed in duplicate.

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<th></th>
<th>B&lt;sub&gt;max&lt;/sub&gt; (fmol/mg)</th>
<th>K&lt;sub&gt;d&lt;/sub&gt; (nM)</th>
<th>B&lt;sub&gt;high&lt;/sub&gt; (%)</th>
<th>B&lt;sub&gt;low&lt;/sub&gt; (%)</th>
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<td>Control</td>
<td>125 ± 10</td>
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<tr>
<td>Lesioned</td>
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<td>8.3 ± 1.1</td>
<td>46 ± 6</td>
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* p < 0.05 compared with that of controls using two-tailed Student’s t test.

Fig. 5. Effect of 6-OHDA lesion on expression of striatal Gα proteins. Striatal membranes prepared from control (C) and lesioned (L) striata were solubilized and subjected to 10% SDS-polyacrylamide gel electrophoresis. The size-fractioned proteins were transferred to nitrocellulose membranes and blotted with specific Gα protein antibodies. Immunoreactive signals were detected using enhanced chemiluminescence and visualized by exposure to X-ray film. The obtained signals were quantified using a Zeineth soft laser scanning densitometry (Zeineth, Fullerton, CA). The experiment was repeated three times and similar results were obtained. No differences in expression of Gα1/2, Gα11, Gai, and Gao proteins were observed between control (C) and lesioned (L) striata.

[3H]raclopride. The competition curves obtained from control and lesioned striatal membranes yielded high- and low-affinity sites for both D<sub>1</sub> and D<sub>2</sub> dopamine receptors. Although the ratio of high- to low-affinity states for the D<sub>1</sub> dopamine receptor was increased by 48% in lesioned compared with control striata, this ratio did not change for the D<sub>2</sub> receptor (Tables 1 and 2).

Direct coupling of dopamine receptors to G proteins was further assessed by co-immunoprecipitation of receptors with Gα proteins. In this series of experiments, striatal membranes were solubilized and Gα proteins were immunoprecipitated using specific Gα protein antibodies, and the D<sub>1</sub> or D<sub>2</sub> dopamine receptor binding sites associated with Gα proteins were assessed, respectively, using [3H]SCH23390 or [3H]raclopride as specific receptor ligands. As shown in Fig. 6, [3H]SCH23390 binding was detected in immunoprecipitates of Gαs/olf and Gαq proteins, whereas [3H]raclopride binding was found only in immunoprecipitates of Gai protein. Stimulation of the receptor with dopamine increased D<sub>1</sub> receptor binding in immunoprecipitates of Gαs/olf and Gαq proteins and D<sub>2</sub> receptor binding in immunoprecipitates of Gai protein. Although basal coupling of D<sub>1</sub> receptors to Gαs/olf or Gαq proteins was similar in control and lesioned striata, dopamine-mediated coupling to Gαs/olf and Gαq proteins was increased 97 and 84%, respectively, in lesioned versus control striata. On the other hand, both basal and dopamine-enhanced couplings of D<sub>2</sub> receptors to Gai protein were increased in the denervated striata by 29 and 59%, respectively.

Discussion
In the present communication, we have demonstrated, as others have before, that unilateral denervation of the nigrostriatal dopaminergic pathway with 6-OHDA elicits heightened dopamine receptor-regulated activities in the ipsilateral striatum, as evidenced by contralateral rotational behavior in response to dopamine receptor stimulation. The sensitiza-
tion of dopamine receptors appears to occur at both D₁ and D₂ dopamine receptors since administration of selective D₁ or D₂ dopamine receptor agonists induced contralateral rotations. The present experiments further demonstrate that the supersensitivity of the dopamine receptors was associated with enhanced transmembrane signaling that results from augmented interactions between the dopamine receptors and their G protein. This is supported by the concurrent increases in dopamine-stimulated adenylyl cyclase activity and dopamine receptor-stimulated GTP binding to and palmitoylation of Gα proteins in denervated striata.

Activation of D₁ dopamine receptors is known to stimulate adenylyl cyclase and result in increased intracellular cAMP levels and activation of cAMP-dependent protein kinase A. The present experiment demonstrates that dopamine-stimulated adenylyl cyclase activity was enhanced in the lesioned striatum when compared with the intact side. Although dopamine activated both D₁ and D₂ dopamine receptors, dopamine-mediated stimulation of adenylyl cyclase was blocked by the selective D₁ dopamine receptor antagonist SCH23390 but not by the selective D₂ dopamine receptor antagonist raclopride, indicating that dopamine stimulates...
cyclase via the D$_1$ dopamine receptor. This is in accord with previous findings that D$_1$ dopamine receptor supersensitivity is associated with enhanced cAMP-dependent signaling (Missale et al., 1989; Pfif et al., 1992; Gnanalingham et al., 1995) and D$_1$ receptor-mediated activation of the protein kinase A pathway is crucial for D$_1$ dopamine receptor-mediated locomotor activity (Cole et al., 1994; Oh et al., 1997). Thus, enhanced D$_1$ dopamine receptor signaling may underlie the supersensitivity of the D$_1$ dopamine receptors that mediates rotational activity in the unilateral lesioned rats. D$_2$ dopamine receptor-enhanced locomotor activity, on the other hand, has previously been shown to be associated with activation of the extracellular signal-regulated protein kinase pathway in the denervated striatum (Cai et al., 2000).

Transmembrane signaling at dopamine receptors, like other G protein-coupled receptors, is mediated via G proteins. Stimulation of the receptors promotes the exchange of GTP for GDP and increases the depalmitoylation/palmitoylation cycle of receptor-associated Go proteins. The binding of GTP to Go proteins is critical for coupling of the receptor to its effector, whereas depalmitoylation/palmitoylation of Go proteins enhances the capability of Go proteins to move within the membrane thus facilitating their interaction with other membrane proteins (Bouvier et al., 1995; Wedegaertner et al., 1995). It has previously been documented that the D$_1$ dopamine receptor couples to Go$_{olf}$ and Go$_{11}$ proteins (Wang et al., 1995; Cai et al., 1999; Corvol et al., 2001; Jin et al., 2001), whereas signaling of the D$_2$ dopamine receptors is mediated via Gi protein (Wang et al., 1995; Jin et al., 2001), although recent reports suggest the possibility that D$_{1A}$ dopamine receptor may interact with Golf protein in striatum (Herve et al., 1993, 2001; Zhuang et al., 2001; Corvol et al., 2001). Inferential evidence presented in these reports suggests that D$_{1A}$ dopamine receptor-mediated cAMP production is compromised but not abolished in Go$_{olf}$ deficient mice (Corvol et al., 2001), whereas data that directly link D$_{1A}$ dopamine receptor to Golf is still lacking. Similarly, the D$_{1A}$ dopamine receptor-mediated c-fos expression and locomotor activity in response to amphetamine or cocaine was attenuated in Go$_{olf}$ knockout animals (Zhuang et al., 2000). Although the antibody used in the present investigation does not differentiate between Go$_{olf}$ and Golf, the data shown here clearly indicate that increased dopamine receptor functions following denervation of dopaminergic afferents to striata correlate directly with increased interaction between D$_1$ dopamine receptors and their associated G proteins. Similarly, enhanced association of D$_2$ dopamine receptors with Gi proteins that is known to negatively regulate adenylyl cyclase was also observed. The increase in dopamine-induced cAMP accumulation in lesioned striatum argues that perhaps a dominant D$_1$ dopamine receptor-mediated signaling, at least in the activation of adenylyl cyclase, mediates the expression of functional supersensitivity following nigrostriatal dopaminergic denervation. The conclusion that augmented dopaminergic function is mediated by a more efficacious interaction between dopamine receptors and their G protein is corroborated by the present data that dopamine-stimulated GTP-$\gamma$S binding and palmitoylation were greatly enhanced in membranes obtained from denervated striata. The changes in signaling efficiency of dopamine receptors appear to be specific since unilateral lesion of the nigrostriatal dopaminergic pathway did not affect cholinergic receptor-stimulated GTP-$\gamma$S binding to G proteins.

To explore whether changes in expression of dopamine receptors and their associated Go proteins were responsible for the heightened dopamine receptor-G protein interaction and the consequent enhanced dopamine receptor signaling, the levels of the dopamine receptors and Go proteins were assessed by receptor binding assays and by immunoblot analysis, respectively. The results show that although there was a 37% increase in D$_2$ dopamine receptor density, no changes in D$_1$ dopamine receptor or Go$_{olf}$ protein levels were observed in denervated striata. This is consistent with previous studies using similar animal models (Missale et al., 1989; Hamdi and Kostrzewa, 1991; Chalon et al., 1999; Araki et al., 2000). Thus, in the absence of changes in D$_1$ dopamine receptor density or in receptor-associated Go$_{olf}$ protein expression, the enhanced sensitivity of D$_1$ dopamine receptors in response to stimulation can be best explained by the increase in coupling of D$_1$ dopamine receptors to their Go$_{olf}$ proteins. On the other hand, supersensitivity of D$_2$ dopamine receptors appears to be mediated by increases in both D$_2$ dopamine receptor expression and D$_2$ dopamine receptor-Gi protein coupling. The conclusion that denervation leads to enhanced dopamine receptor-G protein coupling is strongly supported by the demonstration of 1) increased ratio of high/low-affinity D$_1$ dopamine receptor sites or an increase in total number of high-affinity D$_2$ receptors, which are thought to represent the G protein-coupled state of the receptors, and 2) heightened dopamine-induced increases in co-immunoprecipitation of [$^3$H]SCH23390 binding sites with Go$_{olf}$ and Go$_{11}$ proteins and of [$^3$H]raclopride binding sites with Gi proteins in the denervated striatum.

In summary, data presented here demonstrate that D$_1$ and D$_2$ dopamine receptor-mediated locomotor activity and receptor signaling are sensitized in the unilateral 6-OHDA-lesioned rat. This denervation-induced dopamine receptor functional augmentation is closely related to increased coupling of dopamine receptors with their associated G proteins. These results directly illustrate that the enhanced association of dopamine receptors with G proteins following denervation is a pivotal mechanism in the development of dopamine receptor supersensitivity and may provide a mechanism to explain enhanced dopaminergic function in Parkinson's disease or following chronic blockade of dopamine receptors. Because receptor-regulated cAMP production plays a critical role in the normal physiology of the basal ganglia (Greengard et al., 1999), understanding the mechanism that underlies modification of cAMP levels in pathological conditions or following repeated administration of drugs or of substances of abuse such as cocaine may facilitate the design of new therapeutic strategies that aim at alleviating conditions caused by hyperactive dopaminergic function.

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