Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 311 (2003) 49–54
BBRC
www.elsevier.com/locate/ybbrc

1-Methyl-4-phenylpyridinium-induced down-regulation of dopamine transporter function correlates with a reduction in dopamine transporter cell surface expression

Jaturaporn Chagkutip,[a,c] Roxanne A. Vaughan,[b] Piyarat Govitrapong,[a,c] and Manuchair Ebadi[a,*]

[a] *Department of Pharmacology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, North Dakota 58202, USA*
[b] *Biochemistry and Molecular Biology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, North Dakota 58202, USA*
[c] *Neuro-Behavioural Biology Center, Institute of Science and Technology for Research and Development, Mahidol University, Salaya, Nakornpathom 73170, Thailand*

Received 12 September 2003

Abstract

The mechanisms whereby 1-methyl-4-phenylpyridinium (MPP^+) mediates cell death and Parkinsonism are still unclear. We have shown that dopamine transporter (DAT) is required for MPP^+-mediated cytotoxicity in HEK-293 cells stably transfected with human DAT. Furthermore, MPP^+ produced a concentration- and time-dependent reduction in the uptake of [^{3}H]dopamine. We observed a significant decrease in [^{3}H]WIN 35428 binding in the intact cells with MPP^+. The saturation analysis of the [^{3}H]WIN 35428 binding obtained from total membrane fractions revealed a decrease in the transporter density (B_{max}) with an increase in the dissociation equilibrium constant (K_d) after MPP^+ treatment. Furthermore, biotinylation assays confirmed that MPP^+ reduced both plasma membrane and intracellular DAT immunoreactivity. Taken together, these findings suggest that the reduction in cell surface DAT protein expression in response to MPP^+ may be a contributory factor in the down-regulation of DAT function while enhanced lysosomal degradation of DAT may signal events leading to cellular toxicity.

Keywords: MPP^+; Dopamine transporter; Dopamine uptake; Biotinylation; WIN 35428 binding; Lysosomal degradation; Neurotoxicity; Parkinson's disease

Methyl-4-phenylpyridinium (MPP^+), the active metabolite of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), mediates selective damage to dopaminergic neurons and has been widely used to generate a model of Parkinson's disease. However, the mechanisms of the neurotoxic action of MPP^+ are not fully understood. MPP^+ is transported into cells via the dopamine transporter (DAT) where it mediates cellular toxicity [1–3]. DAT is a presynaptic plasma membrane protein responsible for the regulation of extracellular dopamine levels and termination of its action by mediating the reuptake of dopamine [4,5]. Functional impairment of DAT alters many physiological and behavioral processes that are mediated by dopamine. A dysfunction of dopamine transmission could consequentially interrupt motor neural circuits which control movement as seen in Parkinson's disease (PD).

Recently it has been shown that cellular mRNAs encoding DAT and vesicular monoamine transporters are decreased in PD [6,7]. Indeed, an alteration of dopaminergic neurotransmission by the modulation of DAT activity could have an important implication in the cellular events which lead to PD and could be a target for potential therapeutic intervention.

When MPP^+ enters into the cells, it causes the release of dopamine from secretory vesicles and subsequently generates free radicals [8–11]. Inside the cell, MPP^+ disrupts cellular respiration by inhibiting mitochondrial complex I system [12,13], reducing the level of ATP, and

* Corresponding author. Fax: 1-701-777-4158.
E-mail address: mebadi@medicine.nodak.edu (M. Ebadi).

0006-291X/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2003.09.155

hence contributing to degeneration of dopaminergic neurons.

Several studies have shown that MPP^+ causes a significant decrease in the activity of DAT [14–17]. However, it remained unclear whether the decrease in DAT activity resulted from the selective uptake of MPP^+ through DAT, reduced dopaminergic neurons mediated by the toxin, or changes in the trafficking of the transporter molecules. In the present study, we have hypothesized that a reduction in cell surface expression of DAT may be an underlying mechanism for the down-regulation of DAT function by MPP^+ and subsequent neurotoxicity. To test this hypothesis, we selected HEK-293 cells, which stably express human DAT in order to monitor specially the effects of MPP^+ on DAT function. The data indicate that MPP^+ significantly reduced cell viability, dopamine uptake, and cell surface expression of DAT, thus correlating directly the neurotoxic effects of MPP^+ with alterations in the kinetic parameters of dopamine transporters.

Materials and methods

Cell line and culture. HEK-293 cells stably transfected with cDNA for the human dopamine transporter (hDAT) were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% fetal bovine serum at 37 °C and 5% CO_2. Cells were grown in monolayer cultures for 24–48 h until confluent. Before all assays, cells were thoroughly washed to remove residual MPP^+ after the completed incubation times.

Cell viability assay. The treated cells were plated in a 96-well plate and processed with the 3-(4,5-dimethylthizol-2-yl)-2,5-diphenyltetrazolium bromide reagent (MTT; Sigma) to measure the activity of mitochondrial dehydrogenase enzymes which cleave the tetrazolium ring to produce formazan [18]. MTT prepared in phosphate-buffered saline (PBS) at 5 mg/ml was diluted 10-fold in the culture medium and incubated at 37 °C for 4 h. After incubation, the MTT medium was removed; 100 μl of 0.04 N HCl in isopropanol was added to each well for 15 min in a dark area. The amount of solubilized MTT formazan product was determined by spectrophotometry at 540 nm.

[^{3}H]Dopamine uptake assay. The treated cells were suspended in Krebs bicarbonate buffer (20 mM $NaHCO_3$, 122 mM NaCl, 0.5 mM Na_2HPO_4, 4.8 mM KCl, 1.2 mM $MgSO_4$, 0.2 μg/ml ascorbic acid, 2 mg/ml glucose, and 100 μM pargyline, pH 7.4) and allowed to equilibrate (approximately 75 μg protein was added to each assay tube). The aliquoted cells were then incubated with 20 nM [^{3}H]dopamine (68 Ci/mmol; Amersham, Piscataway, NJ, USA) for 10 min at 37 °C. Non-specific uptake was determined by adding 10 μM nomifensine to the incubation solution before adding radioactive dopamine. The uptake was terminated by rapid vacuum filtration over Whatman GF/C. The cells were washed on the filter rapidly three times with 3 ml of Krebs bicarbonate buffer. Accumulate radioactivity was determined by liquid scintillation counting.

Radioligand binding assay. To assess changes in the density of hDAT in HEK-293 cells, binding assays were carried out on intact cells and membrane fractions using [^{3}H]WIN 35428 (85 Ci/mmol; Dupont-NEN, Boston, MA) as radioligand. Radioligand binding for total cell membrane was carried out as described previously [19]. After treatment, culture medium was removed, washing twice with 5 ml phosphate-buffered saline (PBS), followed by adding 4 ml lysis buffer (2 mM Hepes, 1 mM EDTA) for 10 min at 0 °C. The lysed cells were scraped, centrifuged at 31,000*g* for 20 min, resuspended in 500 μl of Krebs bicarbonate buffer followed by sonication for 5 s, and used immediately. The treated cells were resuspended in Krebs bicarbonate buffer containing 3 nM [^{3}H]WIN 35428 as final concentration and a concentration range of 1–12 nM for saturation assays. Non-specific binding was determined by adding 10 μM nomifensine to the incubation solution before adding the radioactive labeled dopamine. Tubes were incubated for 2 h at 4 °C and the incubations were terminated by rapid vacuum filtration over Whatman GF/C. The filters were rinsed three times with 3 ml of ice-cold buffer. Accumulated radioactivity was determined by liquid scintillation counting. Estimated dissociation equilibrium constant (K_d) and a transporter density (B_{max}) were determined from non-linear plots of the binding data and Scatchard linear transformation plots using Prism 3.0 from Graph Solfware, San Diego, CA.

Cell surface biotinylation and immunoblotting. Confluent cells after treatment were washed three times with 1 ml of ice-cold calcium- and magnesium-supplemented PBS (Ca/Mg-PBS, 138 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 9.6 mM Na_2HPO_4, 1 mM $MgCl_2$, and 0.1 mM $CaCl_2$, pH 7.3). They were then incubated with Sulfo-NHS-biotin solution (1.5 mg/ml, Pierce Chemical, Rockford, IL) in Ca/Mg-PBS for 1 h at 4 °C with agitation. Free sulfo-NHS-biotin was removed by washing with ice-cold 0.1 M glycine in 1 ml Ca/Mg-PBS twice. The reaction was further quenched by incubation with 100 mM glycine for 30 min after which the cells were washed with Ca/Mg-PBS three times. Biotinylated cells were lysed in 0.5 ml radioimmunoprecipitation assay buffer, (10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, and 1% sodium deoxycholate), supplemented with a protease inhibitor mixture tablet (Roche Diagnostics) for 1 h at room temperature with gentle shaking. The lysed samples were then clarified at 20,000*g* for 30 min at 4 °C. Supernates were incubated with immobilized neutravidin beads (3 mg of protein/ml of beads, Pierce) for 1 h at room temperature to separate biotinylated from non-biotinylated protein. The beads were washed three times with 1 ml radioimmunoprecipitation assay buffer and the biotinylated proteins were eluted with 50 μl of 2× Laemmli sample buffer (100 mM Tris–HCl, pH 6.8, 20% glycerol, 10% SDS, 0.1 M dithiothreitol, and 0.2% bromphenol blue) for 30 min at room temperature. Aliquots of total cell lysates representing equal amounts of protein and non-biotinylated proteins were precipitated with TCA (5% final concentration) and the pH was neutralized with 5.0 M Tris-base before being resuspended in Laemmli sample buffer. Biotinylated and non-biotinylated proteins were resolved by SDS–PAGE (10% acrylamide). Western blots were performed. Blots were probed with a rat anti-dopamine transporter monoclonal antibody diluted 1:1000 (Chemicon, Tamecula, CA). Immunoreactive bands were visualized by ECL on Hypersensitive ECL film (Amersham, Arlington Heights, IL) and analyzed with Quantity One software (Bio-Rad Laboratories, Hercules, CA).

Statistical analysis. Indicated data are reported as percentages of control values. Statistical evaluation was performed using Student's paired *t* test and values of $p < 0.01$ were considered statistically significant for all analyses.

Results

Effect of MPP^+ on cell viability of HEK-293 and transfected HEK-hDAT cells

To define MPP^+-mediated cytotoxicity selectively via the dopamine transporter, we assessed the viability of HEK-293 and HEK-hDAT cells following incubation with different concentrations of MPP^+ using the MTT assay. As shown in Fig. 1A, treatment of HEK-hDAT

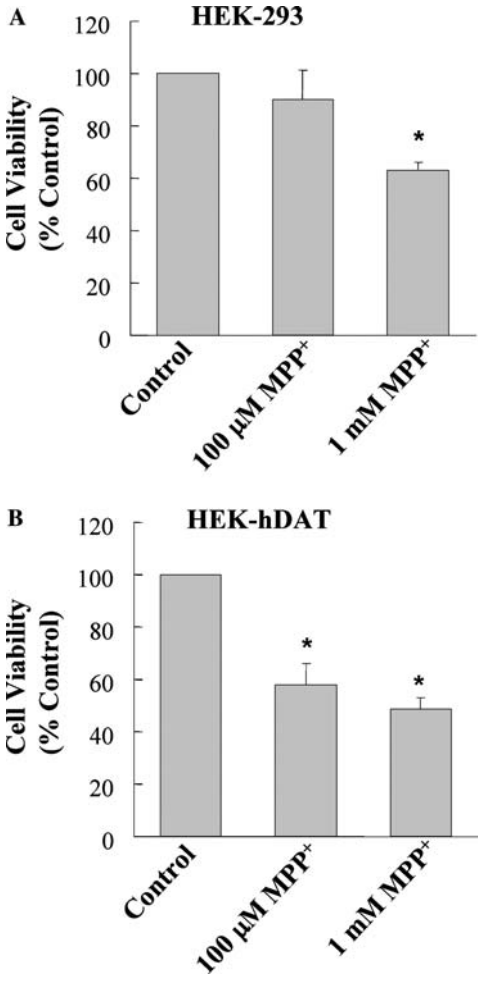

Fig. 1. Toxicity of MPP^+ on viability of HEK-293 cells expressing the hDAT measured by MTT reduction. (A) HEK-293 and (B) hDAT stably expressing HEK-293 cells were treated with MPP^+ (100 μM and 1 mM) for 24 h and cell viability was assessed by the MTT assay. Results are presented as means ± SEM of eight independent experiments. $^*P < 0.001$, compared to control within the group by Student's paired t test.

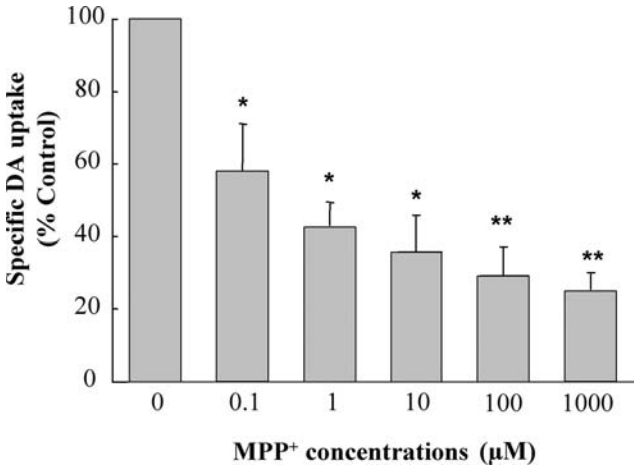

Fig. 2. Effect of MPP^+ on [^{3}H]DA uptake in hDAT-HEK cells. Results are presented as means ± SEM of at least four independent experiments. $^*P < 0.01$ and $^{**}P < 0.001$ compared to control within the group by Student's paired t test.

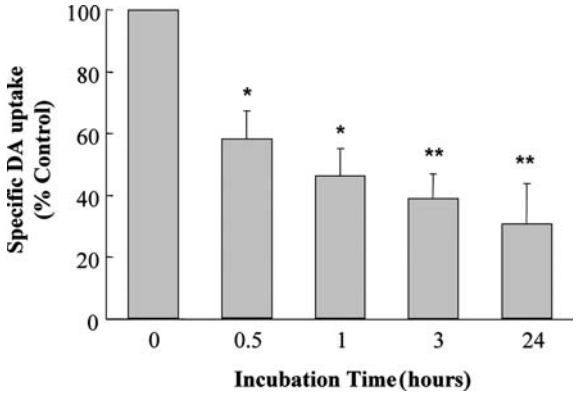

Fig. 3. Time course of MPP^+ decreased [^{3}H]DA uptake in hDAT-HEK cells. Results are presented as means ± SEM of six independent experiments. $^*P < 0.01$ and $^{**}P < 0.001$ compared to control within the group by Student's paired t test.

cells for 24 h at 37 °C with MPP^+ produced a significant reduction in cell viability (100 μM: 66 ± 8% of control, 1 mM: 60 ± 4% of control). MPP^+ at 1 mM produced mild toxic effects on HEK-293 cells with a notable decrease in cell viability, but no significant changes in cell viability at 100 μM.

Effect of MPP^+ on hDAT function

Results presented in Figs. 2 and 3 indicate that exposure of HEK-hDAT to MPP^+ for 24 h caused a concentration- and time-dependent decrease in [^{3}H]dopamine uptake. MPP^+ at concentrations as low as 1 μM significantly reduced [^{3}H]dopamine uptake (58 ± 13% of control) and maximal inhibition was observed at 1 mM concentration (25 ± 5% of control). As shown in Fig. 3, after incubation with MPP^+ (100 μM) for 30 min [^{3}H]dopamine uptake was reduced. After 3 and 4 h incubations, the reduction in [^{3}H]dopamine uptake remained the same (38 ± 8% of control and 30 ± 13% of control, respectively). An incubation time of 3 h was chosen for subsequent experiments since viability of the HEK-hDAT cells was compromised with the longer periods of incubation.

Effects of MPP^+ on [^{3}H]WIN 35428 binding in HEK-hDAT cells

The effect of MPP^+ on [^{3}H]WIN 35428 binding related to the reduction of DAT function was investigated in HEK-hDAT cells. As shown in Fig. 4, after 3 h of incubation with increasing concentrations of MPP^+, there was a concentration-dependent reduction in [^{3}H]WIN 35428 binding sites in the intact cells with a maximal reduction of 100 μM (39 ± 0.8% of control) and 1 mM (32 ± 7% of control). Apparently, there was no

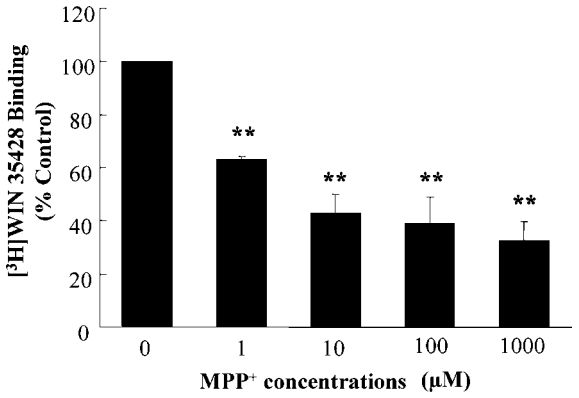

Fig. 4. MPP^+ selective reduced density of [^{3}H]WIN 35,428 binding sites in intact hDAT-HEK cells. Results are presented as means ± SEM of three independent experiments. $^{**}P < 0.001$ compared to control within the group by Student's paired t test.

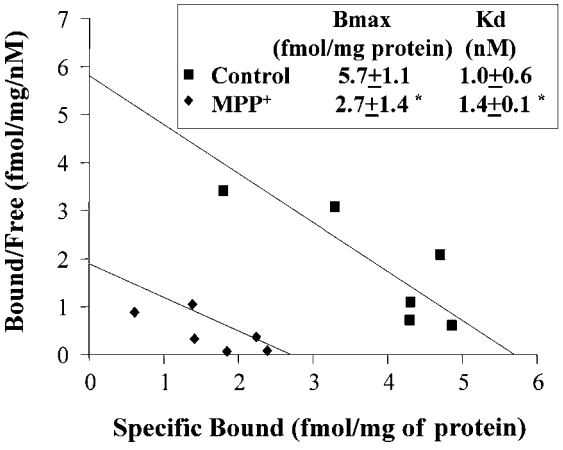

Fig. 5. Saturation curve of [^{3}H]WIN 35428 binding to hDAT-HEK cell membrane. The experiment shown is representative of four identical experiments. The shown B_{max} and K_d of [^{3}H]WIN 35,428 binding are presented as means ± SEM of four independent experiments. $^*P < 0.01$ compared to control within the group by Student's paired t test.

significant difference in the reduction of [^{3}H]WIN 35428 binding between 100 μM and 1 mM MPP^+. Importantly, MPP^+ treatment reduced the density (B_{max}) of [^{3}H]WIN 35428 binding sites (5.7 ± 1.1 and 2.7 ± 1.4 fmol/mg in control and treated groups, respectively), whereas a higher K_d value (1.0 ± 0.6 and 1.4 ± 0.1 nM in control and treated groups, respectively) in the membrane fraction was found by Scatchard analysis (Fig. 5).

Effect of MPP^+ on DAT cell surface expression

We investigated whether the negative effect of MPP^+ on dopamine uptake and [^{3}H]WIN 35428 binding correlates with DAT protein expression. An extensive study using biotinylation assays showed for the first time that MPP^+ significantly reduced the levels of biotinylated and non-biotinylated DAT proteins (52.8 ± 7.4 and 77.2 ± 1.4% of control, respectively; Fig. 6A). This parallels closely the reduction in [^{3}H]dopamine uptake (Fig. 3) and the level of cell surface [^{3}H]WIN 35428 binding after MPP^+ treatment (Fig. 4).

Discussion

In the present study, we investigated the mechanism by which MPP^+ lowers the level of dopamine transporter activity and asserts the role of DAT in MPP^+-mediated cytotoxicity. We have shown that cultured HEK-hDAT cells exposed to MPP^+ exhibit a significant decrease in cell survival. This is consistent with recent reports [17,20,21] which suggest that the dopamine transporter is required for MPP^+-mediated cytotoxicity. For instance, MPP^+-treated HEK-293 cells show minimal cytotoxicity compared to HEK-hDAT cells at a corresponding concentration of MPP^+. HEK-293 cell death occurred at very high MPP^+ concentrations (1 mM), which most likely was caused by changes in pH or osmolarity of the culture medium.

Several studies have demonstrated that MPP^+ elicits a marked decrease in hDAT transport activity, but the mechanisms involved largely remain hypothetical and equivocal. Consistent with earlier studies [7,14–16], we have shown that the down-regulation of hDAT observed in response to MPP^+ (Fig. 2) occurred in a concentration- and time-dependent fashion. MPP^+ at a concentration of 100 μM was used to investigate the time course of dopamine uptake because this concentration produced the highest reduction in dopamine uptake without affecting the cell culture conditions. Since MPP^+ has been shown to impair cell energy metabolism over time [11,16,17,22–24], a 3 h incubation period was chosen to preclude any possible effects of energy deficit and cell death on DAT function and the underlying mechanisms involved.

We used [^{3}H]WIN 35428, which is a specific ligand for DAT, to evaluate the effects of MPP^+ on the expression of DAT proteins. The [^{3}H]WIN 35428 binding assay in intact cells was conducted at low temperature to limit endocytosis of the ligand. A significant reduction in the binding of [^{3}H]WIN 35428 was observed after 3 h incubation with MPP^+ at each of the concentration ranges used (1, 10, 100 μM, and 1 mM) and this was consistent with a decline in DAT activity. Moreover, a notable decrease in B_{max} value for [^{3}H]WIN 35428 binding (Fig. 5) in the total membrane fractions isolated from lysed cells after treatment with 100 μM MPP^+ was observed (Fig. 5), and this indicates that the MPP^+-induced decrease in DAT is correlated with the reduction in the number of available [^{3}H]WIN 35428 binding sites at the cell surface and also in the cytoplasm.

The cell-impermeable biotinylated sulfo-NHS-biotin reagent and Western blot analysis were used to demonstrate that MPP^+ decreased both the biotinylated

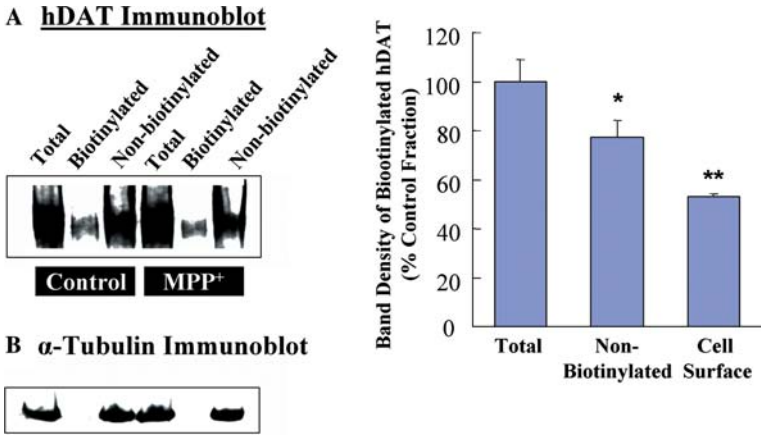

Fig. 6. MPP^+ decreases cell surface expression of hDAT. (A) A representative immunoblot showing total cell extracts, biotinylated extracts, and non-biotinylated extracts after treatment with MPP^+. (B) Immunoblot with anti-α-tubulin antibody. After detection of hDAT, the blot was stripped and probed to identify the cytoskeletal membrane protein α-tubulin. Intensity of α-tubulin-specific band was used to normalize hDAT-specific immunoreactivity in control and MPP^+-treated cell. Quantification of immunoblots obtained from four independent experiments. Results are presented as means ± SEM. $*P < 0.01$ and $*P < 0.001$ compared to control within the group by Student's paired t test.

and non-biotinylated DAT protein levels (Fig. 6), and this is consistent with the data obtained from the Scatchard analysis (Fig. 5). The data further indicate a smaller percentage change for the intracellular DAT, compared with that of the plasma membrane (72% of control and 53% of control, respectively). This probably reflects the greater absolute amounts of measurable intracellular DAT which also includes cell surface membrane DAT. Taken together, these findings suggest that MPP^+-mediated down-regulation of DAT activity correlates with an overall lowering of DAT protein expression.

Another mechanism whereby MPP^+ may cause a reduction in DAT activity is phosphorylation. In recent observations it was proposed that dopamine transporter activity was regulated by phosphorylation of DAT [25–27]. A consequence of phosphorylation is the possible sequestration of the transporter proteins [28–30] and lower activity. Our data however provide evidence that MPP^+ may reduce the activity of DAT by lowering the level of DAT on both plasma membrane and the cytoplasm. It is also possible that MPP^+ mediates neurotoxicity by increasing the sensitivity of DAT to

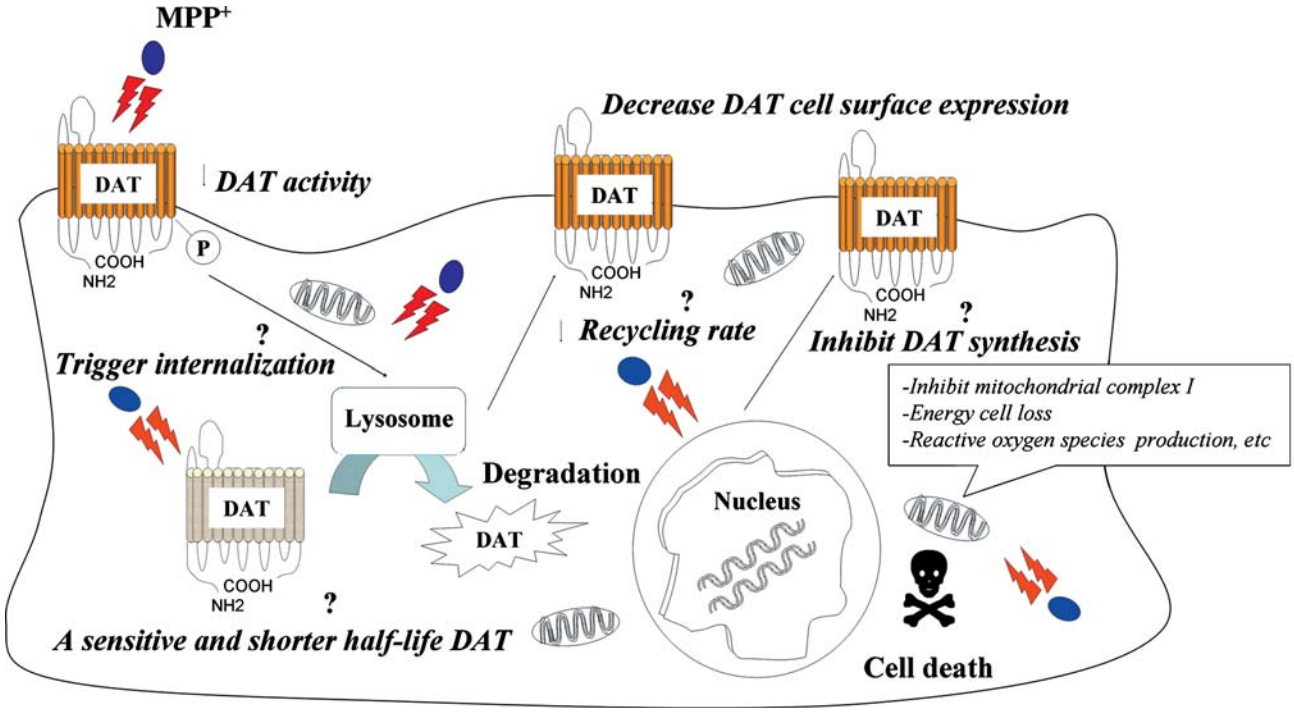

Fig. 7. Schematic representation of how MPP^+ may mediate cellular toxicity by involvement with DAT in HEK-hDAT cells. MPP^+ is selectively taken up into the cell via DAT, triggering internalization and degradation of DAT. A consequential reduction in DAT at the cell surface and in the cytoplasm lowers DAT activity, related to PD, while MPP^+ promotes the development of cytotoxic neurological sequelae.

lysosomal degradation, resulting in a shorter half-life (Fig. 7). The data presented here form the basis for further studies towards understanding the neurotoxic processes that occur in PD. The decrease in DAT function could involve (i) DAT phosphorylation; (ii) enhanced internalization and degradation of DAT, and (iii) reduction in DAT protein expression, or a combination of all three (see Fig. 7). However, these avenues remain to be explored further.

Acknowledgments

The authors express their heartfelt appreciation to Mrs. Dani Stramer for her skill in typing the manuscript. Dr. Chagkutip is the recipient of the Thai Royal Golden Jubilee provided by the Government of Thailand. We are also grateful to Dr. John Swinscoe for careful reading of the manuscript. The studies cited here have been supported by a grant from NINDS #2R01 NS34566-09 (M.E.).

References

[1] K.F. Tipton, T.P. Singer, Advances in our understanding of the mechanisms of the neurotoxicity of MPTP and related compounds, J. Neurochem. 61 (1993) 1191–1206.
[2] R.R. Gainetdinov, F. Fumagalli, S.R. Jones, M.G. Caron, Dopamine transporter is required for in vivo MPTP neurotoxicity: evidence from mice lacking the transporter, J. Neurochem. 69 (1997) 1322–1325.
[3] J.A. Javitch, R.J. D'Amato, S.M. Strittmatter, S. Snyder, Parkinsonism-inducing neurotoxin, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite *N*-methyl-4-phenyl pyridium by dopamine neurons explains selective toxicity, Proc. Natl. Acad. Sci. USA 82 (1985) 2173–2177.
[4] S.G. Amara, M.J. Kuhar, Neurotransmitter transporters: recent progress, Annu. Rev. Neurosci. 16 (1993) 73–93.
[5] M.E. Reith, C. Xu, N.H. Chen, Pharmacology and regulation of the neuronal dopamine transporter, Eur. J. Pharmacol. 324 (1997) 1–10.
[6] G.R. Uhl, D. Walther, D. Mash, B. Faucheux, F. Javoy-Agid, Dopamine transporter messenger RNA in Parkinson's disease and control substantia nigra neurons, Ann. Neurol. 35 (1994) 494–498.
[7] K.A. Harrington, S.J. Augood, A.E. Kingsbury, O.J. Foster, P.C. Emson, Dopamine transporter (DAT) and synaptic vesicle amine transporter (VMAT2) gene expression in the substantia nigra of control and Parkinson's disease, Brain Res. Mol. Brain Res. 36 (1996) 157–162.
[8] S.G. Speciale, C.L. Liang, P.K. Sonsalla, R.H. Edwards, D.C. German, The neurotoxin 1-methyl-4-phenylpyridinium is sequestered within neurons that contain the vesicular monoamine transporter, Neuroscience 84 (1998) 1177–1185.
[9] G.D. Chang, V.D. Ramirez, The mechanism of action of MPTP and MPP^+ on endogenous dopamine release from the rat corpus striatum superfused in vitro, Brain Res. 368 (1986) 134–140.
[10] T. Obata, C.C. Chiueh, In vivo trapping of hydroxyl free radicals in the striatum utilizing intracranial microdialysis perfusion of salicylate: effects of MPTP, $MPDP^+$, and MPP^+, J. Neural. Transm. Gen. Sect. 89 (1992) 139–145.
[11] J. Lotharius, K.L. O'Malley, The parkinsonism-inducing drug 1-methyl-4 phenylpyridinium triggers intracellular dopamine oxidation. A novel mechanism of toxicity, J. Biol. Chem. 275 (2000) 38581–38588.
[12] S. Przedborski, V. Jackson-Lewis, Mechanisms of MPTP toxicity, Mov. Disord. 13 (1998) 35–38.
[13] R.R. Ramsay, J.I. Salach, T.P. Singer, Uptake of the neurotoxin 1-methyl-4-phenylpyridine (MPP^+) by mitochondria and its relation to the inhibition of the mitochondrial oxidation of NAD^+-linked substrates by MPP^+, Biochem. Biophys. Res. Commun. 134 (1986) 743–748.
[14] S. Barc, G. Page, B. Fauconneau, L. Barrier, F. Huguet, A new in vitro approach for investigating the MPTP effect on DA uptake, Neurochem. Int. 38 (2001) 243–248.
[15] C. Fonck, M. Baudry, Toxic effects of MPP^+ and MPTP in PC12 cells independent of reactive oxygen species formation, Brain Res. 905 (2001) 199–206.
[16] C. Fonck, M. Baudry, Rapid reduction of ATP synthesis and lack of free radical formation by MPP^+ in rat brain synaptosomes and mitochondria, Brain Res. 975 (2003) 214–221.
[17] A. Storch, A.C. Ludolph, J. Schwarz, HEK-293 cells expressing the human dopamine transporter are susceptible to low concentrations of 1-methyl-4-phenylpyridine (MPP^+) via impairment of energy metabolism, Neurochem. Int. 35 (1999) 393–403.
[18] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[19] R.E. Whitehead, J.V. Ferrer, J.A. Javitch, J.B. Justice, Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter, J. Neurochem. 76 (2001) 1242–1251.
[20] C. Pifl, B. Giros, M.G. Caron, Dopamine transporter expression confers cytotoxicity to low doses of the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium, J. Neurosci. 10 (1993) 4246–4253.
[21] S. Kitayama, C. Mitsuhata, S. Davis, J.B. Wang, T. Sato, K. Morita, G.R. Uhl, T. Dohi, MPP^+ toxicity and plasma membrane dopamine transporter: study using cell lines expressing the wild-type and mutant rat dopamine transporters, Biochim. Biophys. Acta 1404 (1998) 305–313.
[22] M. Ebadi, P. Govitrapong, S. Sharma, D. Muralikrishnan, S. Shavali, L. Pellett, R. Schafer, C. Albano, J. Eken, Ubiquinone (coenzyme q10) and mitochondria in oxidative stress of Parkinson's disease, Biol. Signals Recept. 10 (2001) 224–253.
[23] S.K. Sharma, M. Ebadi, Metallothionein attenuates 3-morpholinosydnonimine (SIN-1)-induced oxidative and nitrative stress in dopaminergic neurons, Antioxid. Redox Signal. 5 (2003) 231–264.
[24] M. Ebadi, S.K. Sharma, Peroxynitrite and mitochondrial dysfunction in the pathogenesis of Parkinson's disease, Antioxid. Redox Signal. 5 (2003) 319–335.
[25] R.A. Vaughan, R.A. Huff, G.R. Uhl, M.J. Kuhar, Protein kinase C-mediated phosphorylation and functional regulation of dopamine transporters in striatal synaptosomes, J. Biol. Chem. 272 (1997) 15541–15546.
[26] R.A. Huff, R.A. Vaughan, M.J. Kuhar, G.R. Uhl, Phorbol esters increase dopamine transporter phosphorylation and decrease transport Vmax, J. Neurochem. 68 (1997) 225–232.
[27] L. Zhang, L.L. Coffey, M.E. Reith, Regulation of the functional activity of the human dopamine transporter by protein kinase C, Biochem. Pharmacol. 53 (1997) 677–688.
[28] Z.B. Pristupa, F. McConkey, F. Liu, H.Y. Man, F.J. Lee, Y.T. Wang, H.B. Niznik, Protein kinase-mediated bidirectional trafficking and functional regulation of the human dopamine transporter, Synapse 30 (1998) 79–87.
[29] H.E. Melikian, K.M. Buckley, Membrane trafficking regulates the activity of the human dopamine transporter, J. Neurosci. 19 (1999) 7699–7710.
[30] A.L. Bauman, S. Apparsundaram, S. Ramamoorthy, B.E. Wadzinski, R.A. Vaughan, R.D. Blakely, Cocaine and antidepressant-sensitive biogenic amine transporters exist in regulated complexes with protein phosphatase 2A, J. Neurosci. 20 (2000) 7571–7578.