Selenoprotein T Exerts an Essential Oxidoreductase Activity That Protects Dopaminergic Neurons in Mouse Models of Parkinson’s Disease

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Abstract

Aims: Oxidative stress is central to the pathogenesis of Parkinson’s disease (PD), but the mechanisms involved in the control of this stress in dopaminergic cells are not fully understood. There is increasing evidence that selenoproteins play a central role in the control of redox homeostasis and cell defense, but the precise contribution of members of this family of proteins during the course of neurodegenerative diseases is still elusive. Results: We demonstrated first that selenoprotein T (SelT) whose gene disruption is lethal during embryogenesis, exerts a potent oxidoreductase activity. In the SH-SY5Y cell model of dopaminergic neurons, both silencing and overexpression of SelT affected oxidative stress and cell survival. Treatment with PD-inducing neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or rotenone triggered SelT expression in the nigrostriatal pathway of wild-type mice, but provoked rapid and severe parkinsonian-like motor defects in conditional brain SelT-deficient mice. This motor impairment was associated with marked oxidative stress and neurodegeneration and decreased tyrosine hydroxylase activity and dopamine levels in the nigrostriatal system. Finally, in PD patients, we report that SelT is tremendously increased in the caudate putamen tissue. Innovation: These results reveal the activity of a novel selenoprotein enzyme that protects dopaminergic neurons against oxidative stress and prevents early and severe movement impairment in animal models of PD. Conclusions: Our findings indicate that selenoproteins such as SelT play a crucial role in the protection of dopaminergic neurons against oxidative stress and cell death, providing insight into the molecular underpinnings of this stress in PD.

Introduction

Parkinson’s disease (PD) is the most common neurodegenerative movement disorder frequently associated with aging since it affects ~1% of the elderly population (17). There is currently no cure and no effective mechanism-based treatment (41). Clinical symptoms of PD include rigidity, resting tremor, bradykinesia, and postural instability (35, 55). These manifestations are largely attributable to a selective degeneration of midbrain dopaminergic neurons of the nigrostriatal pathway, which leads to progressive decline of motor functions. The basis of the preferential dopaminergic neuron vulnerability is not understood. However, functional analysis of genetic mutations in heritable forms of PD and evidence gained from studies with neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine...
Involvement of oxidative stress in dopaminergic neuron death and in the pathogenic mechanisms of Parkinson's disease (PD) is well established. However, the molecular underpinnings of this stress in dopaminergic neurons are not totally elucidated. The results of our present study reveal the activity of selenoprotein T (SelT) as a novel enzyme highly expressed in PD patients, which protects dopaminergic neurons against oxidative stress and prevents early and severe movement impairment in animal models of PD. These data provide direct evidence for the involvement of a selenoprotein in the control of oxidative stress and survival of dopaminergic neurons and consequently in protection against neurotoxin-provoked motor impairment.

(MPTP) or rotenone in animal models pointed to common pathogenic mechanisms, including mitochondrial complex I impairment and associated oxidative and nitrosative stresses, which are widely viewed as the leading causes of dopaminergic neuronal death in the substantia nigra compacta (SNc) (9, 45, 52). In fact, complex I inhibition and oxidative damage were shown to be relevant to naturally occurring PD since complex I deficiency and reactive oxygen species (ROS) scavenger depletion were established in the SNc of patients with idiopathic and presymptomatic PD (29). In this disease, detoxifying enzymes and reducing agents are unable to maintain intracellular ROS levels at a homeostatic set point, leading to cellular demise (26, 41). Thus, although mitochondria and other cellular compartments hold an extensive antioxidant defense system, it is now well established that this arsenal is overwhelmed by excess ROS in PD (14, 36, 40).

To maintain a redox balance, cells engage several reducing enzymes, including members of the selenoprotein family, which incorporate the selenium atom as a selenocysteine (Sec) residue through highly specialized protein translation machinery (25). Selenoproteins such as glutathione peroxidase (GPx) or thioredoxin reductase (TrxR) play instrumental roles in cell survival and homeostasis (5, 43). These enzymes exert antioxidant activities via a catalytic domain encompassing the Sec residue, which regulate diverse cellular functions, including redox equilibrium, protein folding, or Ca2+ homeostasis, all processes known to be deregulated in neurodegenerative diseases and particularly in PD (22, 41).

In an attempt to uncover new neuroprotective cues relevant for neurodegenerative pathologies, including PD, we have previously identified selenoprotein T (SelT), a selenoprotein with unknown physiological function (32), in a panenogenic screen as a gene regulated by the neurotrophic factor pituitary adenylate cyclase-activating polypeptide (PACAP) in the catecholaminergic PC12 cells (23). SelT contains a thioredoxin-like fold encompassing the Sec residue within a putative catalytic domain (19) and has been shown to be involved in fibroblast cell adhesion (48) and PC12 cell calcium homeostasis (23). The SelT gene is robustly and widely expressed during embryogenesis, but declines postnatally in most tissues, including the brain (42, 53). However, its stimulation in PC12 cells by PACAP, which exerts potent neuroprotective and antioxidant actions, and its potential involvement in the control of redox homeostasis (48) suggested to us that this new selenoprotein could exert an oxidoreductase activity that would protect neurons that are prone to oxidative stress such as the midbrain dopaminergic neurons as evidenced in PD.

Using a recombinant SelT, we first showed that this selenoprotein exerts a potent TrxR-like activity in an in vitro assay. This enzymatic activity was required in the dopaminergic cell model SH-SY5Y to counter 1-methyl-4-phenylpyridinium (MPP+) -induced oxidative stress and cell death. In fact, we found that conditional disruption of SelT in the brain in Nes-Cre/SelTP−/− mice exacerbates the sensitivity to MPTP or rotenone administration, provoking rapid-onset and severe motor impairment. The lack of SelT expression in the nigrostriatal pathway led to enhanced neurotoxin-induced neurodegeneration associated with elevated oxidative stress and altered tyrosine hydroxylase (TH) activity and dopamine production.

**Results**

**Recombinant SelT displays a TrxR-like activity**

Based on its main structural feature, that is, a Sec-containing thioredoxin-like fold, we reasoned that SelT might exert an oxidoreductase activity through its CVSU redox machinery (Fig. 1A). To test this hypothesis, we engineered an SelT form compatible with bacterial protein synthesis machinery by replacing the Sec residue by a Cys in the thioredoxin-like fold (Fig. 1A). However, we failed to purify such a recombinant SelT protein from bacterial extracts (data not shown). Comparison of the SelT protein sequence with that of a soluble member of the thioredoxin family, SelW (1), revealed several structural similarities except for a hydrophobic stretch of amino acids, including several a-helices, present in SelT, but absent in SelW (Fig. 1A). This region has been shown to be involved in the integration of SelT in the endoplasmic reticulum (ER) membranes (23). Therefore, we presumed that this hydrophobic region might hamper the production of SelT in Escherichia coli since recombinant SelW, which lacks this segment, has previously been generated in this heterologous system (1).

We thus engineered the SelT protein without this domain, which could indeed be readily purified at high yield from bacterial extracts (Fig. 1B). We then tested its putative activity as a GPx or a TrxR, which represent two major types of selenoprotein activities due to a CXXU domain (19). In contrast to purified bovine erythrocyte GPx used as a positive control, recombinant SelT did not display a GPx activity assayed using Cayman's GPx assay (data not shown). However, recombinant SelT exhibited an important TrxR-like activity compared with that of a control rat liver TrxR, which was assayed through the reduction of 5,5′-dithio-bis(2-dinitrobenzoic acid) (DTNB) with NADPH to 5-thio-2-nitrobenzoic acid (Fig. 1C). The TrxR-like activity of recombinant SelT was dependent on Cys/Sec residues of the thioredoxin-like fold (Fig. 1C). To ascertain this TrxR-like activity of SelT, we used the TrxR-specific inhibitor, aurintricarboxylic acid (ATM), which totally abrogated the oxidoreductase capacity of SelT as well as that of the liver TrxR used as control (Fig. 1C). SelT exhibited a relative kcat (turnover number) of 3940 min−1, which is comparable with that of TrxR (39). These data demonstrate that SelT could exert strong oxidoreductase activity as a TrxR-like enzyme.
SelT protects dopaminergic cells by inhibiting MPP⁺-induced ROS production

To determine whether SelT could be involved in the protection of dopaminergic neurons in degenerative conditions and whether it may control oxidative stress in these cells, we used the model of MPP⁺-induced neurotoxicity in the neuroblastoma SH-SY5Y cells. We first assessed if the neurotoxin would stimulate SelT expression in these cells. We found indeed that MPP⁺ (50 μM) triggers a twofold increase in SelT immunoreactivity in SH-SY5Y cells after 36 h of treatment, while TH immunoreactivity was not affected in these conditions (Fig. 2A, B), as previously described (12, 44). This result was confirmed by Western blotting, which also revealed a specific twofold increase in SelT protein levels in SH-SY5Y cells following MPP⁺ treatment (Fig. 2C, D). Consistently, MPP⁺ triggered a twofold stimulation of SelT mRNA levels in these neurons as measured by quantitative polymerase chain reaction (PCR) (Fig. 2E), indicating that SelT gene expression is enhanced in conditions of neurotoxicity associated with oxidative stress as obtained with MPP⁺.

To establish a link between increased expression of SelT and higher survival rate of dopaminergic neurons as a consequence of SelT-mediated reduction of ROS levels, we assessed these parameters after overexpression of SelT-eGFP or mutant SelT-eGFP chimeric constructs in SH-SY5Y cells and exposure to MPP⁺. Overexpression of SelT efficiently protected SH-SY5Y cells against the toxicity of MPP⁺ since the number of eGFP-positive cells was several fold higher after transfection with SelT-eGFP compared with an empty vector or a mutant SelT-eGFP where the Sec amino acid has been replaced by a neutral Ser residue (65.7% ± 4.9% vs. 25.3% ± 3.1% or 24.7% ± 5.9%, respectively, mean ± standard error of the mean [SEM] relative to control) (Fig. 3A, B). Concurrently, SelT-eGFP, but not the empty vector or the mutant SelT-eGFP, significantly reduced ROS levels generated in the presence of MPP⁺ (143.6% ± 15.0% vs. 205.8% ± 7.6% or 193.1% ± 5.0%, respectively, mean ± standard error of the mean [SEM] relative to control) (Fig. 3C). In addition, we showed that SelT also protects, through its Sec-containing active site, dopaminergic cells against oxidative stress produced by hydrogen peroxide treatment (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/ars). Conversely, reduction of SelT levels through lentiviral shRNA transduction (Supplementary Fig. S2) led to higher sensitivity of SH-SY5Y cells to MPP⁺ toxicity compared with a scrambled shRNA (54.2% ± 9.4% vs. 85.7% ± 1.7%, mean ± SEM relative to control) (Fig. 3D, E), which was associated with higher ROS levels (260.7% ± 12.1% vs. 130.0% ± 6.1%, mean ± SEM relative to control) (Fig. 3F).
Finally, we analyzed phosphorylated TH (phosphoTH) at Ser31 as a readout of the active enzyme to determine the impact of SelT on TH activity. Silencing of SelT expression provoked a reduction in phosphoTH levels in control and MPP\(^+\)-treated SH-SY5Y, suggesting that SelT is required for optimal TH activity in dopaminergic neurons in basal condition and under oxidative insult. Together, these data demonstrate that SelT protects SH-SY5Y cells and the dopamine-producing enzyme, TH, against excess ROS levels through its Sec-containing, thioredoxin-like catalytic domain.

SelT is induced in neurons and astrocytes of the nigrostriatal pathway in MPTP-treated mice

To investigate the possible involvement of SelT in the dopaminergic system in vivo, we analyzed SelT immunoreactivity in the SNc and striatum of mice intraperitoneally injected with MPTP (15 mg/kg, four doses). In this condition, progressive degeneration of dopaminergic neurons and fibers of the SNc and striatum, respectively, is observed as assessed by TH immunoreactivity (Fig. 4A, B). SelT was not detected in the SNc of control adult animals (Fig. 4C), in agreement with our previous immunohistochemical data showing low expression of SelT in the adult brain (53). By contrast, a dense TH immunolabeling of dopaminergic neurons was observed (Fig. 4C). Administration of MPTP induced intense SelT immunostaining 8 days post-treatment, which colocalized with the TH labeling in the degenerating SNc (Fig. 4C). Similarly, SelT immunoreactivity was not detected in the striatum of control animals, but was strongly increased in the degenerating striatum, as revealed by TH labeling, in MPTP-treated animals (Fig. 4D).

To define more precisely the spatiotemporal induction of SelT expression during the course of neurodegeneration, we analyzed nigrostriatal tissue sections obtained from brains of animals treated with MPTP for 2, 4, and 8 days. Figure 4E shows that SelT immunoreactivity is induced in dopaminergic neurons of SNc 2 days postinjection and increased steadily thereafter during the course of neurodegeneration. In contrast, SelT immunoreactivity was not observed in the few astrocytes present in this area, as revealed by glial fibrillary acidic protein (GFAP) labeling, which were mainly observed at day 8 post-treatment (Fig. 4E) as previously reported (31). In the striatum, SelT induction occurred not only in dopaminergic fibers but also in astrocytes, which are gradually activated during neurodegeneration as revealed by GFAP colabeling (Fig. 4F, see the insets). These data suggest...
FIG. 3. SelT promotes survival and inhibits oxidative stress in SH-SY5Y cells. (A) Confocal microscopic images of SH-SY5Y cells that were transfected with the pCiG2 vector expressing SelT (pCiG2-SelT) or a mutant version where the Sec residue was replaced by the Ser residue (pCiG2-SelTm), or with the empty vector (pCiG2), and challenged by MPP⁺ (100 μM) for 36 h. Transfected cells are stained in green due to eGFP expression thanks to an IRES sequence present in the pCiG2 vector. Nuclei are stained in blue by DAPI. Scale bar: 50 μm. (B) Quantification of the number of eGFP-positive cells in control and MPP⁺-treated conditions. The data are expressed as mean ± SEM and are compared using Student’s t-test, **p < 0.01 (n = 3 independent experiments with three determinations per condition in each experiment). (C) Transfected SH-SY5Y cells were treated or not with MPP⁺ (100 μM) and incubated with the DCFDA probe for 45 min. The fluorescence of ROS-oxidized DCF probe was measured. The data are expressed as mean ± SEM and are compared using Student’s t-test, *p < 0.05 (n = 3 independent experiments with six determinations per condition in each experiment). (D) Photomicrographs of SH-SY5Y cells that were transduced with lentiviral vectors for sh-SelT or scrambled shRNA and treated or not with MPP⁺ (100 μM) for 24 h. Scale bar = 50 μm. (E) Cell viability was assessed after transduction with sh-SelT or scrambled shRNA and treatment with MPP⁺. The data are expressed as mean ± SEM and compared using Student’s t-test, *p < 0.05 (n = 3 independent experiments with six determinations per condition in each experiment). (F) SH-SY5Y cells that were transduced with lentiviral vectors for sh-SelT or a scrambled shRNA and treated or not with MPP⁺ (100 μM) for 24 h were incubated with the DCFDA probe for 45 min, and the fluorescence of ROS-oxidized DCF probe was measured. The data are expressed as mean ± SEM and compared using Student’s t-test, **p < 0.001 (n = 4 independent experiments with six determinations per condition in each experiment). (G) Western blot analysis of phosphoTH-Ser31 in SH-SY5Y cells that were transduced with lentiviral vectors for sh-SelT or a scrambled shRNA and treated or not with MPP⁺ (100 μM) for 24 h. Alpha-tubulin (α-Tub) signal was used to ensure equal protein loading. (H) Quantification of phosphoTH-Ser31 signal observed by Western blot after normalization by quantified α-Tub signal. The data are expressed as mean ± SEM and are compared using Student’s t-test, *p < 0.05 (n = 2 independent experiments with four determinations per condition in each experiment). DAPI, 4,6-diamino-2-phenylindole; DCF, 2′,7′-dichlorofluorescein; ROS, reactive oxygen species. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars
FIG. 4. Induction of SelT expression in the nigrostriatal pathway following MPTP administration. (A) Exposure to MPTP (four doses of 15 mg/kg) provokes a progressive degeneration of dopaminergic neurons in the SNc and fibers in the striatum (Str) as revealed by TH immunolabeling of tissue sections at 2, 4, and 8 days post-treatment. Note that the cortex (Cox) region is devoid of labeling. (B) The number of TH-positive neurons in the SNc at the different times of treatment with MPTP (five sections from each animal were counted, n = 5 per animal group). (C) SelT and TH immunoreactivity in the SNc of control and MPTP-treated (8 days) mice. (D) SelT and TH immunoreactivity in the Str of control and MPTP-treated (8 days) mice. SelT was detected in dopaminergic fibers and astrocytes (Ast) in the Str. (E) SelT and GFAP immunoreactivity in the SNc of control and MPTP-treated mice at 2 (d2), 4 (d4), and 8 days (d8) post-treatment. (F) SelT and GFAP immunoreactivity in the Str of control and MPTP-treated mice at 2 (d2), 4 (d4), and 8 days (d8) post-treatment. The insets depict the colocalization of SelT with GFAP in Ast. Nuclei are stained in blue with DAPI. Similar results were obtained using several animals (n = 5 per animal group) in two different experiments. Scale bars = 50 μm. GFAP, glial fibrillary acidic protein; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SNc, substantia nigra compacta. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars
that SelT induction in the nigrostriatal pathway is part of a defense mechanism that occurs in neurons and astrocytes during neurodegeneration.

SelT deficiency leads to early embryonic lethality and higher sensitivity to MPTP in brain conditional knockout

To study the physiological antioxidant role of SelT in mice, we disrupted its gene either globally, by crossing a mice line carrying loxP-flanked exons 2–3 in the SelT allele (encompassing the sequence encoding the thioredoxin-like fold of SelT) with CMV-Cre transgenic mice, or conditionally in the brain by crossing the former mice with nestin (Nes)-Cre transgenic mice. Genotyping confirmed the occurrence of different alleles in offspring (Fig. 5A, B). Analysis of large numbers of offspring identified only heterozygous (SelT+/−) and wild-type (WT) mice, but no SelT−/− mice were born after SelT gene disruption (Fig. 5C), indicating that global homozygous SelT mutation causes embryonic lethality.

FIG. 5. Disruption of the SelT gene provokes early lethality and higher vulnerability to MPTP in the brain. (A) Schematic representation of WT, floxed, and mutant mouse SelT alleles showing the location of the LoxP sites introduced by homologous recombination to be able to delete exons 2–3, which contain the SelT redox center, including the Sec residue. The location of the primers used (arrows) and the size of the DNA fragments characteristic of the different alleles are also shown. (B) PCR analysis on tail DNA samples showing the different alleles (WT: wild-type; HT: heterozygous; KO: null) obtained after breeding of SelTfl mice with a second mice strain expressing Cre recombinase under the control of the CMV promoter (CMV-Cre) or the nerve cell-specific promoter and enhancer of the rat nestin gene (Nes-Cre). (C) The percentage of pups with different genotypes in 240 born animals was determined after inbreeding of CMV-Cre/SelTWT/fl (SelT+/−). No homozygous mice for the SelT− allele were found after total SelT gene knockout. (D) Western blot analysis of SelT expression in different tissues from Nes-Cre/SelTfl/fl. α-Tub was used as an internal control to ensure equal protein loading. (E) Western blot analysis of the Str showing the very low expression of SelT and its induction in WT, but not Nes-Cre/SelTfl/fl (KO) mice, following MPTP administration (15 mg/kg). α-Tub was used as an internal control to ensure equal protein loading. (F) Quantification of SelT signal observed by Western blot after normalization by quantified α-Tub signal. The data are expressed as mean ± SEM and are compared using Student’s t-test, **p<0.01 (n=3). (G) Motor skills of WT and Nes-Cre/SelTfl/fl (KO) mice were tested between 15 and 60 min after injection of the vehicle or MPTP (15 mg/kg). The forelimbs of the animals were placed on a horizontal bar, and the time spent on the bar was registered for each group. The data are expressed as mean ± SEM and are compared using Student’s t-test, **p<0.01 (n=6–7 per animal group). (H) A Kaplan–Meier curve for animal survival in the two groups in control or MPTP-treated conditions. The data were compared between groups using the Mantel–Cox test. **p<0.01 (n=7–8 per animal group). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars
To determine the embryonic stage of lethality of SelT−/− mice, embryos of time-mated pregnant females were surgically removed and genotyped at different time points of embryonic development (E8, E9, E10, E12, and E14). At these stages, no homozygous embryos could be found (Supplementary Table S1) and empty gestational sacs were observed in each pregnant SelT−/− female. Below E8, the embryos are not sufficiently developed to be collected properly. This analysis revealed that embryos died before embryonic day 8, indicating that SelT is indispensable for early stages of embryonic development.

We then investigated the impact of SelT gene disruption in the brain using conditional knockout mice. Brain SelT-deficient Nes-Cre/SelTfl/fl mice were viable and were observed at a normal Mendelian ratio. The specificity of SelT gene disruption in the brain was verified through the analysis of the expression of SelT in tissues such as the testis or the pancreas (Fig. 5D) where the selenoprotein is expressed in the adult (42, 53). Because SelT cannot readily be detected in the adult brain in basal conditions (53) (Fig. 4), we analyzed its expression and regulation in the striatum of Nes-Cre/SelTfl/fl mice and WT littermates after MPTP treatment. Western blot analysis confirmed the low expression of SelT in the striatum of control mice, likely due to occurrence in non-nerve cells, which did not change in knockout animals (Fig. 5E, F). Consistently, administration of MPTP significantly increased SelT levels in the striatum of WT, but not knockout, mice (Fig. 5E, F), confirming the upregulation of SelT in the striatum under MPTP treatment in WT mice as described above and showing the disruption of this gene in the brain of Nes-Cre/SelTfl/fl animals, including the striatum.

Strikingly, administration of a single dose of MPTP (15 mg/kg) rapidly induced tremor and absence of movement within 2 h post-treatment in Nes-Cre/SelTfl/fl animals, but not in WT littermates (Videos 1–4). To quantify the movement defect, Nes-Cre/SelTfl/fl animals and control littermates were subjected to a horizontal bar test adapted from Deacon (18), an assay consisting of placing the mice forelimbs on a horizontal bar and measuring the elapsed time before the animals leave the bar. This test showed that MPTP-treated Nes-Cre/SelTfl/fl mice remain immobile for a long time (>5 min) compared with untreated mutant mice and MPTP-treated or untreated WT mice, which mostly left the bar within seconds (Fig. 5G). Much to our surprise, the MPTP effect culminated in the death of majority of the knockout animals within 2 h after injection, in contrast to animals of the three other groups, which survived in control and MPTP-treated conditions, as illustrated by the Kaplan–Meier survival curve (Fig. 5H).

**SelT deficiency increases oxidative stress and impacts dopamine levels in the nigrostriatal pathway**

Because the neurotoxin, MPTP, selectively targets dopaminergic neurons *via* its neuroactive metabolite, MPP+, which is known to induce oxidative damage by inhibiting mitochondrial complex I activity, we reasoned that the phenotype observed in Nes-Cre/SelTfl/fl mice is the result of an excess of free radicals in the nigrostriatal structure that would lead to a rapid decline in TH activity and dopamine availability. To test the possibility of an impact of early oxidative stress on dopamine production in Nes-Cre/SelTfl/fl animals, we first analyzed 3-nitrotyrosine (3-NT) production, a biomarker for nitrating oxidants, in brain sections from these animals in comparison with control littermates. We found that a short exposure to MPTP induces a strong immunoreaction for 3-NT products in dopaminergic neurons and fibers of knockout mice in comparison with WT littermates, which exhibited a low level of 3-NT (Fig. 6A–D). Note that increased 3-NT immunoreaction is mainly observed in TH-labeled cells and not in neighboring neurons (indicated by arrows in Fig. 6A, C), confirming the specificity of MPTP action in the conditions used. This result shows that the absence of SelT provokes rapid accumulation of nitrosative species in dopaminergic neurons and fibers, indicative of acute oxidative stress upon MPTP treatment.

To determine whether dopamine biosynthesis is altered in these conditions and thus would account for the motor deficits observed in Nes-Cre/SelTfl/fl mice, we analyzed phosphoTH at Ser31 and Ser40 as a readout of the active enzyme and measured dopamine levels in the striatum of animals from the different groups. Strikingly, loss of SelT resulted in a substantial reduction in phosphoTH (Ser31, 52.8% ± 2.2%, and Ser40, 74.3% ± 3.4%, of control values, mean ± SEM) (Fig. 6E, F) and dopamine content (60.6% ± 8.4%, mean ± SEM) (Fig. 6G) in the striatum of Nes-Cre/SelTfl/fl in comparison with WT littermates. Treatment with MPTP for 1 h decreased phosphoTH (Ser31, 28.2% ± 0.5%, and Ser40, 43.7% ± 1.2%, mean ± SEM) and dopamine levels (57.2% ± 7.1%, mean ± SEM) in the striatum of WT animals and decreased further phosphoTH (Ser31, 23.4% ± 1.1%, and Ser40, 33.4% ± 2.8%, mean ± SEM) and dopamine content (35.8% ± 8.2%, mean ± SEM) in the striatum of Nes-Cre/SelTfl/fl compared with WT animals (Fig. 6E–G). Note that total TH was unchanged in KO mice or after MPTP treatment (Fig. 6E), indicating that total number of TH neurons and fibers are not altered in our conditions (MPTP 15 mg/kg, 1 h).

These results indicate that the severe motor deficits observed in Nes-Cre/SelTfl/fl mice upon MPTP treatment are associated with a decline in TH activity and dopamine content in the striatum after SelT gene disruption, which is exacerbated by excess free radicals present after injection of the neurotoxin.

**SelT deficiency accelerates dopaminergic neuron degeneration and motor dysfunction in rotenone-treated Nes-Cre/SelTfl/fl**

The strong motor defect observed after treatment of Nes-Cre/SelTfl/fl mice with a single dose of MPTP prompted us to test the effect of another neurotoxin such as rotenone, also known to trigger dopaminergic neuron degeneration and to replicate the pathology of PD. Consistent with the results obtained with MPTP, a single dose of rotenone at 1.5 mg/kg, which usually triggers progressive degeneration of nigral neurons in WT mice within few days (11, 50), also caused Nes-Cre/SelTfl/fl animal death (Fig. 7A). We have therefore used a progressive treatment with three lower doses at 0.25, 0.5, and 1 mg/kg of rotenone over 3 weeks, and we performed then a rearing behavioral test to assess the effect of the neurotoxin on motor skills, as previously described (11). Rotenone provoked a significant drop in the number of rears, which was more pronounced in Nes-Cre/SelTfl/fl compared with WT mice (Fig. 7B). At the low doses of rotenone used, only 1 Nes-Cre/SelTfl/fl mouse died after the last injection of...
the neurotoxin at 1 mg/kg (Fig. 7C). These data show that rotenone is more potent in altering motor function in Nes-Cre/SelTfl/fl mice, indicating that this neurotoxin as MPTP affects more severely the nigrostriatal system of the knockout mice compared with WT littermates.

Consistently, immunohistochemical analysis using TH labeling revealed that rotenone induces dopaminergic neuron and fiber degeneration in the SNC and the striatum, respectively, in both SelT mutant and WT mice (Fig. 8A, B), but neurodegeneration was more extensive in Nes-Cre/SelTfl/fl mice.
SelT is dramatically increased in the caudate putamen of postmortem PD patients

Because SelT expression was induced in the nigrostriatal pathway of animal models of PD, we asked whether this increase could also be observed in patients with the disease. We have therefore screened by quantitative PCR the expression of SelT in different human brain samples, including the caudate putamen (CPu) from control subjects and PD patients. Compared with control samples or with other regions of the brain, including the mesencephalon (Mes) or the subthalamic nucleus (STN), SelT mRNA levels were tremendously increased in the CPu samples of PD patients (>200-fold increase) (Fig. 9A). We also performed an immunohistochemical analysis of SelT and TH in the CPu area (Fig. 9B). As could be expected, dense TH immunolabeling was observed in the putamen (PUT) and, to a lesser extent, in the caudate (CD) areas of control subjects (Fig. 9C). SelT immunoreactivity was also detected in the CPu of the control aged subjects, but was markedly increased in this brain region of PD patients (Fig. 9C). Together, these data show that SelT expression at both the mRNA and protein levels is strongly increased in the affected CPu of patients with PD.

Discussion

We have found that the selenoprotein, SelT, plays a crucial role in the protection of dopaminergic neurons against oxidative stress and that its loss is associated with enhanced neurotoxin-induced degeneration of the nigrostriatal system, decreased dopamine production, and impaired motor function. These data are the first to show the occurrence of SelT in the nigrostriatal pathway and the essential role of a selenoprotein to maintain the functionality of the dopaminergic system and to preserve motor function in conditions of oxidative burden. In line with this finding, several studies have previously shown that certain selenoproteins, in particular TrxR, are able to protect neuronal cells (3, 37), but the impact on dopamine production and motor activity was not known yet.

As shown using a recombinant protein, SelT exerts an oxidoreductase activity that resembles that of a TrxR enzyme because SelT was effective, thanks to its thioredoxin-like fold, in catalyzing the reduction of an oxidized substrate in the presence of NADPH and was inhibited by the TrxR-specific inhibitor ATM. This finding indicates that SelT, which is located in the ER (23, 42, 53), represents an essential new component of the thioredoxin system in addition to TrxR1 and TrxR2, which act in the cytosol and mitochondria, respectively (34). This notion is supported by the lethality of SelT-deficient mice occurring early during embryogenesis as has been also observed for TrxR1 and TrxR2 knockout mice (16, 28), highlighting the important role of SelT and the thioredoxin system for embryonic development. We noticed that the percentage of SelT−/− animals is lower than might be expected (50% SelT+/− and 50% SelT−/− instead of 34% SelT+/− and 66% SelT−/− in case of embryonic mortality), suggesting a potential partial lethality of the SelT−/− embryos. Indeed, we found that litters from SelT−/− mouse intercrosses have a smaller size (mean 4.3 pups in 39 litters) compared with WT C57BL/6 mice litters (6.5 pups), which may support the unexpected genetic distribution.
Mice with a conditional knockout of the SelT gene in the brain are viable, as are brain TrxR gene knockout mice. However, our in vitro and in vivo experiments showed that the lack of this selenoprotein interferes with dopaminergic neuron function since SelT-deficient cells and Nes-Cre/SelT<sup>fl/fl</sup> mice exhibited lower phosphoTH and/or dopamine levels in comparison with the controls. Both impaired dopaminergic neuron functionality and dopamine neuron loss could account for the alteration of the dopaminergic system in Nes-Cre/SelT<sup>fl/fl</sup> mice. However, since our results show that total TH and a-tubulin protein levels are unchanged in SelT-deficient compared with WT mice, it is most likely that SelT gene disruption affects the functionality rather than the number of dopaminergic neurons. It should be noted that SelT gene expression in the brain is high during development (24, 53) and that SelT gene knockout leads to neurodevelopmental abnormalities and hyperactivity (13). Therefore, the alterations in TH activity and dopamine levels observed in SelT-deficient mice are most likely due to neurodevelopmental effects of SelT deficiency, which would lead to a perturbed dopaminergic system function in adulthood as observed in the present study. It has previously been shown that...
Selenoproteins support neuron development and prevent brain atrophy and neurodegeneration in rodents and humans, but the role of the different selenoproteins in the development and function of the nigrostriatal pathway is not yet known since these studies reported the effects of total selenoprotein deficiency (47, 51, 57, 59). In particular, it has recently been reported that impairment of total selenoprotein expression in the brain provokes striatal neuronal loss in mice (47), although the nature of the cells lost was not identified.

To directly assess the impact of SelT on dopaminergic neurons, we used different mice models of PD. In a first model, we administered four doses of MPTP (15 mg/kg) at 1-h intervals, which induced progressive dopaminergic neuron and fiber degeneration, thus mimicking, although to some extent, PD. Since it has been shown that different protocols of MPTP administration may lead to various outcomes in terms of PD characteristics (21) and since SelT-deficient mice were highly vulnerable to MPTP treatment, which did not allow measurements beyond 1 day of treatment, we used a different PD model based on progressive injections of low doses of rotenone to confirm the data obtained with MPTP. Using these two different models of PD in mice, we demonstrated here that SelT is required for dopamine production in basal conditions and is induced by neurotoxins in the nigrostriatal system to protect dopaminergic neurons against oxidative stress. Indeed, upon MPTP treatment, Nes-Cre/SelTfl/fl exhibited high levels of oxidative stress in the nigrostriatal system associated with a marked decrease in active TH levels and reduced concentration of dopamine in the striatum. Dopamine levels were obviously insufficient to sustain motor function since mutant mice rapidly exhibited several characteristic symptoms of PD, including tremor and akinesia after administration of a single dose of neurotoxin, indicating that SelT plays a crucial role to ensure appropriate dopamine production and motor skill. Likewise, treatment with low doses of rotenone led to enhanced degeneration of the nigrostriatal system associated with decreased rearing behavior and dopamine levels. Of note, the neurotoxins exerted specific effects in dopaminergic neurons and not in nondopaminergic cells as revealed by the analysis of 3-NT.
immunolocalization and α-tubulin immunoreactivity used as an index of total nerve cells. These results underline the importance of SeIT in sustaining the integrity of dopaminergic neurons in the face of oxidative stress and further emphasize the direct link between oxidative stress, dopaminergic neuron impairment, and motor dysfunction proposed for PD (45). It is interesting to note that SeIT gene induction in response to neurotoxin administration occurs both in neurons and astrocytes of the nigrostriatal pathway, indicating that the selenoprotein is mobilized in both cell types to protect this pathway against oxidative stress.

Dopamine levels were significantly reduced in Nes-Cre/SelTfl/fl mice and further decreased to low levels after neurotoxin administration. In addition, Nes-Cre/SelTfl/fl mice may present an increased turnover of dopamine to compensate for its deficiency, leading to auto-oxidation and further impairment of the neurotransmitter (7, 14, 38). Surprisingly, Nes-Cre/SelTfl/fl mice exhibited hyperactive behavior, although their dopamine levels were decreased compared with WT mice (13). This adaptive phenotype probably arises as a consequence of the neurodevelopmental alterations observed in these mice (13) and is reminiscent of the hyperactivity behavior observed in the attention-deficit hyperactivity disorder where a role of dopamine dysfunction is suspected (58). Rapid dopamine decline below a threshold level could be responsible for sudden Nes-Cre/SelTfl/fl animal death observed after treatment with neurotoxins, in contrast to WT mice, which have a normal concentration of dopamine and which increase their SeIT levels in the nigrostriatal system to counter MPTP toxicity.

The Nes-Cre/SelTfl/fl mice exhibited a higher level of nitrosative species as revealed by the excessive accumulation of 3-NT in the nigrostriatal pathway of these animals within a short period of time after neurotoxin-induced oxidative stress. In addition, SeIT-deficient SY5Y cells also exhibited higher sensitivity to H2O2-induced oxidative stress. Since mutant SeIT lacking the Sec residue and the knockout SeIT gene lacking exon 2, which contains the putative SeIT catalytic center with the Sec residue, failed to protect SY5Y cells or Nes-Cre/SelTfl/fl mice, respectively, our data strongly suggest that dopaminergic neurons lose their resistance to nitrosative/oxidative stress in the absence of SeIT oxidoreductase activity. Several pathogenic mechanisms may account for this high vulnerability to neurotoxin-induced oxidative stress of SeIT-deficient dopaminergic neurons. It has been shown that accumulation of 3-NT, a marker of peroxynitrite-mediated nitration of proteins at their tyrosine residues, provokes dopaminergic neuron dysfunction and loss (8, 30). In particular, it has been demonstrated that tyrosine nitration in response to peroxynitrite or MPP+ challenge causes TH inactivation and consequently dopamine synthesis failure, a finding that explains the extent of dopamine deficit observed in the brain of patients, which exceeds dopaminergic neuron loss (2, 27). Alteration of dopamine production and activity, through TH inactivation and dopamine oxidation, may concur to provoke Nes-Cre/SelTfl/fl vulnerability and motor impairment.

Additionally, since SeIT is located in the ER, which plays a key role in protein folding quality control, this selenoprotein could also be involved in the unfolded protein response (UPR) consequent to ER stress under high free radical levels, which contributes to the pathogenic mechanisms involved in dopaminergic neuron demise in PD (41). A role in the UPR has been recently documented for another thioredoxin-like selenoprotein, Sep15, which interacts under ER stress conditions with multiprotein complexes (including unfolded proteins) that are delivered to the proteasome for degradation (33, 49). The precise mechanism of action of selenoproteins in the UPR and protein degradation is not known yet, but may involve their redox activity (33, 49). It is tempting to speculate that SeIT through its TrxR-like activity may affect thiol redox circuits, including other antioxidant enzymes such as peroxiredoxins or GPx, whose impairment in the absence of SeIT would lead to dysfunctional redox control, ROS accumulation, and cell death. The SeIT knockout mice described in the present study will be instrumental to address this issue.

Quantitative PCR, immunochemical, and Western blot analyses revealed that SeIT expression is significantly increased in PD models in vitro and in vivo, prompting us to investigate the levels of this selenoprotein in the CPu of human patients with PD, as evidenced by Lewy bodies and clinical symptoms, in comparison with age-matched subjects without the disease. We focused our attention on the CPu since striatal dopaminergic denervation is a key pathological event usually used for the diagnosis of PD by analyzing, for instance, the binding activity of dopamine transporters present on dopaminergic fibers. Our pilot study using PD and non-PD human samples uncovered a strong increase in the mRNA and protein levels of SeIT in the CPu of the affected subjects compared with the different controls used. It should be mentioned that TH labeling, which is abundant in the CPu of control subjects, was scarce in PD patients (data not shown) in accordance with the notion that the degeneration of dopaminergic terminals and axons precedes the demise of dopaminergic neurons in the SNC (10). Thus, the increase of SeIT observed in these heavily affected patients is likely contributed also by the induction of its expression in the reactive gliosis usually found at autopsy in these patients (54). This finding is in line with the marked induction of SeIT immunoreactivity in astrocytes in addition to dopaminergic fibers that we found in the striatum of WT mice after 8 days post-treatment with MPTP. Although our results should be substantiated in larger cohorts, the strong increase in the expression of SeIT in the CPu of Parkinson’s patients observed by quantitative PCR and immunohistochemistry and its progressive induction in an MPTP-treated animal model of the disease suggest that its assessment could help in identifying at-risk individuals or to track disease progression and response to therapies. Interestingly, Bellinger et al. (4, 6) also reported an altered expression of GPx4 and SeIP in surviving nigral cells and in dystrophic PUT dopaminergic fibers in Parkinson’s patients, indicating that different selenoproteins may represent complementary biomarkers of PD. Further clinical studies are warranted to establish selenoproteins as reliable markers of PD.

In conclusion, our findings identified a hitherto unknown oxidoreductase activity in the nigrostriatal pathway, which prevents rapid-onset motor impairments in mouse models of PD. Thus, SeIT acts as a gatekeeper of redox homeostasis in the nigrostriatal pathway indispensable for normal dopamine production and therefore for maintaining motor skills in conditions of high oxidative stress. The identified key role of SeIT in the dopaminergic pathway, the phenotype observed...
for Nes-Cre/SelTff/fl knockout mice upon neurotoxin treatment, and the high expression of this oxidoreductase in the CPUs of PD patients provide important clues for further understanding of the etiopathogenic pathways associated with oxidative stress that lead to dopaminergic neuronal death and motor deficits in PD.

Materials and Methods

Animals

All experimental procedures were approved by the Normandy Regional Ethics Committee (Authorization No. N/05-02-13/0103/02-16) and were carried out in accordance with the European Committee Council Directive of November 24, 1986 (86/609/EEC). Mice were housed under a 12-h light/12-h dark cycle (light on at 07:00 am) and had free access to food and water. Ambient temperature was maintained at 22°C ± 2°C. To generate transgenic mice, the SelT gene was floxed by homologous recombination in the genome of C57BL/6J mice to delete the sequence region encoding the thioredoxin-like domain (exons 2 and 3). Mice with SelTfl/fl allele were generated in the Mouse Clinical Institute (Strasbourg, France). They were then bred with Cre recombinase-expressing mice to promote the genetic deletion of the thioredoxin-like motif. The full knockout was obtained by breeding SelTfl/fl mice with CMV-Cre transgenic animals also obtained from the Mouse Clinical Institute, while the brain conditional mutant Nes-Cre/SelTfl/fl (13) was generated by mating SelTfl/fl mice with animals expressing the Cre recombinase under the control of the rat nestin (Nes) promoter (Tg [Nestin-Cre]/1 Klnfl/fl knockin mice), which were acquired from the Jackson Laboratory (56). Genotypes were acquired from the Jackson Laboratory (56). Genotypes were determined by PCR with the following primers: forward, 5′-G GCTTTATGTAAGCAGTCTAAACTGTTTCTGC-3′, and reverse, 5′-CGCCCATTTTAAGACTACTCTTCGTT-3′, for WT (211 bp) and SelTfl/fl (261 bp) alleles; forward, 5′-ATCCGCCAGGCCTTTTCTGACATAC-3′, and reverse, 5′-GCCAGATTACGTATA TCCTGGGAC-3′, for Cre allele (387 bp); and forward, 5′-GGTTATGTAAGCAGTCT TAAACTGTTTCTGC-3′, and reverse, 5′-GCCTAGGTTTT ACCTGAGAAACCAAAG-3′, for SelTfl/fl allele (409 bp).

Human tissue samples

Human brain specimens were collected from six subjects (two women and four men aged between 52 and 83 years) 12–53 h postmortem and were obtained from the Biological Resource Platform GIE Neuro-CEB of the La Pitié-Salpêtrière Hospital (Paris, France). Parkinson’s patients, but not control subjects, presented Lewy bodies and clinical symptoms of the disease.

MPTP and rotenone treatments

MPTP-HCl (Sigma-Aldrich) was dissolved in sterile 0.9% saline and intraperitoneally injected into 3-month-old male C57BL/6J mice in four doses of 15 mg/kg in 100-μl volumes at 1-h intervals, as previously described (15). Mutant male Nes-Cre/SelTfl/fl mice, aged 3–5 months, received only a single dose of MPTP of 15 mg/kg. Rotenone (Sigma-Aldrich) was first dissolved in dimethylsulfoxide and then diluted in the triglyceride, Miglyol 812N (Fagron), before intraperitoneal injection at various doses (11, 50). Control animals were treated with the vehicle only.

Rearing behavior

For this test, the mouse was placed in a clear plexiglass cylinder (height = 30 cm, diameter = 20 cm) for 5 min and the number of rears was quantified as described previously (20). To be classified as a rear, the animal had to raise its forelimbs above shoulder level and make contact with the cylinder wall with either one or both forelimbs (11).

Immunohistochemistry and immunocytochemistry

Animals were anesthetized with sodium pentobarbital (120 mg/kg; Ceva Santé Animale), heparinized, and perfused through an intracardiac cannula with 0.9% NaCl in 0.1 M phosphate buffer (pH 7.4), followed by 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). Brains were removed and postfixed in the same fixative at 4°C, which was changed to PBS azide after 24 h. Tissues were sectioned into 40-μm slices with a vibratome. The sections were incubated with 1% donkey serum diluted in 1% bovine serum albumin and 0.3% Triton X-100 in PBS for 2 h at room temperature, and then exposed overnight at 4°C to primary antibodies, including anti-SelT (23) diluted 1:200, anti-GFAP diluted 1:200 (Santa Cruz Biotechnology), anti-TH diluted 1:200 (Millipore), and anti-3-nitrotyrosine (3-NT) diluted 1:200 (Merck Millipore). Immunostaining was visualized using Alexa Fluor 488 or 594-conjugated secondary antibodies diluted 1:200 (Invitrogen). Counterstaining with 1 μg/ml 4,6-diamino-2-phenylindole (DAPI; Sigma-Aldrich) in PBS for 1 min was performed before mounting the slides with PBS/glycerol 50/50. Samples were analyzed with a Leica SP2 confocal laser scanning microscope (DMRAX-UV) equipped with the Acousto-Optical Beam Splitter system (Leica Microsystems).

For immunocytochemistry, cultured cells were fixed with 2% instead of 4% PFA; all other procedures were the same. Quantification of fluorescence signals was performed using the ImageJ software. When human samples were used, frozen sections were first postfixed in 4% PFA for 20 min and then incubated for 1 h at room temperature with polyclonal rabbit antibodies directed against SelT or TH diluted 1:200. The sections were incubated with a streptavidin–biotin–peroxidase complex (Dako Corporation), and the enzymatic activity was revealed with diaminobenzidine. The slices were then counterstained with hematoxylin. Observations were made under a Nikon E 600 light microscope. As controls for all the immunoreactions, first or secondary antibodies were omitted, and no specific labeling was observed.

Production and activity assessment of recombinant SelT

Rat SelT cDNA devoid of signal peptide sequence was amplified from PC12 cells and cloned in the pGEM-T vector (Promega). Sequence mutagenesis was conducted by using the QuikChange® II XL site-directed mutagenesis kit (Agilent Technologies) to delete the putative transmembrane domain and to convert the Cys-X-X-Sec motive to Cys-X-X-Cys or Ser-X-X-Ser (Fig. 1). The nucleotide sequence of all constructs was verified by DNA sequencing (Beckman Coulter Genomics). The different cDNAs were then subcloned in the pET28 vector (Millipore) for protein expression in BL21 bacteria. Protein synthesis was induced by adding
1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG; Sigma-Aldrich). Bacterial homogenates were centrifuged and incubated for 1 h with equilibrated sepharose beads coupled to Ni-nitroacetic acid (Qiagen). The beads were washed and proteins were eluted in the presence of 250 mM imidazole following the manufacturer’s recommendations. Enzyme activity of recombinant SelT was assessed using thioredoxin reductase and glutathione peroxidase assay kits (Interchim).

**Plasmids, cell culture, transfection, and treatment**

The cDNA encoding SelT was amplified from PC12 cells and inserted into pCIG-eGFP vector (a gift from Dr E. DiCicco-Bloom, Rutgers, NJ), which enables coexpression of SelT with eGFP. Point mutagenesis of SelT was performed as described above to convert the Sec to an Ser residue within the thioredoxin-like domain. All constructs were sequence verified. The human SH-SY5Y neuroblastoma cell line (ATCC) was cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Life Technologies) and supplemented with 10% fetal bovine serum (Lonza), 1% L-glutamine, 50 U/ml of penicillin, and 50 U/ml of streptomycin (Life Technologies). Cells were maintained for 2 days in a humidified 5% CO₂ atmosphere at 37°C and then treated or not with MPP⁺ for 24–36 h. SH-SY5Y cells were transfected with the various pCIG-eGFP plasmid constructs using Lipofectamine 2000 (Life Technologies).

**Lentivirus production and transduction**

The lentiviral constructs containing a shRNA, 5'-CGTG TGATTACAGAAGACTA-3', targeting human SelT or a scramble control shRNA, 5'-GAGAACGATCCTCACC TAC T-3', were generated in the VIRHD-EP vector as described previously (24). The constructs were sequence verified. HEK-293T cells were cotransfected with the shRNA lentiviral vector along with pCMV-dr8.74 (packaging) and pMD2.G (envelope) plasmids (24). Two days after transfection, the conditioned media were collected, supplemented with 8 μg/ml polybrene, and added for overnight incubation to freshly plated SH-SY5Y cells for transduction. Two days after infection, the cells were selected in 2 μg/ml puromycin.

**Measurement of cell viability and intracellular ROS levels**

The number of viable cells was determined using the CellTiter-Blue® cell viability assay (Promega), and the levels of intracellular ROS were measured using the DCFDA Cellular ROS Detection Assay Kit (Abcam) following the manufacturer’s instructions. The fluorescence of ROS-oxidized 2',7'-dichlorofluorescein (DCF) was measured at 530 nm.

**Western blot analysis**

Animals were anesthetized by isoflurane inhalation (AErrane®; Baxter) and the brains were immediately recovered after decapitation and quickly placed in dry ice. Tissue and cell samples were homogenized in a lysis buffer (Ozyme) and analyzed as reported previously (53) after protein transfer onto a nitrocellulose membrane (GE Healthcare). For all experiments, 30 μg of proteins was used. Membranes were probed with polyclonal antibodies against SelT (N-term) (Acris) diluted 1:500, TH (Millipore) diluted 1:400, phosphoTH-Ser31 diluted 1:500, and phosphoTH-Ser40 (Cell Signaling) diluted 1:500. After incubation with horse-radish peroxidase-conjugated secondary antibodies (1:1000), the resulting immune complexes were visualized using the ECL chemiluminescence system (Thermo Scientific). An antibody against α-tubulin (Sigma-Aldrich) diluted 1:200 was used as a control to ensure equal protein loading.

**Quantitative real-time PCR**

Total RNA was prepared using the NucleoSpin® RNA II kit (Macherey-Nagel). Contaminating DNA was removed by DNase I treatment, and 1 μg of total RNA was reverse transcribed using the ImProm II Reverse Transcription kit (Promega). Real-time PCR was performed on cDNA using the Fast SYBR® Green Master Mix (Applied Biosystems) in the presence of 300 nM of primers for SelT, forward 5'-GGTAT CAGACATCCCGATTG-3' and reverse 5'-GGAAAGAT GCTA TGTGTCTATAATGG-3', or Gapdh, forward 5'- CATGGCCTTCCGGTTTCCA-3' and reverse 5'-CCTGCT TCAACACCTTCTGA-3', used as a reference gene. Reactions were carried out using the ABI PRISM 7500 Fast Detection System (Applied Biosystems). Relative gene expression levels were calculated based on the values for the gene of interest normalized with the values of the GAPDH gene.

**Dopamine assay**

Dopamine concentrations in striatum homogenates from the various genotypes treated with the vehicle or MPTP were determined using a mouse dopamine Elisa kit following the manufacturer’s instructions (Mybiosource).

**Statistics**

In the experiments with mice, matched controls from the same litters were used. Results are expressed as mean ± SEM and represent data from a minimum of three independent experiments. Statistical analysis of the data was performed with GraphPad Prism 6 software. Statistical significance of difference between groups was determined by the two-tailed unpaired Student’s t-test or the Mann–Whitney U-test. For Kaplan–Meier curves, statistical comparisons between groups were made using the log-rank Mantel–Cox test.

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**Author Disclosure Statement**

No competing financial interests exist.
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Abbreviations Used

3-NT = 3-nitrotyrosine
ATM = aurothiomalate
CD = caudate
CPu = caudate putamen
DAPI = 4,6-diamino-2-phenylindole
DCF = 2',7'-dichlorofluorescein
DMEM = Dulbecco’s modified Eagle’s medium
ER = endoplasmic reticulum
Fl = flox
GFAP = glial fibrillary acidic protein
GFP = green fluorescent protein
GPx = glutathione peroxidase
IPTG = isopropyl β-D-1-thiogalactopyranoside
KO = knockout
Mes = mesencephalon
MPP+ = 1-methyl-4-phenylpyridinium
MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Nes = nestin

PACAP = pituitary adenylate cyclase-activating polypeptide
PBS = phosphate-buffered saline
PCR = polymerase chain reaction
PD = Parkinson’s disease
PFA = paraformaldehyde
PUT = putamen
ROS = reactive oxygen species
ROT = rotenone
Sec = selenocysteine
SelT = selenoprotein T
SEM = standard error of the mean
SNc = substantia nigra compacta
STN = subthalamic nucleus
Str = striatum
TH = tyrosine hydroxylase
TrxR = thioredoxin reductase
UPR = unfolded protein response
WT = wild-type
α-Tub = alpha-tubulin