Superoxide Dismutase/Catalase Mimetics Are Neuroprotective against Selective Paraquat-mediated Dopaminergic Neuron Death in the Substantial Nigra

IMPLICATIONS FOR PARKINSON DISEASE*

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Exposure of mice to the herbicide paraquat has been demonstrated to result in the selective loss of dopaminergic neurons of the substantia nigra, pars compacta (SNpc) akin to what is observed in Parkinson disease (PD). In this study, we investigate the efficacy of two synthetic superoxide dismutase/catalase mimetics (EUK-134 and EUK-189) in protecting against paraquat-induced dopaminergic cell death in both the rat dopaminergic cell line 1RB6AN57 (N27) and primary mesencephalic cultures in vitro and in adult mice in vivo. Our data demonstrate that pretreatment with either EUK-134 or EUK-189 significantly attenuates paraquat-induced neurotoxicity in vitro in a concentration-dependent manner. Furthermore, systemic administration of EUK-189 decreases paraquat-mediated SNpc dopaminergic neuronal cell death in vivo. These findings support a role for oxidative stress in paraquat-induced neurotoxicity and suggest novel therapeutic approaches for neurodegenerative disorders associated with oxidative stress such as PD.

Parkinson disease (PD)† is a common age-related neurodegenerative disease that is pathologically characterized by the selective loss of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the ventral midbrain and the presence of ubiquinoned protein deposits in residual neurons (Lewy bodies) (1, 2). Epidemiological studies have suggested that exposure to agricultural chemicals is associated with an increased risk of developing PD (3–9). The herbicide 1,1’-dimethyl-4,4’-bipyridium (paraquat, PQ) may contribute to the pathogenesis of PD based on both epidemiological studies of parkinsonism correlated with exposure to the agent (3, 7), and its structural similarity to the active metabolite of the parkinsonism-inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 1-methyl-4-phenylpyridinium ion. Exposure of mice to either paraquat alone or in combination with the dihydrocarbamate fungicide manganese ethylenebisdiethiocarbamate results in preferential degeneration of dopaminergic nigrostriatal neurons, mimicking the cell loss observed in PD (10, 11). Recently, we reported that paraquat administration triggers an apoptotic cell death program through oxidative stress-mediated activation of the JNK signaling pathway, suggesting a possible mechanism for selective dopaminergic neuron loss (12).

The salen manganese complexes (EUKs), synthetic SOD and catalase mimetics, catalytically eliminate both superoxide and hydrogen peroxide (13–15). These compounds have been shown to be neuroprotective in several animal models, including those for inflammatory autoimmune disease (16), ischemia (15), epilepsy (17), and amyotrophic lateral sclerosis (18). The compounds also significantly increase the mean and maximum lifespan in Caenorhabditis elegans (19), alleviate lethal oxidative pathologies in mice with genetically deleted SOD2 (20), and reverse age-related learning deficits and brain oxidative stress (21). Treatment of rats with EUK-134 significantly reduces DNA-binding activity of two transcription factors, activator protein-1 (AP-1) and NF-κB, and attenuates kainate-induced neuropathology (17).

The molecular mechanisms that support the feasibility for the potential use of the salen manganese complexes as therapy for PD are far from being completely elucidated. The results of the current investigation demonstrate, for the first time, the capacity of these potentially therapeutic antioxidant compounds to neuroprotect against selective dopaminergic midbrain cell death not only in vitro but also in vivo in an animal model of the disease.

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‡ The abbreviations used are: PD, Parkinson disease; SNpc, substantia nigra pars compacta; SN, substantia nigra; JNK, c-Jun NH2-terminal kinase; SOD, superoxide dismutase; EUK, synthetic superoxide dismutase/catalase mimetics; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PQ, 1,1’-dimethyl-4,4’-bipyridium (paraquat); TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; TH, tyrosine hydroxylase; MnTBAP, manganese(III) tetrakis (4-benzoic acid) porphyrin.

** Experimental Procedures

Materials—3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1,1’-dimethyl-4,4’-bipyridium dichloride (paraquat), protease inhibitor mixture, lactacystin, and mononclonal anti-β-actin antibody were purchased from Sigma. Polyvinylidene difluoride membrane and SDS-PAGE gels were obtained from Bio-Rad. Rabbit anti-phospho-stress-activated protein kinase/JNK (Thr183/Tyr185), anti-phospho-c-Jun (Ser63), anti-cleaved caspase-3, and anti-caspase-3 antibodies were purchased from Cell Signaling Technology (Beverly, MA). Rabbit and sheep anti-tyrosine hydroxylase polyclonal antibodies were obtained from Chemicon (Temecula, CA). Media and sera were purchased from Invitrogen. Salen manganese complexes were gifts from Eukarion, Inc. (Bedford, MA). Osmotic minipumps (Alzet 2004) were from Alza Scientific Products (Mountain View, CA).

Cell Culture—The rat dopaminergic cell line 1RB6AN57 (N27) was grown in RPMI 1640 medium supplemented with 10% fetal calf serum (Invitrogen), 100 units/ml penicillin, and 100 μg/ml streptomycin. Cell
viability was determined by MTT incorporation (22). DNA fragmentation was examined by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) analysis with an in situ cell death detection kit (Roche Molecular Biochemicals) according to the manufacturer's instructions (22). Stained cells were counted in 10 randomly chosen microscopic fields (at least 500 cells). Data were expressed as the mean ± S.E. of the percentage of total cells that displayed TUNEL staining. To evaluate the effect of the salen manganese complexes on cell death, these compounds were added 1 h prior to paraquat or lactacystin. Caspase-3 activity was performed using a commercially available kit from Bio-Rad (Hercules, CA) as previously described (22). Briefly, cells were pelleted and subsequently lysed. Whole supernatant following sedimentation was incubated with the synthetic substrate cabobenzoxy-Asp-Glu-val-Asp-7-amino-4-trifluoromethylcoumarin for 2 h at 37 °C. Measurements were made on a fluorescent microplate reader using filters for excitation (400 nm) and detection of emitted light (530 nm). Serial dilutions of amino-4-trifluoromethylcoumarin were used as standards. A negative control in which caspase-3 inhibitor (Ac-DEVD-chloromethyl ketone) was added and a positive control containing apopain were used to test the efficacy of the assay.

**Primary Mesencephalic Cultures**—Primary mesencephalic cell cultures were prepared from embryonic gestation day 14–15 mouse embryos as described previously (12). Briefly, dissociated cells were seeded at 10⁵ cells per well onto poly-L-lysine-coated 24-well culture plates. Cultures were maintained at 37 °C in a humidified atmosphere containing 95% air and 5% carbon dioxide, in Neurobasal medium (Invitrogen) containing 2% B27 supplement, 2 mM glutamate, 100 units/ml penicillin, and 100 µg/ml streptomycin. After 4 days, one-half of the medium was replaced with fresh medium. Cells were then grown an additional 2 days and then treated with 40 µM paraquat for 24 h. The number of tyrosine hydroxylase (TH)-positive neurons in mesencephalic cultures was determined as described previously (12). The specificity of neurotoxicity was analyzed by double label immunostaining with the anti-TH antibody and antibodies against phospho-JNK, phospho-c-Jun, and cleaved caspase-3, respectively, as described previously (12). Experiments were repeated with cultures isolated from four independent dissections.

**Immunocytochemistry**—Cultures were fixed with paraformaldehyde in phosphate-buffered saline and permeabilized with 0.3% Triton X-100 in phosphate-buffered saline as described previously (12). Primary antibodies included the following: sheep polyclonal anti-TH (1: 500), rabbit polyclonal anti-phospho-JNK (1:100), rabbit polyclonal anti-phospho-c-Jun (1:100), and rabbit polyclonal anti-cleaved caspase-3 (1:200). The secondary antibodies were rhodamine-conjugated goat anti-mouse IgG (Jackson ImmunoResearch; 1:200) and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Vector, Burlington, CA, 1:200). 4′,6-Diamidino-2-phenylindole (DAPI) (Vector) was used to counterstain nuclei. Control experiments included omitting primary antibody.

Salen Manganese Complex Administration in Vivo—Eight-week-old male Sprague-Dawley (Jackson Laboratory, Bar Harbor, ME) mice were anesthetized with 4% isoflurane in 70% N₂O/O₂ (1:1) and subcutaneously implanted with an osmotic minipump containing either 5% mannitol or 15 mM EUK-189 (dissolved in 5% mannitol). Pumps delivered EUK-189 at a rate of 0.25 µmol/day (21).

**Paraquat Administration**—Mice were intraperitoneally injected with either saline or 7 mg/kg paraquat (dissolved in saline) at 2-day intervals for a total of 10 doses. Animals were killed at day 7 or 8 after the last administration as previously described (12). Experimental protocols were in accordance with the National Institutes of Health Guidelines for Use of Live Animals and were approved by the Animal Care and Use Committee at the Buck Institute of Age Research.

**Stereological TH-positive Neuron Counts**—Littermates were fixed by perfusion as previously described (12). Cryostat-cut sections (40 µm) were taken through the entire midbrain. TH-positive neurons were immunolabeled by incubating the tissue sections successively with a rabbit polyclonal anti-TH antibody (1:200) and biotinylated horse anti-rabbit IgG (1:200, Vector Laboratories) and then following the staining procedure outlined by the manufacturers of Vectastain ABC kit (Vector Laboratories). The avidin-biotin-peroxidase complex (DAB) was used as the substrate. The total number of TH-positive neurons in the substantia nigra pars compacta was counted from four to five littermates per group by using the optical fractionator method, an unbiased stereological technique of cell counting (23), as previously described (12).

**Western Blot Analysis**—Total protein was isolated from brain tissue as described previously (12). Protein concentration of the supernatant following sedimentation was incubated with the synthetic substrate cabobenzoxy-Asp-Glu-val-Asp-7-amino-4-trifluoromethylcoumarin for 2 h at 37 °C. Measurements were made on a fluorescent microplate reader using filters for excitation (400 nm) and detection of emitted light (530 nm). Serial dilutions of amino-4-trifluoromethylcoumarin were used as standards. A negative control in which caspase-3 inhibitor (Ac-DEVD-chloromethyl ketone) was added and a positive control containing apopain were used to test the efficacy of the assay.

**RESULTS**

**Protective Effects of EUK Compounds on Paraquat-induced Neurotoxicity in a Dopaminergic Midbrain-derived Rat Cell Line**—We used two separate salen manganese complexes, EUK-134 and EUK-189, in this study (Fig. 1). EUK-189 has both SOD and catalase activities equivalent to those of EUK-134 but has increased lipophilicity and neuroprotective activity (20). N27 is an immortalized dopaminergic neuronal cell line isolated from fetal rat mesencephalic cultures (24). The N27 cell line produces dopamine and expresses the dopamine-synthesizing enzyme tyrosine hydroxylase (TH) and the dopamine transport (DAT). Recently, we used this cell line as a model to study the potential role of paraquat on the JNK signaling pathway, because it relates to dopaminergic cell death associated with PD (12). As previously shown (12), treatment with 400 µM paraquat for 18–24 h increased caspase-3 activation, cell death, and DNA fragmentation compared with untreated control. However, when EUK-134 or EUK-189 (15 or 30 µM) was added 1 h before addition of paraquat, caspase-3 activation, cell death, and DNA fragmentation were all significantly inhibited (Fig. 2). In the case of 30 µM EUK-189, paraquat-mediated increases in caspase-3 activation, cell death, and DNA fragmentation were all significantly inhibited (Fig. 2). Neither EUK-134 nor EUK-189 at the concentrations used were stimulatory or inhibitory for neuronal survival without paraquat (data not shown).

Lactacystin is a selective proteasome inhibitor; even at high concentration and long treatment times, it does not significantly inhibit other proteases (25). To study whether the effects of the salen manganese complexes were specific for oxidative stress-induced cell death, N27 cells were treated with EUK-134 or EUK-189 (15 or 30 µM) for 1 h prior to treatment with 5 µM lactacystin. Cell death and DNA fragmentation were analyzed by MTT and TUNEL staining methods at 24 h, respectively. As seen in Fig. 3, there was no difference in lactacystin-induced apoptotic death in the absence or presence of EUK compounds.

**Protective Effects of EUK Compounds on Paraquat-induced Dopaminergic Neurotoxicity in Primary Mesencephalic Cultures Is Due to Inhibition of JNK Pathway Activation—**

[Image of structures of the salen manganese (EUK) complexes. The ring ligands (R) of the two compounds differ as shown. The axial ligand (X) is chloride for EUK-134 and acetate for EUK-189. Rad. Equal concentrations of protein extracts were electrophoretically resolved on SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes. Primary antibodies for Western blot analysis were used at the following dilutions: phospho-JNK (1:1000), phospho-c-Jun (1:1000), caspase-3 (1:1000), and β-actin (1:5000). Detection was performed using horseradish peroxidase-conjugated secondary antibody and an ECL kit (Amersham Biosciences). Statistical Analysis—All data are expressed as mean ± S.E. for the number (n) of independent experiments performed. Differences among the means for all experiments described were analyzed using one- or two-way analysis of variance. Newman-Keuls post-hoc analysis was employed when differences were observed by analysis of variance testing (p < 0.05).
previous results suggested that paraquat-generated superoxide may lead to activation of the JNK signaling pathway resulting in subsequent dopaminergic neuronal apoptosis, because these could both be attenuated in vitro by either the SOD mimetic, manganese(III) tetrakis (4-benzoic acid) porphyrin (MnTBAP), or the JNK-specific inhibitor SP600125 (12). To assess the neuroprotective ability of the salen manganese complexes in relation to paraquat-induced cell death on a cellular level in primary dopamine midbrain neurons, we examined the effects of EUK-134 or EUK-189 administration on paraquat-treated primary mesencephalic cultures via dual immunofluorescence with antibodies specific for TH and either phospho-JNK, phospho-c-Jun, or cleaved caspase-3, respectively, coupled with 4',6-diamidino-2-phenylindole staining. Cultures were pretreated with EUK-134 or EUK-189 1 h prior to the addition of 40 μM paraquat (PQ). As shown in Fig. 4A, EUK-134 or EUK-189 both separately reduced the colocalization of phospho-JNK, phospho-c-Jun, and activated caspase-3 with TH-positive neurons after 18 h of paraquat treatment. Cells were next stained for TH at 24 h following paraquat treatment, and TH-positive neurons were counted. Pretreatment with EUK-134 or EUK-189 was found to protect TH-positive neurons from paraquat-induced cell death (Fig. 4B). These data, coupled with our previous findings (12), suggest that the salen manganese complexes attenuate paraquat-induced neuronal cell death via inhibition of JNK activation and c-Jun phosphorylation.

**FIG. 2.** Effects of EUK compounds on paraquat-induced caspase-3 activation and cell death in dopaminergic N27 cells in vitro. N27 cells were treated with EUK-134 or EUK-189 1 h prior to the addition of 400 μM paraquat (PQ). Based on previous studies (12), caspase-3 activity at 18 h (A), cell viability at 24 h (B), and TUNEL-positive cells at 24 h (C–E) were measured. Mean ± S.E., n = 5, *p < 0.01, significantly from paraquat. White bar, PQ alone; striped bar, PQ plus 15 μM EUKs; and black bar, PQ plus 30 μM EUKs.

**FIG. 3.** Effects of EUK compounds on lactacystin-induced cell death in dopaminergic N27 cells in vitro. N27 cells were treated with EUK-134 or EUK-189 1 h prior to the addition of 5 μM lactacystin. Cell viability (A) and TUNEL-positive cells (B) were measured at 24 h. Mean ± S.E., n = 5, *p < 0.05, EUK compound-treated versus lactacystin alone. White bar, lactacystin alone; striped bar, lactacystin plus 15 μM EUKs; and black bar, lactacystin plus 30 μM EUKs.

**FIG. 4.** Protective effects of EUK compounds on paraquat-induced nigral dopaminergic neuronal damage in primary cultures. Mesencephalic cultures were treated with 0.5 μM EUK-134 or EUK-189 1 h prior to the addition of 40 μM paraquat as previously described (12). A, neurons that were positive for phospho-JNK (black bar), phospho-c-Jun (white bar), and activated caspase-3 (striped bar) among TH-positive neurons were counted 18 h after paraquat treatment, respectively, as previously described (12). B, TH-positive neuron counts in paraquat-treated mesencephalic cultures 24 h after paraquat treatment. Mean ± S.E., n = 4, *p < 0.01, significantly from paraquat.
EUK-189 Inhibits Paraquat-induced Reduction in TH-positive SNpc Cell Numbers in Vivo via Inhibition of JNK Pathway Activation—To examine whether EUK-189 attenuates the selective loss of nigrostriatal dopaminergic neurons after paraquat administration in vivo, we implanted mice with pumps containing either 5% mannitol (as vehicle control) or 15 mM EUK-189 1 day prior to paraquat treatment. As previously demonstrated (12), exposure of mice to paraquat produced a substantial loss of nigral dopamine neurons when compared with unlesioned controls (Fig. 5A). However, subcutaneous administration of the EUK-189 significantly attenuated the loss of nigral dopaminergic neurons when examined at day 8 following the last paraquat treatment (Fig. 5A). In keeping with previous studies (11, 12), stereological analysis revealed that paraquat administration resulted in an ~39% loss of substantia nigra pars compacta (SNpc) TH-positive neurons compared with saline-treated controls (Fig. 5B). In contrast, paraquat-treated mice that received EUK-189 displayed a significant reduction in the loss of SNpc dopaminergic neurons compared with animals treated paraquat alone (Fig. 5B). Indeed, the number of SNpc TH-positive neurons in EUK-189-treated animals did not significantly differ from saline-treated controls following paraquat administration.

To investigate whether inhibition of the JNK apoptotic cascade contributed to the neuroprotection conferred by EUK-189 following paraquat injection, the levels of phospho-JNK, phospho-c-Jun, and cleaved caspase-3 were detected by Western blot analysis of SNpc tissues. As previously demonstrated (12), the levels of phosphorylated JNK, phosphorylated c-Jun, and cleaved caspase-3 were all enhanced in SNpc tissues prepared from paraquat-treated mice compared with saline tissues prepared from mice in the saline treatment group (Fig. 6). However, pretreatment with EUK-189 completely suppressed the paraquat-induced increase in the levels of phosphorylation of JNK and c-Jun and caspase-3 cleavage (Fig. 6).

**DISCUSSION**

In the present study, we demonstrate that pretreatment of dopaminergic cultures in vitro or systemic treatment of mice in vivo with synthetic catalytic scavengers of reactive oxygen species, the salen manganese complexes EUK-134 and EUK-189, conferred neuroprotection against selective paraquat-mediated dopaminergic nigral cell death. In vitro and in vivo evidence from both our previous work (12) and this current study indicate that these compounds likely attenuate paraquat-induced neuron damage via inhibition of the activation of JNK-mediated apoptosis.

With increased understanding both of the mechanisms of oxidative stress and the role of antioxidants, it has become apparent that antioxidant defense systems normally exist in a balance with endogenous reactive oxygen species. Disruption of this balance appears to be one of the major factors involved in the selective neuropathogenesis associated with PD (26). Studies have shown, for example, that the levels of the thiol-reducing agent glutathione decrease within the SN of patients with Parkinson disease (27, 28), whereas the concentrations of iron, which can act as a catalyst for detrimental oxidative reactions, are elevated (29, 30). Furthermore, the levels of the DNA oxidation by-product 8-hydroxy-2-deoxyguanosine (31, 32), lipid peroxidation (33), and the protein oxidation by-product 4-hydroxy-2-nonenal (34) are all elevated in the SN of PD patients versus age-matched controls, suggesting an involvement of oxidation stress in the ensuing midbrain dopaminergic cell loss.

Some epidemiological investigations have suggested that increased exposure to agricultural chemicals via living in a rural environment, drinking well water, or occupational exposure may be a potential environmental risk factor for the disease (3–9). The widely used herbicide paraquat has been demonstrated to selectively damage the nigrostriatal dopaminergic system (11) and exposure to this specific agricultural chemical has been postulated to be a prime risk factor for PD (3, 7). Experimental paraquat exposure in vivo causes brain α-synuclein aggregation and formation of Lewy body-like neuronal inclusions (35). The mechanisms of neurotoxicity associated with exposure to paraquat are most likely mediated via oxidative stress. Mutations in the DJ-1 gene are linked with autosomal recessive early onset familial form of PD (36), and paraquat has been shown to oxidize the DJ-1 protein (37).

Superoxide anion radicals can be generated by paraquat through both redox cycling via reaction with molecular oxygen and electron transfer reactions with NADH-dependent oxidoreductases (38–40). Superoxide anion radicals may then be converted to H₂O₂ via one of the cellular SODs, Mn-SOD in the mitochondria and Cu/Zn-SOD in the cytosol. H₂O₂ can, in the
presence of iron, subsequently undergo conversion to highly reactive and harmful hydroxyl radicals via the Fenton reaction. Catalase or glutathione peroxidase normally catalyzes the breakdown of \( \text{H}_2\text{O}_2 \) to \( \text{O}_2 \) and \( \text{H}_2\text{O} \). EUK-134 and EUK-189 are mimetics of both superoxide dismutase and catalase and can therefore act to detoxify hydroxyl radicals completely (13–15). In this study, the administration of EUK-134 or EUK-189 prevented paraquat-induced dopaminergic neuron death, suggesting that reactive oxygen species acts upstream in the cell death pathway following exposure to paraquat.

Our results clearly indicate that oxidative stress is involved in the selective neuronal cell death of dopaminergic neurons following paraquat treatment both in vitro and in vivo. Activation of the JNK signaling pathway is considered as part of an oxidative stress response in a number of cell types (41, 42). We previously demonstrated that activation of JNK and c-Jun is increased within the SNpc of paraquat-mediated adult animals and that MnTBAP administration prevents paraquat-induced JNK activation and subsequent dopaminergic apoptosis in vitro (12). However, MnTBAP does not cross the blood-brain barrier (43) and therefore was not a viable option for assessing oxidative stress in paraquat-induced dopaminergic cell death in vitro. Pretreatment of mice with the bioavailable compound EUK-189 was found to inhibit the phosphorylation of JNK and c-Jun and caspase-3 activation in SNpc dopaminergic neurons in agreement with our previous in vitro experiment data, suggesting that EUK-189 can cross the blood-brain barrier and attenuate dopaminergic nigral cell death. Chronic systemic administration of EUK-189 was previously found to significantly decrease in lipid peroxidation and protein oxidation in the aging brain and to reverse age-related learning impairment (21). EUKs have previously been proven to be effective against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-mediated toxicity at least in vitro, and we now demonstrate that they are protective in vivo in a second model of the disease, paraquat intoxication. This suggests a common mechanism of action, i.e. oxidative stress, in selective dopaminergic SN neurodegeneration associated with two separate models of the disease.

Together, our data provide direct evidence that systemic administration of bioavailable synthetic SOD/catalase mimetics protects against selective paraquat-mediated dopaminergic neuronal cell death through mechanisms involving the elimination of oxidative damage and the modulation of signal transduction pathways. Given the potential for exposure to this herbicide to increase the risk for the development of PD and the recent establishment of its systemic administration as a viable model for the disease, this has obvious therapeutic implications.

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REFERENCES

1. Parno, L. S. (1996) J. Neuropathol. Exp. Neurol. 55, 259–272
2. Lang, A. E., and Lozano, A. M. (1998) N. Engl. J. Med. 339, 1044–1053
3. Hertzman, C., Wiens, M., Bowering, D., Snow, B., and Calne, D. (1990) Am. J. Ind. Med. 17, 349–355
4. Jimenez-Jimenez, F. J., Mateo, D., and Gimenez-Roldan, S. (1992) Movement Disorders 7, 149–152
5. Semchuk, K. M., Love, E. J., and Lee, B. G. (1992) Neurology 42, 1328–1335
6. Hubble, J. P., Cao, T., Hassanein, R. E., Neuberger, J. S., and Koller, W. C. (1993) Neurology 43, 1893–1897
7. Liu, H. H., Tasl, M. C., Chen, C. J., Jeng, J. S., Chang, Y. C., Chen, S. Y., and Chen, R. C. (1997) Neurology 48, 1583–1588
8. Gorell, J. M., Johnson, C. C., Rybicki, B. A., Peterson, E. L., and Richardson, R. J. (1998) Neurology 51, 1355–1360
9. Stephenson, J. (2000) JAMA 283, 3055–3056
10. Thiruchelvam, M., Richfield, E. K., Baggs, R. B., Tank, A. W., and Cory-Slechta, D. A. (2000) J. Neurosci. 20, 9297–9214
11. McCormack, A. L., Thiruchelvam, M., Manning-Bog, A. B., Thiffault, C., Langston, J. W., Cory-Slechta, D. A., and Di Monte, D. A. (2002) Neurobiol. Dis. 10, 119–127
12. Peng, J., Mao, X. O., Stevenson, F. F., Hsu, M., and Andersen, J. K. (2004) J. Biol. Chem. 279, 32626–32632
13. Baudry, M., Etienne, S., Bruce, A., Palucki, M., Jacobsen, E., and Malbry, F. (1995) Biochem. Biophys. Res. Commun. 192, 964–968
14. Gonzalez, P. K., Zhuang, J., Benson, P. F., Menconi, M. J., Fink, M. P., Baudry, M., Etienne, S., Bruce, A., Palucki, M., and Jacobsen, E. (1995) J. Pharmacol. Exp. Ther. 275, 798–806
15. Baker, K., Marcus, C. B., Huffman, K., Kruk, H., Malbry, F., Doctrow, S. R., Gonzalez, P. K., Zhuang, J., Benson, P. F., Menconi, M. J., Fink, M. P., Baudry, M., Etienne, S., Bruce, A., Palucki, M., and Jacobsen, E. (1998) J. Pharmacol. Exp. Ther. 284, 215–221
16. Malbry, F., Doctrow, S. R., Orr, P. L., Toco, G., Fedoseyeva, E. V., and Benichou, G. (1997) Cell. Immunol. 177, 62–68
17. Prasad, K. N., Carvalho, E., Kentroti, S., Edwards-Prasad, J., Freed, C., and Vernadakis, A. (1994) In Vitro Cell Dev. Biol. Anim. 30A, 596–603
18. Adams, J. D., Jr., Chang, M. L., and Klaudian, L. (2001) J. Biol. Chem. 276, 8345–8353
19. Liu, R., Liu, I. Y., Bi, X., Thompson, R. F., Doctrow, S. R., Malbry, F., and Baudry, M. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 8526–8531
20. Peng, J., Wu, Z., Wu, Y., Hsu, M., Stevenson, F. F., Boonpleueang, R., Roffler-Schult, V. K., and Andersen, J. K. (2004) J. Biol. Chem. 279, 44285–44291
21. West, M. J. (1999) Trends Neurosci. 22, 51–61
22. Prasad, K. N., Carvalho, E., Kentroti, S., Edwards-Prasad, J., Freed, C., and Vernadakis, A. (1994) In Vitro Cell Dev. Biol. Anim. 30A, 596–603
23. Adams, J. D., Jr., Chang, M. L., and Klaudian, L. (2001) J. Biol. Chem. 276, 8345–8353
24. Perry, T. L., and Yong, V. W. (1988) Neurosci. Lett. 67, 269–274
25. Pearce, R. K., Owen, A., Daniel, S., Jenner, P., and Marsden, C. D. (1997) J. Neural. Transm. 104, 661–677
26. Sofic, E., Paulus, W., Jellinger, K., Riederer, P., and Youdin, M. B. (1991) J. Neurochem. 56, 978–982
27. Jellinger, K. A., Kienzl, E., Kreutzberg, W., and Di Monte, D. A. (2002) J. Neural. Transm. 661–677
28. Elbek, P. M., Doctrow, S. R., Orr, P. L., Toco, G., Fedoseyeva, E. V., and Benichou, G. (1997) Cell. Immunol. 177, 62–68
29. Prasad, K. N., Carvalho, E., Kentroti, S., Edwards-Prasad, J., Freed, C., and Vernadakis, A. (1994) In Vitro Cell Dev. Biol. Anim. 30A, 596–603
30. Adams, J. D., Jr., Chang, M. L., and Klaudian, L. (2001) J. Biol. Chem. 276, 8345–8353
31. Liu, R., Liu, I. Y., Bi, X., Thompson, R. F., Doctrow, S. R., Malbry, F., and Baudry, M. (2003) Proc. Natl. Acad. Sci. U. S. A. 96, 8526–8531