Mitochondrial Complex I Inhibitor Rotenone-Elicited Dopamine Redistribution from Vesicles to Cytosol in Human Dopaminergic SH-SY5Y Cells

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ABSTRACT
Parkinson’s disease is a chronic neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra. Rotenone, a pesticide, produces selective degeneration of dopaminergic neurons and motor dysfunction in rats. To determine the mechanisms underlying rotenone-induced neuronal death, we investigated whether intracellular dopamine plays a role in rotenone (0.1–0.4 μM)-induced apoptosis, using an in vitro model of human dopaminergic SH-SY5Y cells. The 40% decrease of dopamine content by inhibition of dopamine synthesis suppressed rotenone-induced apoptosis. On the other hand, the 30% increase of dopamine content by inhibition of dopamine metabolism enhanced rotenone-induced apoptosis. Depletion of intracellular dopamine using reserpine (0.1–10 μM) also prevented rotenone-induced apoptosis, and this effect was counteracted by dopamine (10–100 μM) replenishment. Inhibition of dopamine reverse transport increased cytosolic dopamine and enhanced rotenone-induced apoptosis. We examined the intracellular localization of dopamine in rotenone-treated cells immunocytochemically and quantitatively. Rotenone induced dopamine redistribution from vesicles to the cytosol. In this process, rotenone stimulated reactive oxygen species and protein carbonylation and decreased an antioxidant, glutathione. Addition of an antioxidant, N-acetylcysteine (3 mM), prevented dopamine being expelled from vesicles and inhibited rotenone-induced apoptosis. Our findings demonstrate that rotenone-generated reactive oxygen species are involved in dopamine redistribution to the cytosol, which in turn may play a role in rotenone-induced apoptosis of dopaminergic cells.

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ABBREVIATIONS: PD, Parkinson’s disease; MPP⁺, 1-methyl-4-phenylpyridinium ion; PARP-1, poly(ADP-ribose) polymerase-1; ROS, reactive oxygen species; MAO, monoamine oxidase; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, 4-hydroxy-3-methoxy-4-phenylacetic acid; α-MT, α-methyl-p-tyrosine; GBR12935, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine; NAC, N-acetylcysteine; VMAT2, vesicular monoamine transporter 2; FITC, fluorescein isothiocyanate; PI, propidium iodide; PBS, phosphate-buffered saline; HPLC, high-performance liquid chromatography; carboxy-H₂DCFDA, carboxy-2′,7′-dichlorodihydrofluorescein diacetate; DHE, dihydroethidium; CM-FDA, 5-chloromethylfluorescein diacetate.
their function. However, vesicular dopamine is protected from oxidative assault. In fact, at physiological concentrations, dopamine does not induce degenerative processes in the brain and has even been shown to activate the expression of cell survival genes and proteins (Weinreb et al., 2003). In contrast, numerous studies have shown that at higher concentrations, dopamine can cause cell death both in vivo and in cell cultures. It is possible that redistribution of dopamine to the cytosol renders dopamine prone to quinone formation. Even at physiological intracellular concentrations of dopamine, redistribution from vesicles to the cytosol would result in dopamine toxicity. Rotenone may enhance dopamine redistribution from vesicles to the cytosol, although this possibility has not been confirmed. In fact, another dopaminergic toxin, methamphetamine, which, unlike rotenone, binds to the dopamine transporter (DAT) and is devoid of complex I inhibition, causes dopamine redistribution from vesicles to the cytosol (Lotharius et al., 2005). Therefore, this dopamine redistribution is possibly a common step in the mechanisms of dopaminergic neuronal toxins. Human SH-SY5Y cells are a model system of dopaminergic neurons. Indeed, rotenone induced apoptosis in SH-SY5Y cells (Watabe and Nakaki, 2004). The present study was undertaken to assess directly whether rotenone causes dopamine to be expelled from vesicles and, if so, whether rotenone-induced ROS are involved in redistribution in human dopaminergic SH-SY5Y cells.

Materials and Methods

Materials. Rotenone, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylacetic acid (HVA), noradrenaline, reserpine, α-methyl-p-tyrosine (α-MT), iproniazid, GBR12935, N-acetylcysteine (NAC), and anti-actin antibody were purchased from Sigma-Aldrich (St. Louis, MO). Anti-dopamine antibody was obtained from Abcam plc (Cambridge, UK), and anti-vesicular monoamine transporter 2 (VMAT2) was from Chemicon International (Temecula, CA).

Cell Culture. SH-SY5Y cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum at 37°C under 5% CO2 in air.

Quantification of DNA Fragmentation. As described previously (Watabe et al., 2004), DNA fragmentation was measured using a Cell Death Detection ELISA® PLUS kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer’s instruction. This assay is useful for differentiating apoptosis from necrosis, and it can detect internucleosomal degradation of genomic DNA, which is a hallmark of apoptosis, by quantitatively measuring histone-associated DNA fragments.

Apoptosis Assay. In the early phase of apoptosis, cells lose their phospholipid membrane asymmetry and phosphatidylserine is exposed at the cell surface. This process was monitored using annexin V-FITC, which is a phospholipid-binding protein with high affinity for phosphatidylserine. The necrotic cell membrane loses its integrity and becomes leaky. Therefore, necrotic cells are easily stained with propidium iodide (PI). Cells treated with rotenone were incubated for 15 min with annexin V-FITC (MBL International Corporation, Watertown, MA) and PI. After washing with phosphate-buffered saline (PBS), fluorescence was measured using a Multimode Detector DTX800 (Beckman Coulter, Fullerton, CA).

Measurement of Dopamine, DOPAC, HVA, and Noradrenaline. To measure total amounts of dopamine, DOPAC, HVA and noradrenaline in cells, the cells were lysed in 0.01 M hydrochloric acid. After centrifugation (15,000g for 10 min at 4°C), the supernatant was immediately injected into an HPLC system. To measure amounts of dopamine in the vesicle fraction and cytosol fraction, the cells were treated with rotenone in the presence of 0.5 μM GBR12935 to prevent release from inside to outside of the cell via the DAT. The vesicle fraction and cytosol fraction were prepared using a Synaptic Vesicles Isolation Kit (Sigma-Aldrich) according to the manufacturer’s instructions. The vesicle fraction was lysed in 0.01 M hydrochloric acid and the cytosol fraction was added to trichloroacetic acid (final concentration 5% (w/v)). After centrifugation (15,000g for 10 min at 4°C), the supernatant was immediately injected into an HPLC system. Dopamine, DOPAC, HVA, and noradrenaline levels in each sample were quantified by comparing the peak areas with those of standard solutions containing dopamine, DOPAC, HVA, and noradrenaline.

Immunoblot Analysis. Immunoblotting was performed as described previously (Watabe et al., 1998). Cells were lysed in a buffer containing SDS and mercaptoethanol, and the cell lysate was then boiled. Denatured proteins were separated on polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (GE Healthcare, Buckinghamshire, UK). The membrane was incubated with a blocking solution [2% bovine serum albumin (Sigma-Aldrich) dissolved in PBS containing 0.2% Tween 20] for 1 h at room temperature, washed with PBS containing 0.2% Tween 20, and incubated with the first antibody dissolved in the blocking solution overnight at 4°C. After washing, the membrane was incubated for 1 h with horseradish-linked secondary antibody. Immunoactive proteins were detected with an enhanced chemiluminescence system (Amersham Pharmacia Biotech).

Detection of ROS. Detection of ROS was performed as described previously (Watabe and Nakaki, 2007). In brief, cells treated with rotenone were incubated at 37°C for 25 min with 25 μM carboxy-H2DCFDA (Molecular Probes, Eugene, OR) or 10 μM dihydroethidium (DHE) (Molecular Probes) and then for 5 min with 1 μM Hoechst 33342 (Sigma-Aldrich). Upon oxidation by ROS, its fluorescent moiety is unmasked and can be detected by fluorescence microscopy. The amount of emitted fluorescence correlates with the quantity of ROS in the cell. After washing with PBS, the amount of fluorescence was measured using a MultiMode Detector DTX800. Quantitative analysis was performed by normalization of the amount of Hoechst 33358 fluorescence in conjunction with brief treatment with each inhibitor. None of the inhibitors damaged the cell nucleus. Moreover, cells were visualized using fluorescence microscopy (Axio Imager M1; Zeiss, Oberkochen, Germany).

Detection of Glutathione. Cells treated with rotenone were incubated at 37°C for 25 min with 5 μM 5-chloromethylfluorescein diacetate (CMFDA; Molecular Probes) and then for 5 min with 1 μM Hoechst 33342. CMFDA is nonfluorescent until acted upon by intracellular esterases. By the reaction with thiols, the majority of which is reduced glutathione, fluorescence can be detected. The amount of emitted fluorescence correlates with the quantity of glutathione in the cell. After washing with PBS, cells were visualized using fluorescence microscopy (Axio Imager M1).

Quantitative Analysis of Glutathione. The glutathione concentration in cells was determined using ThioGlo-1 (Calbiochem, San Diego, CA), a maleimide reagent that produces a highly fluorescent adduct upon reaction with thiol groups. Glutathione content was estimated from the fluorescence response via the interaction of ThioGlo-1 mainly with intracellular glutathione. Cells were incubated at 37°C for 30 min with 10 μM ThioGlo-1. After washing with PBS to remove excess nonreacted ThioGlo-1, the level of fluorescence was measured using a Multimode Detector DTX800.

Measurement of Protein Carbonyls. Cells were treated with rotenone for 8 h, washed with PBS, and lysed in ice-cold lysis buffer (20 mM Tris-Cl, pH 7.4, 140 mM NaCl, 1% Triton X-100, and 2 mM EDTA containing a protease inhibitor mixture). Protein carbonyls were measured with an Oxyblot protein oxidation detection kit (Chemicon International, Temecula, CA). In brief, total cell lysates were incubated with an equal volume of 12% SDS and 2 volumes of 2,4-dinitrophenylhydrazine solution for 15 min at room temperature.
and then with 1.5 volumes of neutralization solution to stop the reaction. The samples obtained were immunblotted using an antibody against dinitrophenol.

**Immunofluorescence Microscopy.** The immunocytochemical study was performed as described previously (Watabe et al., 1997, 2000). Cells were washed with PBS and fixed with 3.7% formaldehyde for 20 min. Cells were permeabilized with PBS containing 0.2% Triton X-100 for 5 min and then washed three times with PBS. Primary antibody was incubated for 1 h at room temperature. Excess antibody was washed out three times with PBS followed by incubation with an appropriate fluorophore-labeled secondary antibody for 1 h at room temperature in an area protected from light. After the excess antibody was washed out three times with PBS, mounting was performed using a ProLong Antifade Kit (Molecular Probes). Images were obtained by fluorescence microscopy (Axio Imager M1).

**Statistics.** Values are means ± S.E. from three experiments. Statistical analysis of the data were performed using analysis of variance followed by Fisher’s test. *p* < 0.05 was considered significant.

**Results**

We examined the effects of rotenone on apoptosis of dopaminergic and non-neuronal cells of human origin. As shown in Fig. 1A, rotenone dose-dependently increased histone-associated DNA fragmentation. However, dopaminergic SH-SY5Y cells are far more vulnerable to rotenone than breast cancer MCF-7 cells and hepatoma HepG2 cells despite the fact that rotenone inhibits mitochondrial complex I in these cells and produces ROS (Rowlands and Casida, 1998; Greenamyre et al., 2003). We also ascertained that apoptosis of SH-SY5Y cells was induced by rotenone using another technique. As shown in Fig. 1B, experiments using annexin V-FITC (an apoptosis-monitoring agent) and PI (an indicator of necrosis) produced effects on the degree of apoptosis similar to those of DNA fragmentation.

To determine the role of endogenous intracellular dopamine in rotenone-induced neurotoxicity, we first examined

![Fig. 1. Apoptosis induction by rotenone in various cell lines. A, after SH-SY5Y, MCF-7, and HepG2 cells had been treated with rotenone at various concentrations for 24 h, a DNA fragmentation assay was performed. B, after SH-SY5Y cells had been treated with rotenone at various concentrations for 16 h, an apoptotic assay using annexin V and PI was performed. Results are presented as means ± S.E. of three independent experiments.](image)

![Fig. 2. Effect of intracellular dopamine on rotenone-induced apoptosis in SH-SY5Y cells. A, after SH-SY5Y cells had been treated with 1 mM α-MT or 10 mM iproniazid for 24 h, intracellular dopamine amount was measured. B, after pretreatment with 1 mM α-MT, SH-SY5Y cells were treated with 0.2 μM rotenone for 24 h and DNA fragmentation assay was performed. C, after pretreatment with 10 mM iproniazid, SH-SY5Y cells were treated with 0.1 μM rotenone for 24 h, and a DNA fragmentation assay was performed. Results are presented as means ± S.E. of three independent experiments. *p < 0.05 compared with control; ***, p < 0.05 compared with rotenone treatment.](image)
the effect of α-MT, an inhibitor of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, on rotenone-induced apoptosis. α-MT decreased the amount of intracellular dopamine and suppressed DNA fragmentation induced by treatment with 0.2 μM rotenone (Fig. 2, A and B). We also examined the effect of iproniazid, an inhibitor of MAO, a dopamine-metabolizing enzyme, on rotenone-induced apoptosis. In contrast with α-MT, iproniazid increased the amount of intracellular dopamine and accelerated DNA fragmentation induced by treatment with 0.1 μM rotenone (Fig. 2, A and C). Next, we examined the effect of reserpine on rotenone-induced apoptosis in regulation of the amount of intracellular dopamine. Reserpine is known to cause dopamine release from neurons in the acute stage of treatment, whereas it causes dopamine depletion in the late stage. As shown in Fig. 3A, reserpine time dependently decreased in-
tracellular dopamine content in the early stage. We examined dopamine content in the cytosol and vesicle fractions at 30 min after reserpine treatment, which is the time that dopamine content began to decrease. Reserpine decreased dopamine in vesicles and increased it in the cytosol (Fig. 3B). On the other hand, rotenone did not affect dopamine content in the cytosol and vesicle fractions at the same treatment times (Fig. 3B). Reserpine alone did not trigger apoptosis (Fig. 3C). When cells were treated simultaneously with rotenone and reserpine, rotenone-induced apoptosis was enhanced (Fig. 3C). In contrast to simultaneous treatment, rotenone-induced apoptosis was suppressed in cells with dopamine depletion by reserpine (Fig. 3D). To examine whether this inhibition by reserpine was caused by dopamine depletion, we added dopamine after reserpine treatment and medium removal. Exogenous dopamine treatment of SH-SY5Y cells dose-dependently increased DNA fragmentation (Fig. 4A). This effect was caused by uptake of dopamine via the DAT, which is dopamine reverse transport, because exogenous dopamine-induced apoptosis was completely suppressed by GBR12935, a DAT inhibitor (Fig. 4B). The addition of dopamine after reserpine treatment and medium removal counteracted the suppressive effect of reserpine on rotenone-induced apoptosis (Fig. 4C).

Rotenone treatment for 8 h decreased the amount of intracellular dopamine, but not that of DOPAC, HVA, or noradrenaline (Fig. 5A). To examine whether this decrease of intracellular dopamine by rotenone was caused by ejection outside cells via DAT, we used GBR12935 again. Inhibition of dopamine reverse transport by GBR12935 prevented the rotenone-induced decrease of intracellular dopamine via the DAT (Fig. 5B). Furthermore, we examined whether this inhibition by GBR12935 caused the increase in cytosolic dopamine. When the leaking of cytosolic dopamine outside cells via the DAT was prevented by GBR12935, rotenone decreased the amount of dopamine in the vesicle fraction and increased it in the cytosol fraction (Fig. 5, C and D). It should be noted that the experimental conditions used in Figs. 3B and 5, C and D, were different in terms of the treatment time with rotenone and the use of GBR12935. Moreover, we examined the intracellular redistribution of dopamine in rotenone-treated cells immunocytochemically. In control cells, dopamine showed the same intracellular redistribution as VMAT2, which is localized in vesicles (Fig. 6A). No dopamine signal was detected in reserpine-pretreated cells. With rotenone treatment, dopamine diffused throughout the cells, in contrast to the distribution of VMAT2 (Fig. 6A). The amount of VMAT2 did not change with rotenone or reserpine treatment, as demonstrated by an immunoblotting technique (Fig. 6B). When rotenone induced an increase of cytosolic dopamine in the presence of GBR12935, rotenone-induced apoptosis was enhanced (Fig. 6C).

These results indicate that increased cytosolic dopamine is crucial to rotenone-induced apoptosis. However, an increase in cytosolic dopamine is necessary but not enough to induce apoptosis, because reserpine induced an increase of cytosolic dopamine content without DNA fragmentation (Fig. 3B). The toxicity of dopamine may result from oxidation of itself. Reserpine is also known to be an uncoupler of the mitochondrial respiration chain (Mania, 1974; Weinbach et al., 1983), and the uncoupler does not generate ROS (Watabe and Nakaki, 2007). Therefore, we examined whether ROS were produced by rote-

![Fig. 5. Change in intracellular dopamine by rotenone in SH-SY5Y cells. A, after SH-SY5Y cells had been treated with 0.4 μM rotenone for 8 h, amounts of intracellular dopamine, DOPAC, HVA, and noradrenaline were measured. B to D, after pretreatment with 500 nM GBR12935, SH-SY5Y cells were treated with 0.4 μM rotenone for 8 h. Whole cell (B), vesicle (C), and cytosol (D) fractions were prepared, and the amount of dopamine was measured. Results are presented as means ± S.E. of three independent experiments. *p < 0.05 compared with control.](image-url)
none under our experimental conditions. The membrane-permeable probe H$_2$DCFDA was used to measure ROS produced in live cells and acquired fluorescent images. ROS in the cells converted the nonfluorescent dye into fluorescein, which emitted green fluorescence. Without rotenone treatment, the amount of ROS was significantly reduced in cells (Fig. 7A). After rotenone treatment, ROS production was increased in cells (Fig. 7, A and B). We examined whether rotenone-induced ROS generation depends on the amount of intracellular dopamine. α-MT failed to attenuate rotenone-induced ROS generation (Fig. 7B), showing that the site of action of α-MT is beyond ROS generation. Using the fluorescence probe DHE, which specifically detects superoxide among ROS, we attempted to detect superoxide in rotenone-treated cells. Rotenone induced the expression of superoxide (Fig. 7C). To examine whether rotenone increases protein oxidation, we measured protein carbonyls in total cell lysates. As shown in Fig. 7D, many protein carbonyls were formed by rotenone treatment. Anti-actin immunoblotting of the same set of samples was used to normalize the quantity of protein carbonyls (Fig. 7E). The significant difference in these parameters between control and rotenone-treated cells suggests that rotenone produced oxidative stress in the cell. Because it was considered to decrease a defensive function against oxidative stress, we examined the amount of intracellular glutathione, which is an antioxidant. As shown in Fig. 8, A and B, rotenone induced a decrease in the amount of intracellular glutathione. NAC, which is an ROS scavenger and a substrate for glutathione synthesis, reduced rotenone-induced glutathione depletion and partially attenuated apoptosis (Fig. 8, B and C). Moreover, NAC inhibited the rotenone-induced dopamine redistribution from vesicles to cytosol (Fig. 6A). These results indicate that rotenone-generated ROS shift dopamine localization from vesicles to the cytosol, and cytosolic dopamine and/or quinone is involved in rotenone-induced apoptosis.

**Discussion**

There is evidence to suggest that nigral neurodegeneration in PD involves apoptosis (Mochizuki et al., 1996; Tompkins et al., 1997; Ayala et al., 2007). In this study, we demonstrated
that rotenone, which inhibits mitochondrial complex I and causes parkinsonian motor dysfunction in rats, leads to dopamine redistribution from vesicles and that this redistribution is blocked by the ROS scavenger NAC.

To rule out nonspecific rotenone toxicity in human cultured cells, we examined the effects of rotenone on nondopaminergic cells. Dopaminergic SH-SY5Y cells were more sensitive to rotenone than breast cancer MCF-7 cells and hepatoma HepG2 cells. Intracellular dopamine could be a factor making the cells more vulnerable to rotenone.

Rotenone dose-dependently increased DNA fragmentation, which is a marker of induction of apoptosis. In this process, the amount of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, did not change, as shown previously (Watabe and Nakaki, 2004). In vivo experiments, the decrease in the amount of tyrosine hydroxylase is generally used as a marker of damage to dopaminergic neurons. It was shown that the decrease in tyrosine hydroxylase was induced later than the induction of DNA fragmentation. We focused on the early events in rotenone-induced apoptosis. To clarify the role of intracellular dopamine in rotenone-induced apoptosis, we used inhibitors of each key event. First, α-MT, an inhibitor of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, significantly inhibited rotenone-induced apoptosis. Second, iproniazid, an inhibitor of MAO, a dopamine-metabolizing enzyme, enhanced rotenone-induced apoptosis. Third, reserpine, which expels dopamine in the acute phase and depletes it in the late stage, accelerated rotenone-induced apoptosis in the acute phase and suppressed it in the late stage. In the acute phase, reserpine expelled dopamine. However, dopamine increased in cytosol on the dopamine-expelled process by reserpine. Therefore, we considered the possibility that the acceleration of rotenone-induced apoptosis by simultaneous treatment with rotenone and reserpine was caused by cytosolic dopamine. Addition of dopamine after reserpine treatment and medium removal counteracted the suppressive effect of reserpine on rotenone-induced apoptosis. Because apoptosis induced by the addition of dopamine alone was suppressed when dopamine reverse transport was blocked by the DAT inhibitor GBR12935, dopamine taken up by the DAT caused induction of apoptosis. As vesicular dopamine is not toxic, we considered the possibility that this effect was caused by cytosolic dopamine. Rotenone decreased the amount of intracellular dopamine, but not that of DOPAC, HVA, or noradrenaline. When DAT was blocked by GBR12935 to prevent the release from inside to outside of the cell via the DAT and to maintain dopamine accumulation in the cytosol, this decrease in cytosolic dopamine was suppressed and rotenone-induced apoptosis was enhanced. Dopamine may need to be expelled from the vesicles, thereby making them vulnerable to ROS and enzymatic metabolism. Therefore, we determined whether rotenone influenced the intracellular distribution of dopamine. Using both an immunocytochemical technique and an HPLC system, we established that rotenone caused dopamine redistribution from vesicles to the cytosol under apoptotic conditions. These results suggest that cytosolic dopamine may play a role in the apoptosis of dopaminergic SH-SY5Y cells induced by rotenone. Mitochondrial impairment and metabolic stress cause striatal dopamine efflux via the DAT (Moy et al., 2007). Our findings may explain the intermediate processes between mitochondrial impairment and dopamine release from the cytosol to the extracellular compartment.

Rotenone inhibits complex I and generates ROS in mitochondria (Li et al., 2003; Panov et al., 2005). Upstream of the rotenone binding site in complex I is a site of electron leakage that can enhance ROS formation (Hensley et al., 1998). Dopamine may not be involved in this ROS generation process, because α-MT failed to attenuate rotenone-induced ROS generation and rotenone inhibits complex I and generates ROS in nondopaminergic cells (Li et al., 2003). Dopamine itself can be autoxidized to form a reactive species, dopamine qui-
none, which is probably covalently incorporated into a variety of molecules including proteins and nucleic acids (Fornstedt, 1990). We demonstrated the characteristics of rotenone-generated ROS, one species being compatible with superoxide, with the capacity to cause protein carbonylation. These findings are consistent with the notion that rotenone-induced ROS in dopaminergic cells is derived from some sources other than dopamine, but probably from mitochondrial complex I/II as suggested by Panov et al. (2005). There is oxidative damage to DNA, lipids, and protein, and oxidative stress may contribute to the neurodegeneration observed in PD (Jenner, 1998). In fact, the brains of patients with PD have decreased levels of reduced glutathione (Dexter et al., 1989; Sian et al., 1994; Alam et al., 1997; Pearce et al., 1997). We have shown that rotenone causes a decrease in intracellular glutathione. Rotenone caused both the production of oxidative stress and a decrease in defensive function. Furthermore, NAC, which is a ROS scavenger and a substrate for glutathione synthesis, reduced rotenone-induced glutathione depletion and attenuated apoptosis. Surprisingly, NAC also prevented rotenone-induced dopamine redistribution from vesicles to the cytosol under apoptotic conditions. A D3/D2 receptor agonist, pramipexole, exerts a neuroprotective action via ROS scavenging (Danzeisen et al., 2006; Sayeed et al., 2006; Joyce and Millan, 2007), and it remains to be seen whether pramipexole has an inhibitory effect on dopamine redistribution. Therefore, the results suggest that rotenone-generated ROS shifted dopamine localization from vesicles to the cytosol and that dopamine outside vesicles, possibly via quinone formation, may cause apoptosis of dopaminergic SH-SY5Y cells.

In conclusion, cytosolic dopamine is possibly a sensitizing factor in rotenone-induced apoptosis. However, the cytosolic dopamine increase alone is not enough to induce apoptosis in dopaminergic SH-SY5Y cells, because reserpine transiently induced an increase in cytosolic dopamine content in the dopamine expulsion process but did not induce apoptosis. Oxidation of dopamine itself in the cytosol is a key event in dopamine toxicity. Reserpine is also known to be an uncoupler of the mitochondrial respiration chain (Mania, 1974; Weinbach et al., 1983), and the uncoupler does not generate ROS (Watabe and Nakaki, 2007). Therefore, in rotenone-induced apoptosis, it is significant that dopamine redistribution to the cytosol is caused by rotenone when ROS levels are
elevated by rotenone. Furthermore, as ROS cause dopamine mobilization from vesicles to the cytosol, ROS may play an important role in rotenone-induced apoptosis of dopaminergic SH-SY5Y cells. A critical problem awaiting resolution is how rotenone and possibly rotenone-induced ROS elicit dopamine redistribution. Finding the answer to this question may help to elucidate not only rotenone toxicity but the pathogenesis of PD.

References


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