Paraquat-induced intracellular Zn2+ dysregulation causes dopaminergic degeneration in the substantia nigra, but not in the striatum

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Abstract

Parkinson's disease (PD) is characterized by a selective death of nigrostriatal dopaminergic neurons, while the difference in the vulnerability to the death between the substantia nigra pars compacta (SNpc) and the striatum is poorly understood. Here we tested the difference focused on paraquat (PQ)-induced intracellular \(\text{Zn}^{2+}\) toxicity via extracellular glutamate accumulation. When PQ was locally injected into the SNpc and the striatum, dopaminergic degeneration was observed in the SNpc, but not in the striatum. Intracellular hydrogen peroxide (H\(_2\)O\(_2\)) produced by PQ was increased in both the SNpc and the striatum. In contrast, extracellular glutamate accumulation was observed only in the SNpc and rescued in the presence of N-(p-amylcinnamoyl)anthranilic acid (ACA), a blocker of the transient receptor potential melastatin 2 (TRPM2) cation channels. PQ increased intracellular \(\text{Zn}^{2+}\) level in the SNpc, but not in the striatum. The increase was rescued by 1-naphthyl acetyl spermine (NASPM), a selective blocker of \(\text{Ca}^{2+}\)- and \(\text{Zn}^{2+}\)-permeable GluR2-lacking AMPA receptors. PQ-induced dopaminergic degeneration in the SNpc was rescued by ACA, NASPM, and GBR, a dopamine reuptake inhibitor. The present study indicates intracellular H\(_2\)O\(_2\) produced by PQ, which is taken up through dopamine transporters, is retrogradely transported to presynaptic glutamatergic terminals, activates TRPM2 channels, accumulates glutamate in the extracellular compartment, and induces intracellular \(\text{Zn}^{2+}\) dysregulation via \(\text{Ca}^{2+}\)- and \(\text{Zn}^{2+}\)-permeable GluR2-lacking AMPA receptor activation, resulting in dopaminergic degeneration in the SNpc. However, H\(_2\)O\(_2\) signaling is not the case in the striatum. Paraquat-induced \(\text{Zn}^{2+}\) dysregulation plays a key role for neurodegeneration in the SNpc, but not in the striatum.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder and has a characteristic of a selective death of nigrostriatal dopaminergic neurons. The resultant deficiency of dopamine release from the nigrostriatal pathway induces a movement disorder, which is characterized by classical parkinsonian motor symptoms [1, 2]. PD is also characterized by a variety of non-motor symptoms prior to the motor symptoms, which are emerged over a decade [3, 4]. However, the exact cause of the PD pathogenesis remains to be clarified [5, 6]. The general standard for PD diagnosis is the presence of nigrostriatal dopaminergic degeneration and Lewy pathology, which consists of abnormal aggregates of \(\alpha\)-synuclein protein, in post-mortem pathological observation [5].

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride, PQ) are widely used to prepare PD model (7,8). PQ has a similar structure to MPP+ (1-methyl-4-phenylpyridinium), a toxic metabolite of MPTP. PQ and MPP+ induce a selective nigrostriatal dopaminergic degeneration (9–12). The herbicide PQ, which naturally exists as PQ\(^{2+}\), a divalent cation, undergoes redox cycling with cellular diaphorases, e.g., NADPH oxidase and nitric oxide synthase. PQ produces PQ\(^+\), a monovalent cation, which passes through dopamine transporters followed by the production of superoxide and reactive oxygen species (ROS) via the redox cycling in dopaminergic neurons to lead to oxidative stress-mediated neurotoxicity (13,14). The neurotoxicity has been reported in
both in the substantia nigra pars compacta (SNpc) and the striatum, while the difference in the vulnerability to the neurodegeneration between the SNpc and the striatum is poorly understood.

PQ induces a transient increase in extracellular glutamate in the striatum of freely moving rats, followed by Ca\(^{2+}\) influx via N-methyl-D-aspartate (NMDA) receptor activation (15). On the basis of the involvement of glutamate excitotoxicity in PQ-induced pathophysiology, we have reported a unique mechanism of nigrostriatal dopaminergic degeneration: PQ-induced ROS activates the transient receptor potential melastatin 2 (TRPM2) cation channel, accumulates glutamate in the extracellular compartment, induces intracellular Zn\(^{2+}\) dysregulation by the rapid influx of extracellular Zn\(^{2+}\) via \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor activation, resulting in nigrostriatal dopaminergic degeneration (16–18).

Here we report the distinct difference in vulnerability to dopaminergic degeneration between the SNpc and striatum and the mechanism of the difference focused on nigrostriatal intracellular Zn\(^{2+}\) dysregulation induced by glutamate excitotoxicity. The difference in vulnerability is important to understand the PD pathogenesis.

**Materials And Methods**

**Animals and chemicals**

Male Wistar rats (10-15 weeks of age, Japan SLC, Hamamatsu, Japan) were housed under the standard laboratory conditions (23 ± 1°C, 55 ± 5% humidity) and had access to food and water freely. The present experiments were done according to the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka, which refer to American Association for Laboratory Animals Science and the guidelines laid down by the NIH in the USA (NIH Guide for the Care and Use of Laboratory Animals). The ethics committee has approved all experimental protocols in the University of Shizuoka.

ZnAF-2DA (Sekisui Medical Co., LTD, Hachimantai, Japan), a membrane-permeable Zn\(^{2+}\) fluorescence probe, readily passes through the cell membrane and is hydrolyzed by esterase in the cytosol compartment followed by generation of ZnAF-2, which cannot permeate the cell membrane. ZnAF-2 is selectively bound to Zn\(^{2+}\), but not bound to other divalent cations such as Ca\(^{2+}\), Mg\(^{2+}\), Fe\(^{2+}\), and Cu\(^{2+}\). The probe was dissolved in dimethyl sulfoxide and then diluted to Ringer solution that consists of 119 mM NaCl, 2.5 mM KCl, 1.3 mM MgSO\(_4\), 1.0 mM NaH\(_2\)PO\(_4\), 2.5 mM CaCl\(_2\), 26.2 mM NaHCO\(_3\), and 11 mM D-glucose (pH 7.3).

HYDROP\(^{TM}\), a membrane-permeable hydrogen peroxide (H\(_2\)O\(_2\)) fluorescence probe (Goryochemical, Sapporo, Japan) readily passes through the cell membrane and is hydrolyzed in the cytosolic compartment followed by generation of HYDROP-EX\(^{TM}\), which cannot permeate the cell membrane. HYDROP-EX is selectively bound to H\(_2\)O\(_2\), but not bound to other ROS such as \(\cdot\)OH, O\(_2\)\(^{\cdot -}\), ClO\(^{-}\), \(\cdot\)O\(_2\), \(\cdot\)NO, and ONOO\(^{-}\). The probe was dissolved in N, N-dimethylformamide and then diluted to Ringer solution.
**Surgical operation**

The rats were anesthetized by intraperitoneal injection with chloral hydrate (400 mg/kg) and individually placed in a stereotaxic apparatus. The skull was exposed and a burr hole was drilled. An injection cannula (internal diameter, 0.15 mm; outer diameter, 0.35 mm) was carefully and slowly inserted into the right SNpc (5.3 mm posterior to the bregma, 2.0 mm lateral, 7.0 mm inferior to the dura) and the right striatum (0.48 mm anterior to the bregma, 4.1 mm lateral, 3.7 mm inferior to the dura) to prevent cellular damages. Thirty minutes after the surgical operation, 40 μM PQ, 40 μM PQ + 50 μM N-(p-amylcinnamoyl)anthranilic acid (ACA), a blocker of TRPM2 cation channels, 40 μM PQ + 10 mM 1-naphthyl acetyl spermine (NASPM), a selective blocker of Ca^{2+}- and Zn^{2+}-permeable GluR2-lacking AMPA receptors, or 2 μM GBR 13069 dihydrochloride (GBR), a dopamine reuptake inhibitor in saline were injected into the right SNpc via the cannula at the rate of 0.2 μl/min for 5 min. Ten minutes after injection, the injection cannula was carefully and slowly removed from the brain in approximately 10 min. In another experiment, 40 μM PQ in saline was injected into the right striatum in the same manner.

**Tyrosine hydroxylase (TH) immunostaining**

The rats were anesthetized with chloral hydrate 2 weeks after PQ injection and perfused with ice-cold 4% paraformaldehyde in PBS. The brain was removed from the rats followed by overnight fixation at 4°C in 4% paraformaldehyde in PBS. Fixed brains were cryopreserved in 30% sucrose in PBS for 2 day and frozen in Tissue-Tek Optimal Cutting Temperature embedding medium. Coronal brain slices (30 mm) were prepared at -20°C in a cryostat and picked up on slides followed by adhering at room temperature for 30 min. For TH immunostaining, the slides were incubated in blocking solution (3% BSA, 0.1% Triton X-100 in PBS) for 1 h and rinsed with PBS for 5 min followed by overnight incubating at 4°C with anti-tyrosine hydroxylase antibody (Abcam). The slides were rinsed with PBS for 5 min and incubated in blocking buffer containing Alexa Fluor 633 goat anti-rabbit secondary antibody (ThermoFisher) for 3 h at room temperature. The slides were rinsed with PBS for 5 min six times, mounted with Prolong Gold antifade reagent, and placed at 4°C for 24 h. Alexa Fluor 633 fluorescence was measured in the SNpc and the striatum using a confocal laser-scanning microscopic system.

**In vivo imaging of intracellular H$_2$O$_2$**

The rats anesthetized with chloral hydrate were treated in the same manner. Injection cannulae were carefully and slowly inserted into the both sides of the SNpc and the striatum to prevent cellular damages. Thirty minutes later, 40 μM PQ in saline containing 50 μM HYDROP was bilaterally injected into the SNpc and the striatum via cannulae at the rate of 0.2 μl/min for 5 min. Ten minutes later, the injection cannulae were slowly moved from the brain in approximately 3 min. The rats were decapitated. The brain was quickly excised from the rats and bathed in ice-cold choline-Ringer containing 124 mM choline chloride, 2.5 mM KCl, 2.5 mM MgCl$_2$, 1.25 mM NaH$_2$PO$_4$, 0.5 mM CaCl$_2$, 26 mM NaHCO$_3$, and 10 mM glucose (pH 7.3) to inhibit excessive neuronal excitation. Horizontal slices (400 μm) of the brains were prepared in an ice-cold choline-Ringer solution in a vibratome ZERO-1 (Dosaka Kyoto, Japan) and
then bathed in an ice-cold choline-Ringer solution. The brain slices were transferred to a recording chamber filled with Ringer solution. Intracellular HYDROP-EX fluorescence was measured in the SNpc and the striatum with a confocal laser-scanning microscopic system. All solutions used in the experiments were continuously bubbled with 95% O₂ and 5% CO₂.

**In vivo microdialysis**

The rats anesthetized with chloral hydrate were treated in the same manner. A microdialysis probe (1-mm membrane, Eicom, Kyoto) was inserted into the right SNpc (5.3 mm posterior to the bregma, 2.0 mm lateral, 7.8 mm inferior to the dura) and the striatum (0.48 mm anterior to the bregma, 4.1 mm lateral, 4.2 mm inferior to the dura) of anesthetized rats. The SNpc and the striatum were preperfused with ACSF (127 mM NaCl, 2.5 mM KCl, 1.3 mM CaCl₂, 0.9 mM MgCl₂, 1.2 mM Na₂HPO₄, 21 mM NaHCO₃ and 3.4 mM D-glucose, pH 7.3) at 2.0 µl/min for 180 min to stabilize the region, perfused with ACSF for 60 min in the same manner to determine the basal concentration of extracellular glutamate and then perfused with 40 µM PQ in ACSF, 40 µM PQ + 50 µM ACA in ACSF, or 40 µM PQ + 50 µM HYDROP in ACSF for 180 min.

The perfusate was collected for 15 min and analyzed for glutamate content by high-performance liquid chromatography (HPLC) [column, CAPCELL PAK C18 UG120A (1 mm x 150 mm) (Shiseido Co Ltd, Tokyo, Japan); mobile phase, 0.1 M potassium dihydrogen phosphate, 0.1 M di-sodium hydrogen phosphate, 10% acetonitrile, 0.5 mM EDTA-2Na, 3% tetrahydrofuran, pH 6.0] using the pre-column derivatization technique with o-phthaldialdehyde and a fluorescence detector (NANOSPACE SI-2, Shiseido Co Ltd). The basal levels and the levels during perfusion with PQ were averaged.

**In vitro imaging of intracellular Zn²⁺**

The brain was quickly excised from anesthetized rats and bathed in ice-cold choline-Ringer to inhibit excessive neuronal excitation. Horizontal brain slices (400 µm) were prepared in the same manner. The brain slices were immersed in 10 µM ZnAF-2DA in Ringer solution for 30 min, rinsed in choline-Ringer solution for 15 min, placed in a chamber filled with 40 µM PQ or 40 µM PQ + 50 µM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an AMPA receptor antagonist in Ringer solution containing 10 nM ZnCl₂ for 10 min, rinsed in choline-Ringer solution for 15 min, and transferred to a recording chamber filled with Ringer solution. Intracellular ZnAF-2 fluorescence (laser, 488.4 nm; emission, 500–550 nm) was observed in the SNpc and the striatum with a confocal laser-scanning microscopic system.

**In vivo imaging of intracellular Zn²⁺**

The rats were anesthetized with chloral hydrate and treated as described above. Injection cannulae were carefully and slowly inserted into the both sides of the SNpc to prevent cellular damages. Thirty minutes later, 40 µM PQ and 40 µM PQ + 10 mM NASPM in saline containing 100 µM ZnAF-2DA were bilaterally injected into the SNpc via cannulae at the rate of 0.2 µl/min for 5 min. Ten minutes later, the injection cannulas were slowly removed from the brain in about 3 min. The rats were decapitated 60 min after the start of the injection and the brain was quickly removed from the rats. Horizontal brain slices (400
μm) were prepared in the same manner and transferred to a recording chamber filled with Ringer solution. The fluorescence of intracellular ZnAF-2 was measured in the SNpc.

Data analysis

Student's paired t-test was used for comparison of the means of paired data. For multiple comparisons, differences between treatments were assessed by one-way ANOVA followed by post hoc testing using the Tukey's test (the statistical software, GraphPad Prism 5). A value of p < 0.05 was considered significant. Data were expressed as means ± standard error. The results of statistical analysis are described in each figure legend.

Results

PQ induces dopaminergic degeneration in the SNpc, but not in the striatum

Loss of nigrostriatal dopaminergic neurons induced by PQ was determined by TH immunostaining. In the case of local injection of PQ (12 mM, 1 µl) in the SNpc, TH staining intensity is drastically reduced even in the ipsilateral striatum, while PQ (40 µM, 1 µl) injected into the SNpc reduces TH staining intensity in the SNpc, but not in the striatum (16). Thus, the SNpc and the striatum were exposed to this low dose and the vulnerability to PQ was compared between the SNpc and the striatum under the same condition.

When PQ was locally injected into the SNpc and the striatum of rats, TH staining intensity was reduced in the SNpc, but not in the striatum (Fig. 1).

PQ-mediated H$_2$O$_2$ preferentially induces glutamate accumulation in the SNpc followed by intracellular Zn$^{2+}$ dysregulation

PQ-mediated ROS production accumulates glutamate in the extracellular compartment of the SNpc via TRPM2 cation channel activation and induces intracellular Zn$^{2+}$ dysregulation (18). Thus, we postulated that PQ taken up through dopamine transporters produces H$_2$O$_2$, which is retrogradely transported to presynaptic TRPM2 channels on glutamatergic terminals through the plasma membranes of dopaminergic neurons in the SNpc and accumulates glutamate in the extracellular compartment via TRPM2 channel activation, followed by intracellular Zn$^{2+}$ toxicity.

Intracellular H$_2$O$_2$ produced by PQ, which was determined by HYDROP, was increased in both the SNpc and the striatum (Fig. 2). In contrast, the increase in glutamate concentration in the extracellular compartment was observed in the SNpc, but not in the striatum, and rescued in the presence of ACA (Fig. 3A and 3B). Furthermore, PQ-mediated increase in extracellular glutamate was also rescued in the presence of HYDROP used as an intracellular H$_2$O$_2$ scavenger (Fig. 3C).
PQ increased intracellular Zn$^{2+}$ level in the SNpc, which was determined by ZnAF-2DA and the increase was rescued in the presence of CNQX (Fig. 4A), while PQ decreased intracellular Zn$^{2+}$ level in the striatum (Fig. 4B). When Zn$^{2+}$ dynamics was checked after PQ injection into the SNpc in vivo, PQ also increased intracellular Zn$^{2+}$ level in the SNpc and PQ-mediated increase in intracellular Zn$^{2+}$ was rescued by NASPM (Fig. 4C).

**Extracellular glutamate-mediated intracellular Zn$^{2+}$ toxicity causes dopaminergic degeneration in the SNpc**

PQ-induced dopaminergic degeneration in the SNpc is rescued in the presence of intracellular (ZnAF-2DA) and extracellular (CaEDTA) Zn$^{2+}$ chelators (16, 18), suggesting that the rapid influx of extracellular Zn$^{2+}$ is linked with the degeneration. When PQ was locally co-injected with ACA or NASPM into the SNpc, PQ-induced decrease in TH staining intensity was rescued by either co-injection of ACA or NASPM (Figs. 5 and 6).

Furthermore, PQ-induced dopaminergic degeneration in the SNpc was completely rescued by co-injection of a dopamine reuptake inhibitor (GBR) (Fig. 7).

**Discussion**

Glutamate excitotoxicity has been reported in numerous neurodegenerative disorders including PD and is associated with the PD pathophysiology in both the SNpc and the striatum. The SNpc is innervated from the subthalamic nucleus and the amygdala via glutamatergic neurotransmitter system. It has been reported that excess activation of glutamate receptors on dopaminergic neurons in the SNpc may be involved in the PD pathophysiology (19–23). On the other hand, the striatum is innervated by glutamatergic neurotransmitter system, i.e., corticostriatal input and thalamostriatal input. Glutamate transporters play a key role in glutamate clearance and protect neurons from glutamate excitotoxicity. Dysfunctional glutamate transporters contribute to the PD pathogenesis (24). In association with reduced expression of the glutamate transporter, GLT-1, altered glutamate release in the dorsal striatum also contribute to the PD pathogenesis (25). Striatal glutamate induces a retrograde excitotoxicity and is involved in neurodegeneration in the intralaminar nuclei of the thalamus generally observed in PD (26). While a selective death of nigrostriatal dopaminergic neurons is well known in the PD pathogenesis, the difference in the vulnerability to the death is poorly understood between the SNpc and the striatum.

In the present study, we tested the difference focused on PQ-induced intracellular Zn$^{2+}$ toxicity via extracellular glutamate accumulation. When PQ was locally injected into the SNpc and the striatum, dopaminergic degeneration was observed in the SNpc, but not in the striatum. PQ readily passes through dopamine transporters and produces a number of ROS via the redox cycling in dopaminergic neurons followed by oxidative stress-mediated neurodegeneration (13,14). Among intracellular ROS derived from PQ, H$_2$O$_2$ readily passes through cell membranes through aquaporin channels (27,28). The increase in H$_2$O$_2$ in the extracellular compartment excites glutamatergic neuron terminals via H$_2$O$_2$-sensitive TRPM2...
channel activation (29–31). Intracellular H$_2$O$_2$ produced by PQ was increased in both the SNpc and the striatum.

On the other hand, extracellular glutamate accumulation was observed only in the SNpc and rescued in the presence of ACA, a blocker of TRPM2 channels and HYDROP, an intracellular H$_2$O$_2$ scavenger, suggesting that intracellular H$_2$O$_2$ produced by PQ is retrogradely transported to presynaptic TRPM2 channels on glutamatergic terminals through the plasma membranes of dopaminergic neurons in the SNpc, and accumulates glutamate in the extracellular compartment via TRPM2 channel activation in the SNpc. However, H$_2$O$_2$ signaling is not the case in the striatum (Fig. 8).

PQ increased intracellular Zn$^{2+}$ level in the SNpc, but not in the striatum. The increase was rescued by NASPM, a selective blocker of Zn$^{2+}$-permeable GluR2-lacking AMPA receptors. The expression of TRPM2 channels is elevated in substantia nigra of PD mouse model after exposure to MPTP and also PD patients (30). On the other hand, the TRPM2 channels are expressed in hippocampus, cortex and striatum and play a crucial role in brain damage by ischaemia-reperfusion, chronic cerebral hypo-perfusion and neonatal hypoxic-ischaemia (32). The present study indicates that PQ is taken up through dopamine transporters and produces H$_2$O$_2$, which is retrogradely transported to presynaptic TRPM2 channels in the SNpc and accumulates glutamate in the extracellular compartment via TRPM2 channel activation, followed by intracellular Zn$^{2+}$ toxicity via Zn$^{2+}$-permeable GluR2-lacking AMPA receptor activation (Fig. 8). In the striatum, on the other hand, PQ is also taken up through dopamine transporters and produces H$_2$O$_2$. However, it is estimated that H$_2$O$_2$ cannot activate presynaptic TRPM2 channels on glutamatergic terminals because glutamatergic terminals are connected with GABAergic neurons, but not with dopaminergic neurons (Fig. 8). More production of H$_2$O$_2$ might be required to activate the TRPM2 channels in the striatum. Activation of TRPM2 channels, functionally expressed in primary cultures of rat striatum, contributes to Amyloid β- and H$_2$O$_2$-induced striatal cell death (33).

Dopaminergic degeneration in the SNpc induced by PQ was rescued by ACA, suggesting that TRPM2 channel activation is linked with neurodegeneration. Dopaminergic degeneration in the SNpc induced by PQ was also rescued by NASPM, suggesting that the rapid influx of extracellular Zn$^{2+}$ through Zn$^{2+}$-permeable GluR2-lacking AMPA receptors is linked with neurodegeneration. It has been that Zn$^{2+}$-permeable GluR2-lacking AMPA receptors are linked with neurodegeneration via intracellular Zn$^{2+}$ toxicity (34–36) It is likely that Zn$^{2+}$-permeable GluR2-lacking AMPA receptors, which are induced by TRPM2 channel activation, play a crucial role in intracellular Zn$^{2+}$ dysregulation followed by neurodegeneration in the SNpc. Furthermore, PQ-induced dopaminergic degeneration in the SNpc was completely rescued by co-injection of a dopamine reuptake inhibitor (GBR), strongly suggesting that involvement of PQ-mediated H$_2$O$_2$ production in the intracellular compartment in neurodegeneration in the SNpc.

In conclusion, intracellular H$_2$O$_2$ produced by PQ, which is taken up through dopamine transporters, is retrogradely transported to presynaptic glutamatergic terminals, activates TRPM2 channels, accumulates glutamate in the extracellular compartment, and induces intracellular Zn$^{2+}$ dysregulation via Zn$^{2+}$-
permeable GluR2-lacking AMPA receptor activation, resulting in dopaminergic degeneration in the SNpc. In contrast, H₂O₂ signaling is not the case in the striatum (Fig. 8). Paraquat-induced intracellular Zn²⁺ dysregulation plays a key role for neurodegeneration in the SNpc, but not in the striatum. The striatum is less vulnerable to PQ than the SNpc.

**Declarations**

**Conflict of interest**

The authors declare that they have no conflict of interest.

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**Author contributions**

Conceptualization: Atsushi Takeda; Data curation: Atsushi Takeda, Haruna Tamano; Formal analysis: Haruna Tamura, Ryusuke Nishio, Nana Saeki, Misa Katahira, Hiroki Morioka; Investigation: Haruna Tamura, Ryusuke Nishio, Nana Saeki, Misa Katahira, Hiroki Morioka; Methodology: Atsushi Takeda, Haruna Tamura, Ryusuke Nishio, Nana Saeki, Misa Katahira, Hiroki Morioka; Project administration: Atsushi Takeda; Resources: Atsushi Takeda; Software: Atsushi Takeda; Supervision: Haruna Tamano; Validation: Atsushi Takeda; Roles/Writing - original draft: Atsushi Takeda; Haruna Tamura: Writing - review & editing; Atsushi Takeda

**Availability of data and material**

Not applicable

**Compliance with ethical standards**

The authors declare that they have no conflict of interest. The Ethics Committee for Experimental Animals has permitted the present study in the University of Shizuoka. There is no informed consent because the present study does not deal with human study.

**Consent to participate**

The present paper has been approved by all named authors.

**Consent for Publication**

The present paper, which is original, has not been published before and is not currently being considered for publication elsewhere.

**Acknowledgments**
Not applicable.

References


Figures

Figure 1
Neuronal loss is induced after injection of PQ into the SNpc, but not after injection of PQ into the striatum. Saline (n=4) or PQ in saline (n=8) were unilaterally injected into the SNpc of rats. Saline (n=3) or PQ in saline (n=5) were unilaterally injected into the striatum of rats. Two weeks later, TH immunostaining with Alexa Fluor 633 fluorescence was measured in the SNpc and the striatum surrounded by the dotted line. Each bar and line represent the ratio of Alexa Fluor 633 fluorescence in the ipsilateral SNpc to Alexa Fluor 633 fluorescence in the contralateral SNpc, which was expressed as 100%. ***, p<0.001, vs. each contralateral side and each ipsilateral side injected with vehicle (t-test).

Figure 2

PQ-mediated increase in intracellular H2O2 in the SNpc and the striatum Saine containing HYDROP (control) and PQ in saline containing HYDROP were bilaterally injected into the SNpc and the striatum and then brain slices were prepared. Intracellular HYDROP-EX fluorescence was measured in the SNpc (saline, n=8; PQ=9) and the striatum (saline, n=4; PQ=4) surrounded by the dotted line. ***, p<0.001, **, p<0.01, vs. saline (t-test).
Figure 3

PQ accumulates glutamate in the extracellular compartment of the SNpc but not of the striatum. The SNpc was perfused with PQ or PQ + ACA (A) and the striatum was perfused with PQ (B). The SNpc was also perfused with PQ or PQ + HYDROP (C). Each bar and line represent the ratio of glutamate concentration in the perfusate with PQ (SNpc, n=6; striatum, n=4), PQ + ACA (n=4), PQ + HYDROP (n=3) to the basal glutamate concentration in the perfusate, which was perfused with ACSF (SNpc, n=6; striatum, n=4) and expressed as 100%. **, p<0.01, ***, p<0.001, vs. the base (ACSF), #, p<0.05, vs. PQ (Tukey's test).
Figure 4

PQ increases intracellular Zn2+ concentration in the SNpc but not in the striatum. Brain slices loaded with ZnAF-2DA were bathed in Ringer (control), PQ, or PQ + CNQX for 10 min. Intracellular ZnAF-2 fluorescence was imaged in the SNpc (A) and the striatum (Str, B) surrounded by the dotted line. SNpc, control, n=4; PQ, n=8; PQ + CNQX, n=6. Striatum, control, n=7; PQ, n=7. Each bar and line represent the ratio of ZnAF-2 fluorescence to the control ZnAF-2 fluorescence, which was expressed as 100%. *, p<0.05, ***, p<0.001, vs. control, ###, p<0.001, vs. PQ (Tukey’s test). Saine containing ZnAF-2DA (control, n=6), PQ in saline containing ZnAF-2DA (n=7), and PQ + NASPM in saline containing ZnAF-2DA (n=7) were bilaterally injected into the SNpc and then brain slices were prepared. Intracellular ZnAF-2 fluorescence was measured in the SNpc surrounded by the dotted line. **, p<0.01, vs. control, ##, p<0.01, vs. PQ (Tukey’s test).
Figure 5

PQ-mediated neuronal loss in the SNpc is rescued in the presence of a TRPM2 channel blocker. Saline (control, n=5), PQ in saline (n=9), and PQ + ACA in saline (n=4) were unilaterally injected into the SNpc of rats. Two weeks later, TH immunostaining with Alexa Fluor 633 fluorescence was measured in the SNpc surrounded by the dotted line. Each bar and line represent the ratio of Alexa Fluor 633 fluorescence in the ipsilateral SNpc to Alexa Fluor 633 fluorescence in the contralateral SNpc, which was expressed as 100%. ***, p<0.001, vs. each contralateral side and each ipsilateral side injected with vehicle, ###, p<0.001, vs. each ipsilateral side injected with PQ (Tukey’s test).
Figure 6

PQ-mediated neuronal loss in the SNpc is rescued in the presence of a Zn2+-permeable GluR2-lacking AMPA receptor blocker Saline (control, n=9), PQ in saline (n=8), and PQ + NASPM in saline (n=5) were unilaterally injected into the SNpc of rats. Two weeks later, TH immunostaining with Alexa Fluor 633 fluorescence was measured in the SNpc surrounded by the dotted line. Each bar and line represent the ratio of Alexa Fluor 633 fluorescence in the ipsilateral SNpc to Alexa Fluor 633 fluorescence in the contralateral SNpc, which was expressed as 100%. ***, p<0.001, vs. each contralateral side and each ipsilateral side injected with vehicle, #, p<0.05, vs. each ipsilateral side injected with PQ (Tukey’s test).
Figure 7

PQ-mediated neuronal loss in the SNpc is rescued in the presence of a dopamine reuptake inhibitor Saline (control, n=5), PQ in saline (n=9), and PQ + GBR in saline (n=6) were unilaterally injected into the SNpc of rats. Two weeks later, TH immunostaining with Alexa Fluor 633 fluorescence was measured in the SNpc surrounded by the dotted line. Each bar and line represent the ratio of Alexa Fluor 633 fluorescence in the ipsilateral SNpc to Alexa Fluor 633 fluorescence in the contralateral SNpc, which was expressed as 100%. **, p<0.01, ***, p<0.001, vs. each contralateral side and each ipsilateral side injected with vehicle, ###, p<0.001, vs. each ipsilateral side injected with PQ (Tukey’s test).
Figure 8

Proposed mechanism on difference in vulnerability to dopaminergic degeneration between the striatum and the SNpc. H2O2 signaling produced by PQ leads to neurodegeneration in the SNpc, but not in the striatum.