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# Inhibition of Protein Tyrosine/Mitogen-Activated Protein Kinase Phosphatase Activity Is Associated with $D_2$ Dopamine Receptor Supersensitivity in a Rat Model of Parkinson's Disease

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## ABSTRACT

Previous work demonstrated that stimulation of $D_2$ dopamine receptors ($D_2$DRs) in the unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rat enhanced striatal extracellular signal-regulated kinase (ERK) activity ipsilateral to the lesion. The present work was designed to explore the mechanism underlying the activation of ERK in the denervated striatum. Stimulation of $D_2$DR induced a 60% inhibition in protein tyrosine phosphatase (PTP) activity but not in PSP activity in lesioned striata. The $D_2$DR antagonist spiperone blocked quinpirole-elicited PTP inhibition, and the $D_1$ receptor agonist 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine (SKF38393) did not inhibit PTP activity, indicating that PTP inhibition is a specific effect mediated by stimulation of $D_2$DR. We further discovered that striatal mitogen-activated protein kinase phosphatase (MKP), a protein phosphatase that is responsible for ERK dephosphorylation, is inhibited in response to $D_2$DR stimulation in 6-OHDA-lesioned rats. More specifically, MKP1 was identified to be the isozyme affected by $D_2$DR stimulation. In PC12 cells that express $D_2$DR, quinpirole elicited no change in PTP or MKP activity, whereas ERK was activated by $D_2$ dopamine receptor stimulation. The results indicate that 6-OHDA-induced striatal denervation leads to abnormal coupling between $D_2$DR and PTP/MKP pathway. Moreover, unilateral inhibition of striatal PTP by an intrastriatal injection of vanadate induced contralateral rotation in control rats in response to $D_2$DR stimulation, thus mimicking the response observed in the unilateral 6-OHDA-lesioned rat. The results indicate that attenuation of the PTP/MKP pathway may be responsible for the development of $D_2$DR supersensitivity.

Parkinson's disease (PD) is a neurodegenerative disease characterized by progressive loss of dopaminergic neurons in substantia nigra, pars compacta. An important aspect of the pathophysiology of PD is the development of supersensitivity of postsynaptic dopamine receptors to dopamine. Supersensitivity is considered to be a compensatory mechanism, i.e., an adaptive increase in responsiveness of striatal postsynaptic dopamine neurons to the neurotransmitter (Abrous et al., 1990; Agid, 1991; Schwarting and Huston, 1996; Bezard and Gross, 1998; Blandini et al., 2000). Development of supersensitivity not only contributes to functional abnormalities and clinical symptoms in PD but also may limit the therapeutic benefit of L-dopa because of its contribution to unacceptable motor and psychiatric side effects that occur in many patients (Bezard and Gross, 1998). Thus, understanding the mechanism of dopamine receptor supersensitivity has become critical for a complete understanding of the pathology of PD and in discovering and designing new therapeutic strategies for this disorder.

Selective lesion of the nigrostriatal pathway that follows the intracerebral injection of the neurotoxin 6-hydroxydopamine (6-OHDA) has been widely used as an animal model that mimics some aspects of the pathology of PD (Ungerstedt, 1968; Arnt and Hyttel, 1984; Blum et al., 2001). Unilateral striatal dopaminergic denervation elicited by this toxin results in an imbalance in dopaminergic transmission in basal ganglia output pathways in the two cerebral hemispheres that develops in response to ipsilateral postsynaptic dopamine receptor supersensitivity (Carey, 1986). The increase in striatal postsynaptic receptor activity may be due to an alteration in receptor density or in receptor-associated second messenger systems. Increased $D_2$ dopamine receptor binding

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**ABBREVIATIONS:** PD, Parkinson's disease; 6-OHDA, 6-hydroxydopamine; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; MKP, mitogen-activated protein kinase phosphatase; MBP, myelin basic protein; MEK, mitogen-activated protein kinase kinase; PTP, protein tyrosine phosphatase; PSP, protein serine/threonine phosphatase; *p*-NPP, *p*-nitrophenyl phosphate; PAGE, polyacrylamide gel electrophoresis; MAPK, mitogen-activated protein kinase; SKF38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine.

has been reported in both PD and in experimental models of PD (Pierot et al., 1988). However, in some studies the increase in the dopaminergic functional response was not always correlated with $D_2$ dopamine receptor up-regulation (Mileson et al., 1991), suggesting that other mechanisms may be involved in this process. An increase in $D_2$ dopamine receptor-Gi protein coupling in rat striata ipsilateral to a 6-OHDA lesion was demonstrated previously (Rubinstein et al., 1990; Radja et al., 1993; Butkerait et al., 1994), indicating that altered $D_2$ dopamine receptor-mediated intracellular signaling may underlie striatal denervation-induced hypersensitivity.

Protein phosphorylation and dephosphorylation play essential roles in regulation of neuronal activity and function. Aberrant protein serine/threonine and tyrosine phosphorylations have been shown to be associated with neurodegeneration (Bennecib et al., 2000; Zhen et al., 2001a). It is of interest that increased protein tyrosine phosphorylation was reported in rat striatum after a 6-OHDA lesion (Girault et al., 1992). Recently, we demonstrated that stimulation of $D_2$ dopamine receptors increased phosphorylation (activation) of striatal extracellular signal-regulated protein kinases (ERKs) ipsilateral to a 6-OHDA injection. It was further demonstrated that inhibition of ERK phosphorylation significantly attenuated $D_2$ dopamine receptor stimulation-mediated locomotor responses in 6-OHDA-lesioned rats (Cai et al., 2000), suggesting that altered phosphorylation plays a role in the development of dopaminergic receptor supersensitivity. However, the mechanism underlying the elevation in ERK phosphorylation in the denervated striatum is not clear. It has been suggested that the up-regulation of $D_2$ receptors and enhanced coupling of the receptor to Gi protein after 6-OHDA lesions may contribute to the aberrant activation of ERKs (Cai et al., 2000). It is known that MAP kinase phosphatase (MKP) plays an essential role in the regulation of MAP kinase activity. MKP is a dual-specificity protein phosphatase that inactivates phosphorylated ERKs by removal of phosphate groups from both tyrosine and/or threonine residues of phosphorylated ERKs (Keyse, 1998). The brain is enriched with MKPs (Stoker and Dutta, 1998). It is thus of interest to investigate whether the MKP pathway is altered after a 6-OHDA lesion and whether this contributes to the enhanced activation of ERKs in response to $D_2$ dopamine receptor stimulation. Herein, we report that stimulation of $D_2$ dopamine receptors selectively inhibits PTP and MKP in 6-OHDA-lesioned striata. Moreover, we found that $D_2$ receptor stimulation-mediated reduction in MKP activity is likely to be the responsible signaling event that mediates $D_2$ receptor supersensitivity and leads to contralateral rotation in the unilateral 6-OHDA-lesioned rat.

## Materials and Methods

**Materials.** Protein kinase A, ABI kinase, phosphatase assay kits, and anti-active ERK antibody were purchased from New England Biolabs (Beverly, MA). Myelin basic protein (MBP) and sodium vanadate were obtained from Sigma-Aldrich (St. Louis, MO). Quinpirole and SKF38393 were purchased from Tocris Cookson (Baldwin, MO). Okadaic acid, MEK, and ERK2 antibodies were purchased from Calbiochem (La Jolla, CA). Electrophoresis reagents were obtained from Bio-Rad (Hercules, CA). Anti-MKP1, MKP2, and MKP3, and horseradish peroxidase-linked secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). [$\gamma$-$^{32}$P]ATP (3000 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). Other reagents were purchased from standard laboratory suppliers

**Animal Surgery and Behavioral Assessment (Cai et al., 2000).** Male Sprague-Dawley rats weighing 175 to 200 g were purchased from Harlan (Indianapolis, IN). Animals were anesthetized with 50 mg/kg i.p. sodium pentobarbital and received a single stereotactic injection of 8 $\mu$g of 6-OHDA in 4 $\mu$l of artificial cerebrospinal fluid and 0.05% ascorbic acid into the mid-forebrain bundle using the following coordinates: AP, −2.5 mm; Lat, +2.0 mm; and Dev, −8.5 mm using bregma as the starting point. To limit damage to adrenergic neurons, 25 mg/kg desipramine-HCl was administrated i.p. 30 min before 6-OHDA.The success of the lesion was assessed by the number of contralateral rotations in response to an injection of 0.2 mg/kg s.c. apomorphine 3 weeks after surgery. Lesioned rats were placed in 50-cm bowls and acclimated to the environment for 30 min before apomorphine injection. Animals demonstrating fewer than 20 rotations/5 min were eliminated from further experiments. Dopamine levels were measured by high-performance liquid chromatography. Ipsilateral striatal dopamine levels were found to be less than 10% of the control side in all selected rats, indicating a severe loss of striatal dopaminergic nerve terminals. To assess responses to select stimulation of dopamine receptor subtypes, the specific $D_2$ dopamine receptor agonist quinpirole (1 mg/kg i.p.) or the $D_1$ receptor agonist SKF 38393 (5 mg/kg i.p.) was administered. Rats were killed by decapitation at designated times, striata were collected from both lesioned and control sides, and the samples were rapidly frozen in liquid nitrogen and stored at −80°C before use.

**Protein Tyrosine Phosphatase (PTP) Inhibitor Treatment.** To investigate the possible role of PTP in $D_2$ dopamine receptor supersensitivity, the selective PTP inhibitor $Na_3VO_4$ was used. Rats were anesthetized with inhaled halothane and received a single injection of 5 or 50 $\mu$mol in 5 $\mu$l of $Na_3VO_4$ or 5 $\mu$l of vehicle (phosphate-buffered saline buffer) directly into the left lateral dorsal striatum using the following coordinates: AP, −0.5 mm; Lat, +5 mm; and Dev, −5 mm. The protein serine/threonine phosphatase (PSP) inhibitor okadaic acid (5 nmol) was used as control. Behavioral studies were conducted 2 h after the intrastriatal injections. Contralateral rotations in response to a subcutaneous injection of 1 mg/kg quinpirole were assessed, and striatal tissues from both brain hemispheres were taken for PTP and PSP activity determinations.

**Lysate Preparation.** For immunoblot assays, frozen striata were homogenized in 2 ml of ice-cold 50 mM NaCl, 1 mM EGTA, 10 mM NaF, 1 mM $Na_3VO_4$, 40 mM $\beta$-glycerophosphate, 1 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, 10 $\mu$g/ml aprotinin and leupeptin, and 1% Nonidet P-40 (buffer A). The lysate was allowed to stand for 30 min on ice and was centrifuged at 12,000*g* for 15 min at 4°C. The protein content in the supernatant was determined by the Bradford assay using bovine serum album as standard. The supernatant was stored at −80°C before use. For the PTP or PSP assays, tissues were homogenized in buffer B containing 10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and proteinase inhibitors. The homogenates were extracted on ice for 30 min before centrifugation (12,000*g* at 4°C). Protein content in the supernatants was determined and aliquots of lysates were used for measurement of protein phosphatase activity or in the immunoprecipitation of MKPs (see below).

**Protein Phosphatase Activity Assays.** PTP and PSP in cellular or tissue lysates were assessed as described previously (Tonks, 1993; Zhen et al., 2001a) by measuring the release of $^{32}$P from labeled substrates. $^{32}$P-labled MBP was prepared by phosphorylating serine/threonine residues of MBP using protein kinase A or tyrosine residues by ABI kinase in the presence of [$\gamma$-$^{32}$P]ATP. Protein phosphatase activity assays were performed in 50 $\mu$l of buffer (50 mM Tris-HCl, pH 7.0, 1 mM EDTA, 5 mM dithiothreitol, 0.01% Brij 35, and 1 mg/ml bovine serum albumin) by incubating 5 $\mu$g of extract protein with $^{32}$P-labeled MBP. A titration assay indicated linear release of labeled $^{32}$P by protein tyrosine phosphatase in striatal

lysates with 0.5 to 10 $\mu$g of lysate protein tested for 10 min at 30°C. The reaction was terminated by adding 150 $\mu$l of ice-cold 20% trichloroacetic acid and was allowed to incubate on ice for 15 min. After centrifugation, the supernatant was subjected to liquid scintillation counting. Background phosphate release was determined by incubating $^{32}$P-labeled MBP in the absence of extract protein. A unit of phosphatase activity was defined as release of 1 nM phosphate from labeled substrate under standard conditions.

**MKP Activity Assay.** Total MKP activity was assessed as $^{32}$P released from labeled ERK2 in striatal lysates (Misra-Press et al., 1995). The substrate (phospho-ERK2) was prepared by incubating 10 ng of recombinant ERK2 with 0.1 $\mu$g of MEK in reaction buffer (25 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol, and 50 $\mu$M [$\gamma$-$^{32}$P]ATP at 30°C) for 30 min. The activation of ERK2 was confirmed by Western blots using anti-active ERK antibody. MKP activity assay was performed by incubating 10 ng of labeled ERK2 with 20 $\mu$g of striatal lysate protein or cell lysate in buffer containing 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 nM okadaic acid, and 2 nM inhibitor-2 for 20 min at 30°C. The reaction was terminated as described above, and released $^{32}$P was monitored by scintillation counting. Alternatively, reaction was stopped by adding 2× the volume of the sample buffer and boiling for 5 min. Samples were loaded onto 12% SDS-PAGE. Phospho-ERK was detected by Western blotting using anti-active ERK antibody. For analyses of activities of individual MKP isoforms, 200 $\mu$l of buffer (10 mM imidazole, pH 7.5, 0.1% $\beta$-mercaptoethanol, and 10 mM *p*-NPP) was added to MKP immunoprecipitate and the reaction was conducted at 30°C for 10 min. The reaction was stopped by the addition of 500 $\mu$l of 0.25 M NaOH and absorbance, measured at 410 nM, was obtained in a microplate reader (Bio-Rad, Hercules, CA). Nonspecific hydrolysis of *p*-NPP by lysates was assessed in nonimmune IgG immunoprecipitates and subtracted from the values obtained for enzyme immunoprecipitates (Brondello et al., 1999). In control experiments, Western blots of aliquots of the MKP1/MKP2 precipitates were conducted. These were probed with MKP1 or MKP2 antibodies to confirm the efficiency of MKP immunoprecipitation and to ensure that the same amount of MKP1/MKP2 was precipitated from either control or treated striata. The results indicated that the MKP antibodies efficiently immunoprecipitated the respective MKP and no differences were noted in the amounts of enzyme precipitated from control or lesioned strata.

**Immunoprecipitation and Immunoblotting.** Three hundred micrograms of supernatant proteins was incubated at 4°C with 3 $\mu$g of anti-MKP1, MKP2, or MKP3 antibody or with nonimmune IgG, respectively, for 2 h, followed by incubation with protein A/G PLUS for 1 h. Immunoprecipitates were washed three times with buffer B. The precipitates were used to measure MKP activity. For immunoblotting, samples were size-separated on 12% SDS-PAGE and transferred to nitrocellulose membranes. The respective proteins were detected after incubation with anti-MKP or with other antibodies for 2 h or overnight followed by incubating the membranes with 1:10,000 dilution of a secondary antibody for 1 h. The signals were visualized with enhanced chemiluminescence (Supersignal Western Blot Detection; Pierce Chemical, Rockford, IL) and exposed to X-ray film.

**Cell Culture and Transfection.** PC12 cells were cultured in RPMI 1640 medium supplemented with 10% horse serum, 5% fetal calf serum, 50 pg/ml streptomycin, and 50 U/ml penicillin. The cells were transfected with DNA when they reached 40 to 60% confluence. Lipofectin was mixed with an equal volume of plasmid DNA coding the $D_2$ dopamine receptor in buffer containing 10 mM Tris, 1 mM EDTA, pH 8.0, and allowed to stand for 20 min at room temperature. Cells were incubated with the DNA-Lipofectin mixture for 24 h. The DNA-containing medium was removed and replaced with 3 ml of RPMI 1640 medium supplemented with 20% fetal bovine serum and incubated for additional 24 h. The cells were then cultured in low-serum medium for 2 h before treatment with the $D_2$ receptor agonist.

**Data Analysis.** Two-tailed analysis of variance followed by the Newman-Keuls test was used in the analyses of the data. Significance was considered at $p < 0.05$.

## Results

**Selective Inhibition of Striatal PTP Activity by $D_2$ Dopamine Receptor Stimulation.** Successfully lesioned rats received an injection of the $D_2$ dopamine receptor agonist quinpirole at various times before sacrifice and total striatal PTP activity was measured. As shown in Fig. 1, PTP

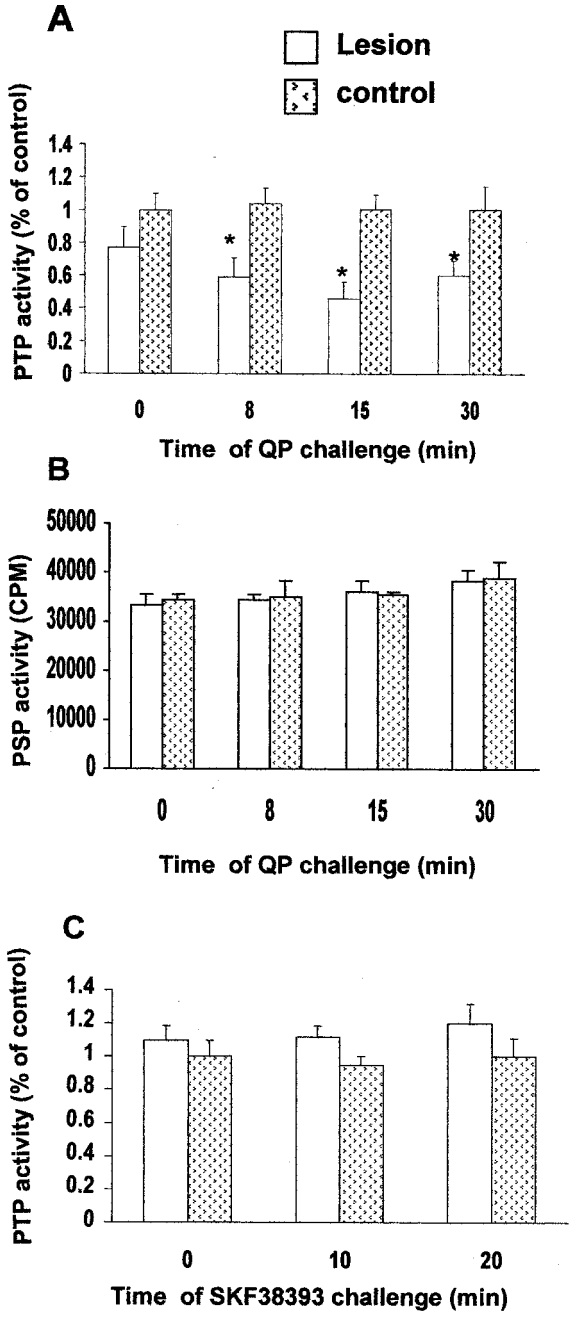


**Fig. 1.** $D_2$ dopamine receptor agonist administration selectively reduced PTP activity in lesioned striatum. Unilateral 6-OHDA-lesioned rats were injected subcutaneously with vehicle (saline), 1 mg/kg quinpirole, or 5 mg/kg SKF38393. Animals were decapitated at the indicated times after injection, and striata from both hemispheres were removed. Striatal lysates were prepared and PTP or PSP activity was assayed. A, striatal PTP activity in response to quinpirole (QP) stimulation. B, striatal PSP activity in response to QP. C, striatal PTP activity in response to SKF38393. Data are expressed as mean ± S.E. of at least five animals in each experimental condition. QP: quinpirole. *, $p < 0.01$, compared with PTP activity in the respective intact striata.

activity on the lesioned side was inhibited ($p < 0.01$) in response to receptor stimulation. The specificity of the PTP assay was tested by responses to 100 $\mu$M vanadate or 1 $\mu$M okadaic acid. The selective PTP inhibitor $Na_3VO_4$ abolished the dephosphorylation of [$^{32}$P]tyrosine-labeled MBP, whereas okadaic acid did not affect dephosphorylation (data not shown). It was also noted that PTP activity, in intact striata, was not altered significantly by the injection of quinpirole. Furthermore, $D_2$ dopamine receptor stimulation exhibited no significant effect on PSP activity either on the lesioned or the intact sides. Stimulation of $D_1$ dopamine receptors with the specific receptor agonist SKF38393 did not significantly change striatal PTP activity (Fig. 1C), suggesting that the quinpirole-elicited inhibition of PTP in lesioned striata is selective for $D_2$ dopamine receptor stimulation. Indeed, pretreatment of 6-OHDA-lesioned rats with spiperone, a selective $D_2$ dopamine receptor antagonist, attenuated quinpirole-mediated PTP inhibition in denervated striata (Fig. 2). As expected, spiperone pretreatment also blocked quinpirole-mediated contralateral rotations (data not shown; Cai et al., 2000). Taken together, the data indicate that stimulation of $D_2$ dopamine receptors selectively inhibits striatal PTP activity ipsilateral to a unilaterally 6-OHDA lesion.

**Stimulation of $D_2$ Dopamine Receptors Enhances Striatal Tyrosine Phosphorylation in 6-OHDA-Lesioned Rats.** Because a change in striatal PTP activity may be expected to result in altered protein tyrosine phosphorylation, we tested whether the level of phosphorylated tyrosine was changed in response to $D_2$ receptor stimulation. Basal levels of phosphotyrosine were not significantly altered in unstimulated denervated striata (Fig. 3, left). However, $D_2$ dopamine receptor stimulation elevated phosphotyrosine levels in denervated striata. Enhanced tyrosine phosphorylation was found in proteins with the following molecular masses: ~180, ~120, and ~40 kDa. Using antibodies that recognize active MAPK, we identified one of these protein bands to be phosphorylated ERK1/2 (Fig. 3, right). However, phospho-p38 MAPK was not altered. Considering the fact that $D_2$ dopamine receptor stimulation inhibits PTP activity (Fig. 1), it is conceivable that the elevation in phosphotyrosine (Fig. 3) that is induced by receptor stimulation in denervated striata is the result of PTP inhibition.

**Stimulation of $D_2$ Dopamine Receptors Inhibits Striatal MKP Activity in the Unilateral 6-OHDA-Lesioned Rat.** We have observed that $D_2$ receptor stimulation increases ERK phosphorylation in lesioned striata, a finding that is in accord with a decline in PTP activity (Fig. 3; Cai et al., 2000). It is conceivable that a change in MKPs, a family of dual-specificity protein tyrosine phosphatases that is responsible for dephosphorylating and inactivating ERK, may contribute to the elevation of phosphorylated ERK in the present context. We, therefore, compared total MKP activity in lesioned and intact rat striata. Striatal MKP activity, ipsilateral to the lesion, was significantly inhibited in response to $D_2$ dopamine receptor stimulation. The activity of MKP in the intact striatum, however, was not altered by stimulation of $D_2$ dopamine receptors (Fig. 4A). The dephosphorylation of phospho-ERK2 was confirmed by Western blotting with the anti-active ERK antibody (Fig. 4B). Stimulation of $D_1$ dopamine receptors with the selective agonist SKF38393 (5 mg/kg), which was shown to induce contralateral rotations (Cai et al., 2000), did not alter MKP activity (data not shown). The results, therefore, indicate that reduced MKP activity in response to $D_2$ dopamine receptor stimulation may, at least in part, be responsible for the decline in total PTP activity that was noted in lesioned striata (Fig. 1). Because there are multiple MKP isoforms, we attempted to identify the specific isoform that mediates the response to $D_2$ dopamine receptor stimulation. Western blot analyses indicated that MKP-1 and MKP-2 are the major MKP forms expressed in striatum, whereas MKP-3 was below the detection limit (Fig. 5A). In agreement with the protein expression data, the enzymatic activities that immunoprecipitated with the MKP isoforms indicated that MKP-1 represents the majority of striatal MKP activity. Moreover,

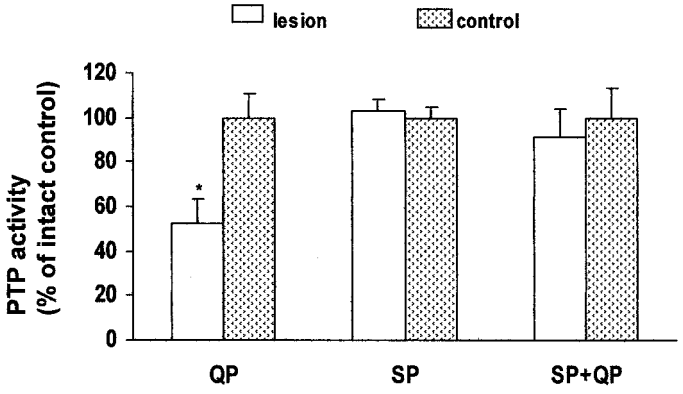


**Fig. 2.** Quinpirole-induced inhibition of PTP in 6-OHDA-lesioned striatum is mediated by the $D_2$ dopamine receptor. Unilateral 6-OHDA-lesioned rats were injected intraperitoneally with vehicle or 2 mg/kg spiperone (SP) 30 min before the subcutaneous administration of 1 mg/kg quinpirole (QP). QP-mediated rotational behavior was recorded for a 5-min period, starting 10 min after QP injection, animals were killed, and striatal PTP activity was measured. Data are expressed as mean ± S.E. of at least five animals in each experimental condition. *, $p < 0.01$, compared with PTP activity in the respective intact striata.

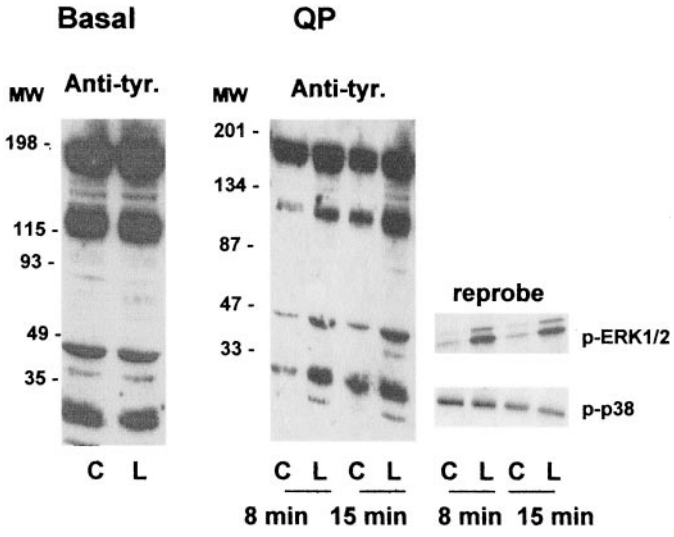


**Fig. 3.** $D_2$ dopamine receptor stimulation enhanced protein tyrosine phosphorylation in the denervated striatum. Unilateral 6-OHDA-lesioned rats were injected subcutaneously with 1 mg/kg quinpirole (QP) 0, 8, or 15 min before decapitation. Striata from both hemispheres were removed and 40 $\mu$g of striatal lysate proteins were separated on 10% SDS-PAGE and transferred to nitrocellulose membranes and probed with anti-phosphotyrosine antibody (PY 20). Some membranes were reprobed with anti-active p38 MAPK or anti-active ERK antibodies. The experiments were repeated at least three times with similar results. A representative blot is shown. C, control striatum; L, lesioned striatum.

we detected a significant decrease in MKP-1 activity in lesioned striata in response to $D_2$ dopamine receptor stimulation (Fig. 5B). This is accompanied by an increase in tyrosine phosphorylated ERKs (Figs. 1 and 3), suggesting that reduced MKP activity contributes to the elevation in phosphorylated ERKs.

**Stimulation of $D_2$ Dopamine Receptors Does Not Elicit PTP or MKP Inhibition in $D_2$ Dopamine Receptor-Expressing PC12 Cells.** The absence of a change in PTP or MKP activity in response to $D_2$ dopamine receptor stimulation in the intact striatum suggests that, under physiological conditions, the $D_2$ dopamine receptor does not modulate PTP or MKP activity. This possibility was tested in PC12 cells that transiently express $D_2$ dopamine receptors. Stimulation of these receptors with quinpirole for up to 60 min did not affect MKP activity although activation of ERK was clearly observed (Fig. 6). Similarly, total PTP activity in the cells was also unchanged by the treatment (data not shown). These, in vitro, data also suggest that $D_2$ dopamine receptors do not regulate MKP or PTP activity under normal conditions, although the receptor couples to the ERK pathway.

**Unilateral Striatal Inhibition of PTP Elicited by a Direct Intrastriatal Injection of Vanadate Induces Contralateral Turning in Control Rats Injected with Quinpirole.** We have noted that stimulation of $D_2$ dopamine receptors inhibits striatal PTP/MKP in 6-OHDA-lesioned rats. This change in PTP/MKP activity, ipsilateral to the lesion, is correlated with increases in striatal tyrosine phosphorylation and with ERK activation that is associated with $D_2$ dopamine receptor-mediated contralateral rotations

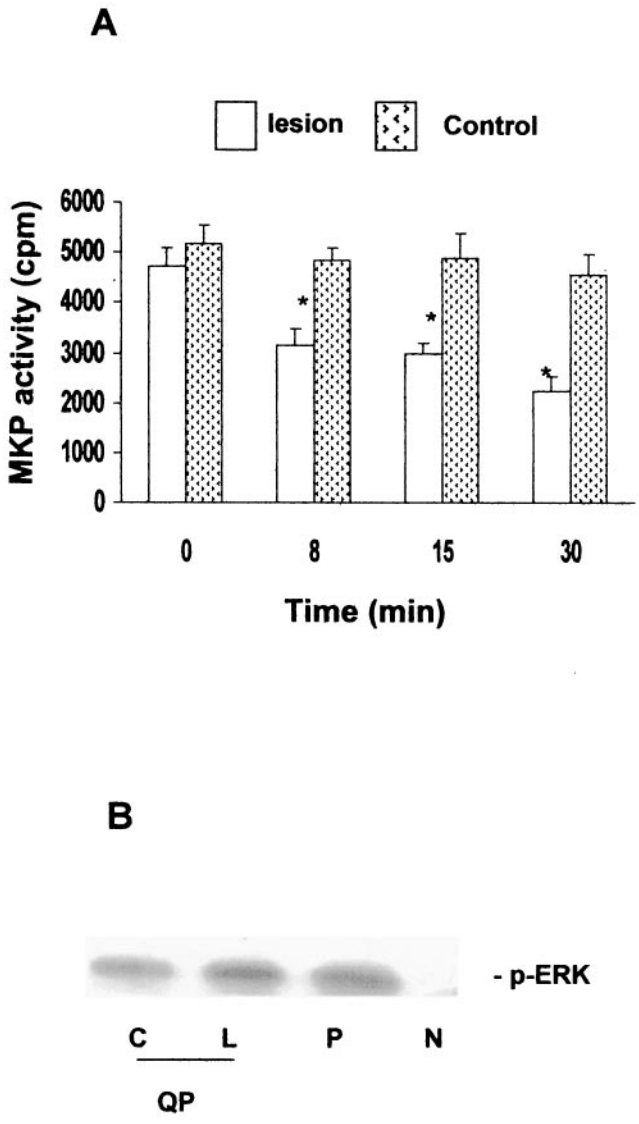


**Fig. 4.** $D_2$ dopamine receptor stimulation decreased MKP activity in 6-OHDA-lesioned striata. Unilateral 6-OHDA-lesioned rats were challenged with quinpirole (QP) and killed 8, 15, or 30 min later. SKF38393 was injected 15 min before killing. Striatal MKP activity was assessed as the $^{32}P$ released from phospho-ERK. A, MKP activity modulated by QP. Data expressed as mean ± S.E. were obtained from four to six rats per group. *, $p < 0.01$, compared with intact control. B, MKP activity reactions were stopped by the addition of 2× sample buffer and the mixture was boiled for 5 min. Sample proteins were separated on 12% SDS-PAGE and the proteins transferred to nitrocellulose membranes and probed with anti-active ERK. A representative blot of three independent experiments is shown. C, intact striatum; L, lesioned striatum; P, positive control (in absence of striatal extracts); N, negative control (in absence of labeled substrate).

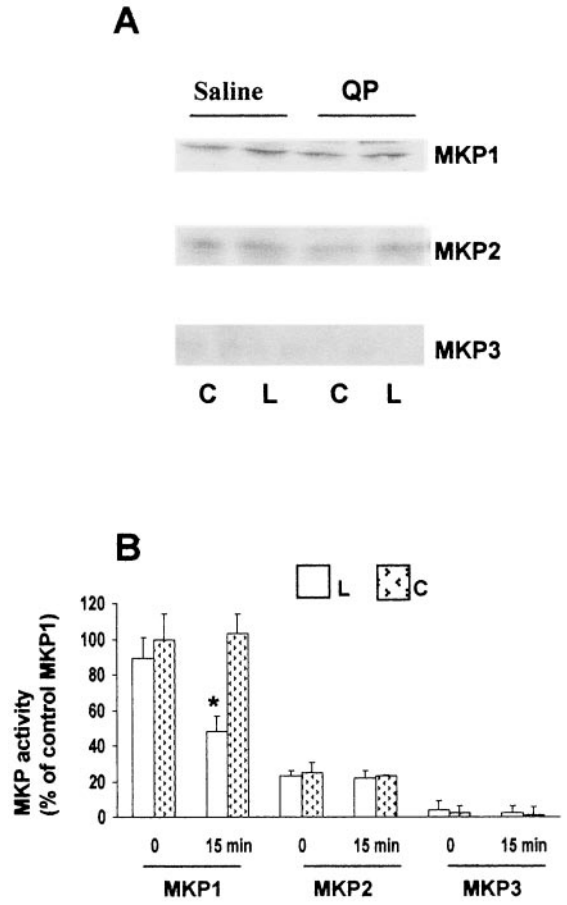


**Fig. 5.** MKP1 activity is regulated by $D_2$ dopamine receptors in 6-OHDA-lesioned rats. Rats were injected with vehicle or quinpirole (QP), and striata were obtained 15 min after the injection. Expression of striatal MKP in tissue lysates (20 $\mu g$/lane) was assessed by Western blotting using anti-MKP antibodies (1:1000). A representative result is shown in A. The experiments were repeated in tissues obtained from three to four animals with similar results. C, intact striatum; L, lesioned striatum. The activities of specific MKP isozymes were assayed in immunoprecipitates of MKP1, MKP2, or MKP3 using *p*-NPP as substrate. The data summarized in B were obtained from four to six rats for each condition and are expressed as mean ± S.E. *, $p < 0.01$, compared with intact control.

(Figs. 1 and 3; Cai et al., 2000). These findings lead us to hypothesize that inhibition of PTP/MKP activity may be the primary mechanism responsible for $D_2$ dopamine receptor-elicited protein tyrosine phosphorylation, ERK activation, and ultimately, receptor supersensitivity in the 6-OHDA-denervated rat. If this is the case, direct inhibition of striatal PTP/MKP unilaterally should elicit contralateral turning in response to $D_2$ receptor stimulation in intact control rats. This postulate was tested in rats that were injected intrastriatally with vanadate on the left side 2 h before a challenge injection of quinpirole or SKF38393. As shown in Fig. 7A, quinpirole elicited contralateral rotations in animals that received a unilateral intrastriatal injection of the PTP inhibitor. As expected, these animals exhibited an elevation in striatal phospho-ERK (Fig. 7B) and an inhibition of striatal PTP activity (Fig. 7C). In contrast, unilateral inhibition of protein serine/theronine phosphatases with okadaic acid in control rats exhibited no turning in response to quinperole, and administration of the $D_1$ dopamine receptor agonist SKF38393 did not induce significant contralateral rotations in vanadate-treated control rats (data not shown). These results indicate that inhibition of striatal PTP/MKP enhances $D_2$ dopamine receptor-mediated ERK activation, which, in turn, leads to contralateral rotation in the lesioned rat. The data therefore, imply that an intrastriatal remodeling occurs in response to dopamine neuron denervation, which results in an abnormal coupling between $D_2$ dopamine and the PTP/MKP pathway.

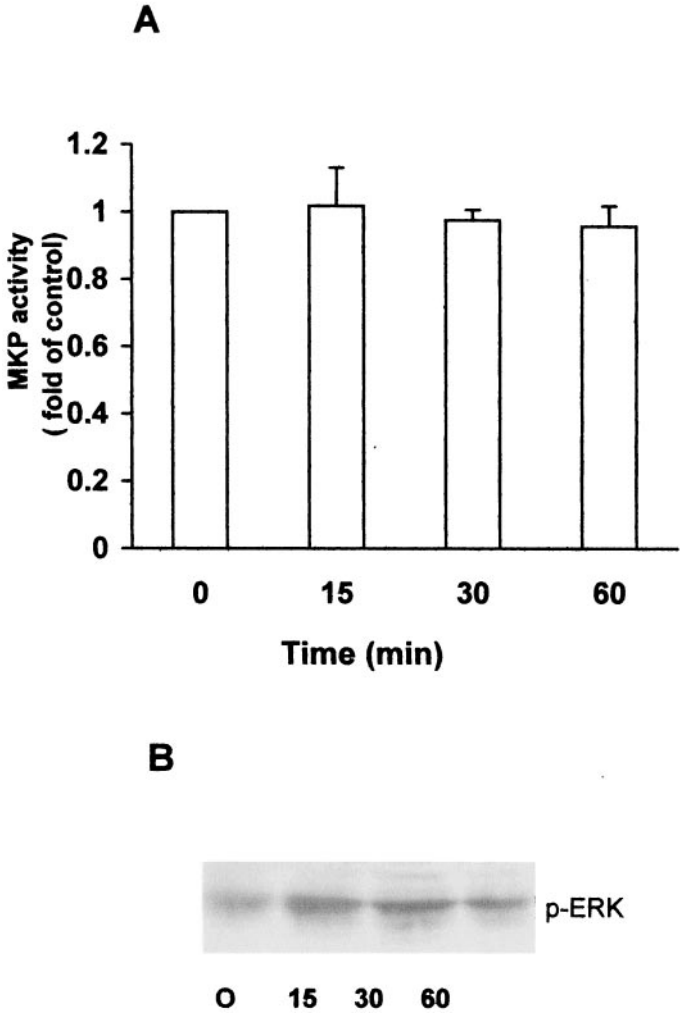


**Fig. 6.** Stimulation of $D_2$ dopamine receptors does not change MKP activity in $D_2$ dopamine receptor-expressing PC12 cells. PC12 cells that transiently express $D_2$ dopamine receptors were stimulated with 1 $\mu$M quinpirole (QP) for 15 to 60 min. Cells were collected, lysed, and 20 $\mu$g of lysate proteins was incubated with substrate (labeled-active ERK2) as described under *Materials and Methods*. MKP activity is expressed in fold stimulation of the activity obtained in control, unstimulated cells. Data presented are the summary of three experiments (A). For D2 receptor-mediated activation of ERKs, 40 mg of lysate protein was loaded onto 12% SDS-PAGE gel and blotted with anti-phospho ERK antibody. A representative blot of three independent experiments that yielded similar results is shown (B).

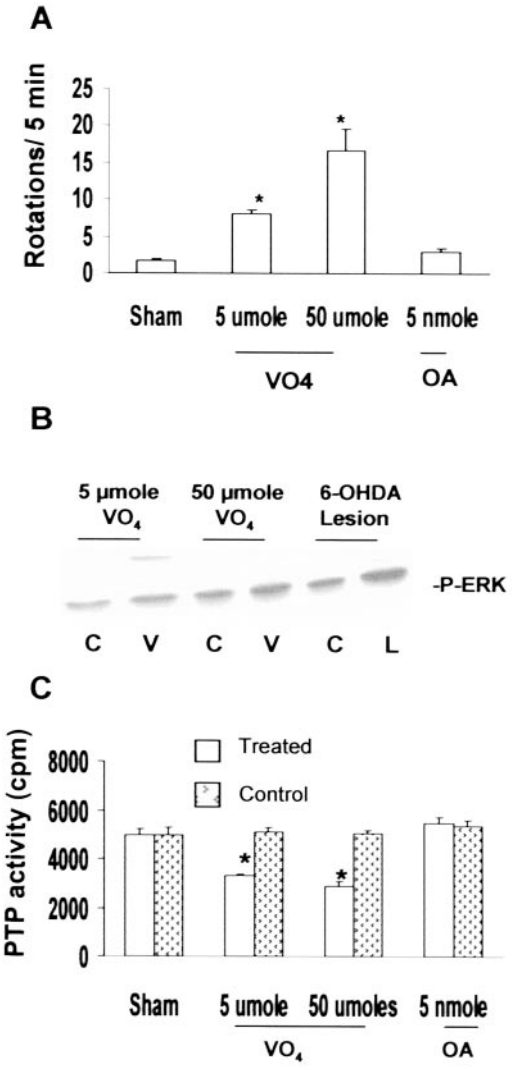


**Fig. 7.** Inhibition of PTP by unilateral intrastriatal injection of vanadate induces contralateral turning in response to $D_2$ dopamine receptor stimulation in control rats. Control rats received intrastriatal injections, aimed to the right hemisphere, of vehicle (sham), 5 or 50 $\mu$mol of $Na_3VO_4$ ($VO_4$), or 5 nmol of okadaic acid (OA). Two hours after injection, the rats were challenged with 1 mg/kg quinpirole and contralateral rotations were recorded for 5 min starting 10 min postdrug. A, data are the summary obtained form at least five animals in each group. *, $p < 0.01$, compared with sham control. B, 40 $\mu$g of striatal lysate proteins was separated on 12% SDS-PAGE, and the proteins were transferred to a nitrocellulose membrane and probed with anti-active ERK antibody. A representative blot is shown. The experiment was repeated in tissues from four animals per group and similar results were observed. C, intact striatum; V, vanadate-treated striata. C, PTP activity in striata. PTP activity was analyzed as described under *Materials and Methods*. Data are expressed as mean ± S.E. ($N$ = 5–6). *, $p < 0.01$, compared with the control side.

## Discussion

The present results demonstrate that stimulation of $D_2$ dopamine receptors in the unilateral 6-OHDA-lesioned/"hemi-parkinsonian" rat model induces selective inhibition of striatal PTP activity ipsilateral to the lesion. Blockade of $D_2$ dopamine receptors prevents the receptor-mediated inhibition of PTP. This receptor stimulation-elicited decrease in PTP activity is correlated with enhanced levels of phosphotyrosine in striatal proteins. Furthermore, we found that MKP1 activity is inhibited by $D_2$ dopamine receptor stimulation. In addition, direct inhibition of striatal PTP via unilateral intrastriatal injection of $Na_3VO_4$ in control rats induced ERK activation and contralateral turning after systemic injection of a $D_2$ dopamine receptor agonist, a pattern that is similar to that observed in 6-OHDA-lesioned rats. The results, therefore, indicate that inhibition of PTP/MKP activity may be a primary event in the emergence of $D_2$ dopamine receptor supersensitivity after dopamine neuronal denervation.

Supersensitivity of dopamine receptors represents a state of enhanced physiological or behavioral responsiveness to receptor stimulation (Kostrzewa, 1995). This phenomenon is particularly relevant to the pathophysiology of Parkinson's disease and to its pharmacotherapy. Animals with unilateral 6-OHDA lesions of the nigrostriatal dopamine system develop supersensitivity of ipsilateral postsynaptic dopamine receptors. This is expressed as contralateral turning behavior when the animal is challenged with a dopamine receptor agonist. Such a lesion leads to an increase in $D_2$ dopamine receptor density that is generally thought to underlie supersensitivity of this receptor. However, the development of $D_2$ receptor supersensitivity is not always associated with upregulation of receptors and recent reports have suggested that a change in intracellular signaling cascades contributes to the enhanced sensitivity of the $D_2$ dopamine receptor (Mileson et al., 1991; Sandstrom and Bruno, 1997; Bezard and Gross, 1998). In recent studies, it has been demonstrated that MAP kinase pathways play an important role in $D_2$-like dopamine receptor signaling (Yan et al., 1999; Zhen et al., 2001b). We have further shown that stimulation of $D_2$ dopamine receptors induces an activation of ERK in striata of 6-OHDA-lesioned rats. This abnormal activation of ERK was shown to be associated with $D_2$ dopamine receptor-mediated contralateral turning (Cai et al., 2000). It is well know that activation of ERKs requires the dual phosphorylation of tyrosine and threonine residues in ERK by MEK, whereas inactivation of ERKs is mediated by MKP. Multiple isoforms of MKP are expressed in the brain and these may be differentially regulated under pathological and physiological conditions (Wiessner et al., 1995; Stoker and Dutta, 1998; Winter et al., 1998). An imbalance between MEK and MKP activities may result in an abnormal phosphorylation state of ERK (Keyse, 1998). Impairment in ERK dephosphorylation via inhibition of PTP/MKP1 activity as noted in the present communication may, therefore, result in enhanced phosphorylation of ERKs that ultimately mediates the increase in responsiveness of $D_2$ dopamine receptors in the denervated striatum of the rat. Thus, $D_2$ receptor-mediated reduction in PTP/MKP activity seems to be an important signaling event that underlies denervation-mediated receptor supersensitization in the $D_2$ dopamine receptor system. This formulation is supported by the following findings: 1) administration of the $D_2$ dopamine receptor agonist quinpirole to 6-OHDA-lesioned rats induced inhibition of striatal PTP and MKP1 activities, 2) the time course of the inhibition of MKP1 is well correlated with elevation in striatal phospho-ERKs (Cai et al., 2000), and 3) in the intact striatum, stimulation of $D_2$ dopamine receptors did not elicit changes in PTP or MKP1 activity. Similarly in PC12 cells that express $D_2$ receptor, stimulation induced ERK activation without any change in PTP/MKP (Figs. 1, 4, and 6), and most significantly, 4) contralateral turning was observed in response to $D_2$ dopamine receptor stimulation after a unilateral intrastriatal injection of a selective PTP inhibitor in control intact rats. However, with regard to the last point, it should be noted that the results of the experiment with the PTP inhibitor are not conclusive due to the fact that $Na_3VO_4$ may have other actions. Notwithstanding, this caveat, the results provide the first evidence that alteration of PTP/MKP activity plays an important role in $D_2$ dopamine receptor-mediated behavioral sensitization.

Although $D_2$ dopamine receptor stimulation alters PTP/MKP1 activity in 6-OHDA-denervated striata (Figs. 1 and 4), the activities of the enzymes were not changed either in intact striata or in $D_2$ dopamine receptor-expressing PC12 cells. It thus seems that under basal conditions, $D_2$ dopamine receptors do not regulate PTP or MKP activity. At the present time, we do not know how PTP and MKP are engaged by the $D_2$ dopamine receptor in the denervated striatum. It seems that intracellular changes, which emerge after denervation of the striatum, facilitate the coupling between $D_2$ dopamine receptors and the PTP/MKP pathway; this coupling seems to be absent under normal physiological conditions. Thus, denervation-induced remodeling of signaling pathways seems to contribute to $D_2$ dopamine receptor stimulation-mediated activation of ERK and to the supersensitivity of this receptor.

The MKPs are inducible early response genes that are under the control of factors such as stress and ischemia (Wiessner et al., 1995; Winter et al., 1998; Soriano et al., 2000). Whether changes in MKP transcriptional processing play a role in $D_2$ receptor-mediated MKP inhibition remains unknown. Moreover, the expression of MKP is regulated by the ubiquitin-directed proteasome complex (Brondello et al., 1999), and recent studies have suggested that an alteration of proteasome activity may contribute to the pathology of Parkinson's disease (Chung et al., 2001; Sequeira, 2001). Whether 6-OHDA alters the MKP degradation system and how such changes contribute to the observed inhibition of MKP1 that follows $D_2$ dopamine receptor stimulation in the denervated striatum remain unknown.

The present findings indicate that unilateral inhibition of striatal PTP by vanadate mimics the behavioral changes that occur in 6-OHDA-denervated rats. In contrast, unilateral inhibition of serine/threonine phosphatase did not induce rotations in response to $D_2$ receptor stimulation (Fig. 7), indicating that tyrosine phosphorylation is selectively involved in $D_2$ receptor-mediated rotational behavior. Although the magnitude of the rotational response in vanadate-treated rats was not as high as that in 6-OHDA-lesioned rats in response to $D_2$ receptor stimulation (Fig. 7; Cai et al., 2000), it seems to correlate with the magnitude of striatal MKP inhibition (Figs. 1 and 7C). Alternatively, this differ-

ence may be due to the fact that vanadate is a nonselective MKP inhibitor, whereas the $D_2$ receptor-mediated effect in the 6-OHDA-lesioned animal is selective for the MKP1 isoform. Notwithstanding this small difference, the collective data clearly indicate that regulation of protein tyrosine phosphorylation plays an essential role in $D_2$ dopamine receptor supersensitivity in the 6-OHDA-lesioned rat that is an animal model that mimics some of the pathology of Parkinson's disease. Therefore, the present findings provide novel insights for the understanding of dopamine receptor supersensitivity and may suggest new approaches for the development of novel treatments for Parkinson's disease.

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