Neurochemistry International 129 (2019) 104492

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

Enhancement of ATP production ameliorates motor and cognitive impairments in a mouse model of MPTP−induced Parkinson's disease

Hidaka Haga[a], Kazuya Matsuo[a], Yasushi Yabuki[a], Chen Zhang[b], Feng Han[c], Kohji Fukunaga[a,*]

[a] Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan
[b] College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang, 31005, China
[c] School of Pharmacy, Nanjing Medical University, Nanjing, 211166, PR China

ARTICLE INFO

Keywords:
Ethyl pyruvate
SA4503
Parkinson's disease
Cognitive function
Neuroprotection
ATP

ABSTRACT

Approximately 30–40% of patients with Parkinson's disease (PD) exhibit cognitive impairments. However, there are currently no clinically effective drugs for the treatment of cognitive impairment in patients with PD. Previous studies have suggested that mitochondrial dysfunction such as decreased adenosine triphosphate (ATP) production triggers dopaminergic neurodegeneration in patients with PD and that mitochondria represent a potential target for the development of novel treatments for preventing PD. Therefore, in the present study, we investigated the cognition-enhancing effects of ethyl pyruvate (EP) and 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (SA4503) in mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism. PD model mice were generated via treatment with MPTP (25 mg/kg, i.p.) once a day for 5 consecutive days. Twenty-four hours after the final injection of MPTP, mice were intraperitoneally injected with EP (25, 50, 100 mg/kg) or SA4503 (1 mg/kg) once a day for 4 weeks. Chronic administration of EP (100 mg/kg i.p.) or SA4503 (1 mg/kg, i.p.) improved both motor deficits and cognitive impairments in MPTP-treated mice. Furthermore, treatment with EP or SA4503 attenuated decreases in the levels of ATP and tyrosine hydroxylase (TH) in the substantia nigra pars compacta (SNpc)/ventral tegmental area (VTA), striatum, and hippocampal CA1 region. Administration of EP or SA4503 protected the dopaminergic neurons from MPTP-induce toxicity and restored the dopamine levels in the striatum. Elevated 4-hydroxy-2-nonenal- (4-HNE-) and nitrotyrosine-reactive protein levels induced by MPTP-treatment were suppressed by EP or SA4503 treatment in the SNpc-VTA, striatum, and hippocampal CA1 region. These observations suggest that EP and SA4503 attenuate cognitive impairments and motor dysfunction in mice with MPTP-induced PD.

1. Introduction

Treatment with levodopa (L-DOPA) temporarily attenuates motor deficits in patients with Parkinson's disease (PD). However, chronic L-DOPA administration often results in adverse effects such as wearing-off and dyskinesia (Stocchi et al., 2013; Fukae et al., 2015). In addition, there are currently no clinically effective drugs for the treatment of cognitive impairment in patients with PD. Due to the growing number of older adults in the population, the need for novel therapeutic strategies for PD remains urgent.

PD is a progressive disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Olanow and Olanow, 1999; Dauer and Przedborski, 2003; Savitt et al., 2006). While the precise mechanism underlying the development of PD remains unclear, several genetic mutations have been identified in patients with familial PD. Mitochondrial damage associated with the mutation of *Parkin* and *PINK1* activates the ubiquitin pathway and recruits Parkin on the surface of damaged mitochondria, leading to selective mitophagy (Chu, 2018; Pickrell and Youle, 2015). In patients with familial PD harboring *Parkin* and *PINK1* mutations, the mutated Parkin and Pink1 proteins accumulate in the damaged mitochondria, subsequently resulting in cell death (Ordureau et al., 2018).

Although several hypotheses are proposed about cause of disease in idiopathic PD, the strong etiological evidences have not been established. When Shamir et al. (2017) carried out gene expression analyses in idiopathic PD patients, many genetic dysregulations are appeared in the mitochondria-localized genes such as *COX4I1*, the terminal enzyme of the mitochondrial respiratory chain, *ATP5A1*, a subunit of mitochondrial ATP synthase, and *VDAC3*, a voltage-dependent anion channel located on the outer mitochondrial membrane. In addition, the

* Corresponding author. Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aramaki-Aoba Aoba-ku, Sendai, 980-8578, Japan.
E-mail address: kfukunaga@m.tohoku.ac.jp (K. Fukunaga).

https://doi.org/10.1016/j.neuint.2019.104492
Received 5 October 2018; Received in revised form 29 May 2019; Accepted 17 June 2019
Available online 20 June 2019
0197-0186/

recruitment of Parkin protein to depolarized mitochondria causes autophagic degradation of mitochondria.

Edgar and Trifunovic (2009) demonstrated that mice with mutation of mitochondrial DNA (mtDNA) exhibit advanced aging phenotypes. Likewise, aging affects mitochondrial function in human (Short et al., 2005; Conley et al., 2000; Crane et al., 2010). The substantial reduction of mtDNA copy number was also observed with aging in the skeletal muscles and liver in rats (Barazzoni et al., 2000). Taken together, the aging is associated with decrease in mtDNA copy number and impairment of mitochondrial ATP production.

Furthermore, previous studies have indicated that ATP levels are decreased in the midbrain of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD model mice, and in SNpc cells from *Parkin* knock-out (KO) mice (Nakano et al., 2017; Giguere et al., 2018). Additional studies have demonstrated that mitochondrial complex I activity and coenzyme Q10 content, the latter of which is an electron acceptor for complex I and complex II, are reduced in the SNpc of patients with PD (Schapira, 1993; Shults et al., 1997). These observations suggest that mitochondrial dysfunction (e.g., decreased ATP production) triggers dopaminergic neurodegeneration in patients with PD and that mitochondria represent a potential target for the development of novel treatments for preventing PD.

Pyruvate is a substrate of the tricarboxylic acid (TCA) cycle that plays an important role in the production of ATP (Vander Heiden et al., 2009; Dang, 2010). Ethyl pyruvate (EP) is hydrolyzed to pyruvate and ethanol under physiological conditions (Kao and Fink, 2010), and several studies have reported that EP can enhance ATP biosynthesis *in vivo* and *in vitro* (Woo et al., 2004; Zeng et al., 2007). EP treatment increases ATP levels in rat myocardial tissue following ischemia-reperfusion injury (Woo et al., 2004) and superfused cerebrocortical slices from neonatal rats (Zeng et al., 2007). Furthermore, previous studies have indicated that EP exerts neuroprotective effects in mice with MPTP-induced PD (Satpute et al., 2013; Huh et al., 2011). Satpute et al. (2013) reported that EP (100 mg/kg, p.o.) treatment improves motor dysfunction and mitochondrial complex I activity in such mice and that the effects are comparable to those observed following combined treatment with levodopa and benserazide. Similarly, Huh et al. (2011) reported that EP injection 12 h after the final injection of MPTP (50 mg/kg, i.p.) attenuated MPTP-induced loss of tyrosine hydroxylase (TH)-positive neurons in the SN and ameliorated motor dysfunction. However, whether EP exerts cognition-enhancing effects remains to be fully elucidated.

Previous research has also demonstrated that the sigma-1 receptor agonist 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (SA4503) exerts neuroprotective effects against hypoxia in rat primary neurons (Nakazawa et al., 1998) and in rat models of stroke (Ruscher et al., 2011). The sigma-1 receptor is located in the membrane of the mitochondria-associated endoplasmic reticulum (ER), where it interacts with the inositol 1,4,5-trisphosphate receptor (IP_3R). Recently, we reported that overexpression of the sigma-1 receptor in neuro2A cells promotes calcium influx and ATP production, suggesting that the sigma-1 receptor mediates mitochondrial function in a positive manner (Tagashira et al., 2014). However, it remains unclear whether and to what extent EP and SA4503 exert cognition-enhancing effects in MPTP-treated mice. In order to determine whether enhancers of mitochondrial function may improve cognitive function in patients with PD, the present study investigated the cognition-enhancing effects of EP or SA4503 treatment in MPTP-treated mice.

2. Experimental procedures

2.1. Animals

Male C57BL/6N mice (9 weeks old) were purchased from Clea Japan Inc. (Tokyo, Japan). Mice were housed in a room held at a constant temperature (23 ± 1 °C) and humidity (55 ± 5%) under a 12-h light–dark cycle (light: 9–21 h), with *ad libitum* access to food and water. All animal procedures were approved by the Committee on Animal Experiments at the Tohoku University. Efforts were made to minimize suffering and decrease the number of animals used.

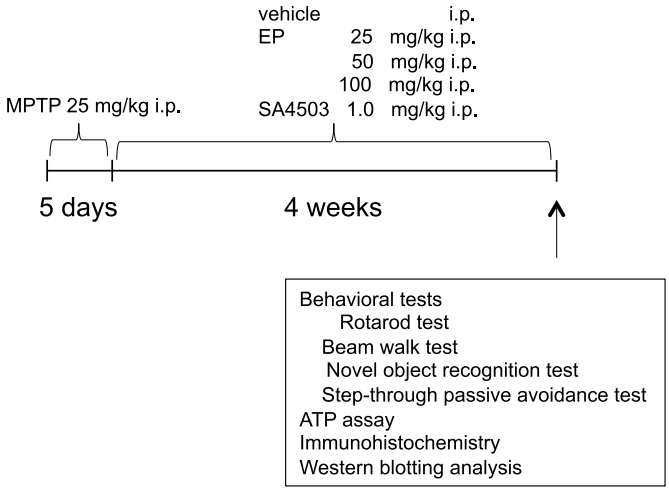

Fig. 1. Experimental schedule for the present study. Mice were injected with MPTP (25 mg/kg, i.p.) once a day for 5 consecutive days. Animals were treated with EP or SA4503 24 h after the final MPTP injection.

2.2. Experimental design and drug administration

MPTP-treated PD model mice were prepared as previously described (Moriguchi et al., 2012). Mice were injected with MPTP (25 mg/kg body weight, i.p.; Sigma, St Louis, MO, USA) or the same volume of saline once a day for 5 days.

EP (Sigma-Aldrich, St-Louis, MO, USA) or SA4503 was dissolved in saline. SA4503, a sigma-1 receptor agonist, was synthesized in the Laboratory of Medicinal Chemistry at Zhejiang University. Control mice were treated with the same volume of saline, EP (100 mg/kg), or SA4503 (1 mg/kg). Twenty-four hours after the final MPTP injection, mice were intraperitoneally injected with EP (25, 50, 100 mg/kg), SA4503 (1 mg/kg), or saline once per day for 28 consecutive days. Twenty-four hours after the final treatment with EP and SA4503, mice were subjected to behavioral tests to assess motor and cognitive functions (Fig. 1). The EP solution was prepared immediately before administration.

2.3. Rotarod test

The rotarod test was conducted as previously described (Moriguchi et al., 2012) using an apparatus consisting of an iron rod (3 cm in diameter, 30 cm in length) with a non-slip surface. Before MPTP treatment, a mouse was placed on the rotating rod (20 rpm) for training. The test was repeated until the fall latency exceeded 100 s. For the test session, the mouse was placed on the rotating rod (20 rpm), and the latency to fall was recorded for a maximum of 300 s.

2.4. Beam walk test

The beam walk test was conducted following previously described methods (Yabuki et al., 2014). During the training session before MPTP treatment, mice were placed 10 cm from the goal and allowed to reach the goal box. During the next several training sessions, mice were placed 30, 50, and 80 cm from the goal. In the test session, mice were placed 80 cm from the goal, and mice that could reach the goal within 60 s were considered to meet the criteria. We also counted the number of foot-slips (missteps) during the test session.

2.5. Novel object recognition test

The novel object recognition test was conducted as previously described by an observer blinded to the treatment group (Yabuki and Fukunaga, 2013). In the 10-min trial session, the mouse was placed in a chamber with two similar objects made of the same material, which were placed in symmetric positions. One hour later, one of the objects was replaced with a novel object, and the test was repeated using the same mouse. During the testing session, searching behavior was recorded for 5 min. The ratio of the number of exploratory behaviors for each object (i.e., sniffing at a distance of less than 1 cm) was calculated as an index of spatial memory.

2.6. Step-through passive avoidance test

The step-through passive avoidance test was conducted as previously described (Yabuki and Fukunaga, 2013). Trial and test sessions were conducted in an apparatus consisting of dark (25 × 25 × 25 cm) and light (14 × 10 × 25 cm) compartments. The floor was composed of steel rods, which were connected to an electronic stimulator (Nihon Kohden, Tokyo, Japan) in the dark compartment. Animals were habituated to the apparatus prior to testing. In the trial session, a mouse was placed in the light compartment with its back facing the dark compartment. Upon the mouse's entrance to the dark compartment, the door was closed, and the stimulator delivered a shock at 0.3 mA for 2 s. The mouse was allowed to remain in the dark compartment for 30 s and removed. Twenty-four hours later, the mouse was placed in the light compartment for the test session, and the latency to enter the dark compartment was recorded for a maximum of 300 s as an index of retention level.

2.7. Immunohistochemistry

Immunohistochemistry was conducted as previously described (Fujita et al., 2009). Following behavioral testing, mice were anesthetized and then perfused with 4% paraformaldehyde. After 24 h of fixation at 4 °C, the brains were removed and sliced with to a thickness of 50 μm using a vibratome (Dosaka EM Co. Ltd., Kyoto, Japan). Coronal sections were incubated with 0.01% Triton X-100 in phosphate-buffered saline (PBS; pH 7.4) for 30 min. After 1 h of incubation with 3% bovine serum albumin (BSA) in PBS (blocking solution), sections were incubated overnight with primary antibody (rabbit polyclonal antibody against TH, 1:1000; Immunostar, Hudson, WI) diluted with blocking solution. After washing, sections were incubated in PBS containing 3% H_2O_2 for 30 min at room temperature and secondary antibody (biotinylated anti-rabbit IgG, 1:200; Vector Laboratories Inc.) diluted with blocking solution for 2 h, following which they were processed using a Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA, USA). The peroxidase reaction product was detected using 3′3′-diaminobenzidine-tetrahydrochloride (DAB; Sigma). After washing with PBS, sections were mounted in Entellan (MERCK, Dannstadt, Germany) and dehydrated using a graded series of ethanol. The sections were then rinsed with xylene and cover-slipped. For each animal, TH-positive cells in two randomly selected regions (500 × 500 μm each) from both sides of the brain were counted and represented as percentages relative to the number observed in control mice. Ventral tegmental area (VTA) and SNpc positions (3.08–3.52 from bregma) were identified following the criteria outlined by Paxinos and Franklin (2001).

2.8. Western blotting analysis

Following decapitation, tissues from the SNpc-VTA, striatum, or hippocampal CA1 region were dissected and frozen in liquid nitrogen, and stored at −80 °C until use. The western blotting analysis was conducted as previously described (Yabuki and Fukunaga, 2013). Frozen tissues were homogenized with homogenization buffer (50 mM Tris-HCl, 40 mM sodium pyrophosphate, 1 mM Na_3VO_4, 4 mM EGTA, 10 mM EDTA, 50 mM NaF, 50 μg/mL trypsin inhibitor, 25 μg/mL pepstatin A, 1 mM dithiothreitol, 100 nM calyculin A, 50 μg/mL leupeptin, 0.5% Triton X-100, pH 7.4). After centrifugation (10 min, 20,400 *g*) to remove insoluble material, Laemmli's sample buffer (Laemmli, 1970) was added, and the solution was boiled at 100 °C for 3 min. Because nitrotyrosine is reduced to aminotyrosine when 2-mercaptoethanol is contained in Laemmli's sample buffer, the supernatant for the nitrotyrosine assay was mixed with Laemmli's sample buffer without 2-mercaptoethanol and heated at 60 °C for 3 min as described (Hashiguchi et al., 2003). Bradford's assay was used to determine protein concentrations in the samples (Bradford, 1976). Equal volumes of sample were loaded onto SDS–polyacrylamide gels and transferred to Immobilon polyvinylidene difluoride membranes. After 1 h of blocking at room temperature with 5% skim milk powder dissolved in Tris with Tween 20 Buffered-Saline (TTBS) solution (50 mM Tris–HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.5), membranes were incubated with anti-β-tubulin (1:5000; Sigma), anti-tyrosine hydroxylase (1:1000; Millipore), anti-choline acetyltransferase (ChAT; 1:1000; Millipore), anti-nitrotyrosine (1:1000; Millipore), anti-4-hydroxy-2-nonenal (4-HNE) antibodies (1:500; JaICA) overnight at 4 °C. After washing with TTBS solution, membranes were immersed in TTBS solution containing the suitable horseradish peroxidase-conjugated secondary antibody. Blots were detected using an ECL immunoblotting detection system (Amersham Biosciences, NJ, USA) and quantified using Image Gauge version 3.41 (Fuji Film, Tokyo, Japan). The density of TH and ChAT were analyzed by normalizing to that of β-tubulin.

2.9. ATP assay

SNpc-VTA, striatal, or hippocampal CA1 tissue was dissected and frozen in liquid nitrogen, and stored at −80 °C. The concentration of ATP in each tissue extract was assessed using an ATP assay kit (Toyo Ink, Tokyo, Japan) following the manufacturer's protocol. Briefly, frozen tissues were homogenized with the homogenization buffer (10 mM HEPES-NaOH, 0.25 M sucrose, pH 7.4), following which they were centrifuged at 1000 *g* for 10 min at 4 °C. The supernatant was then collected. Protein concentrations were aligned using Bradford's assay (Bradford, 1976), and add the extraction buffer to each equivalent concentration sample. After 30 min at room temperature, samples were mixed with luciferin buffer, and oxyluciferin was immediately detected and measured using a luminometer (Gene Light 55, Microtec, Funabashi, Japan).

2.10. Determination of dopamine levels in the striatum using HPLC

In 4 weeks after MPTP injection, the dorsal striatal tissues were dissected out, flash-frozen and stored at −80 °C until use. Measurement of dopamine levels in the dorsal striatum was conducted as described previously (Yabuki et al., 2014). Tissues were weighed and homogenized in 200 μL of ice-cold buffer containing 0.2 M perchloric acid and 100 ng/mL isoproterenol as an internal standard. The homogenate was kept on ice for 30 min to be deproteinized then centrifuged (20,000 *g* for 15 min at 4 °C). After dilution of the supernatant with 9 vol of homogenizing buffer to avoid peak saturation of the measurement, 10 μL of sample was applied to HPLC-electrochemical detection (ECD) system (HTEC-500; Eicom, Kyoto, Japan) and data were calculated as ng/g tissue weight.

2.11. Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Significant differences were determined using Student's *t*-test for two-group comparisons (discrimination index). For all other analyses, a one-way analysis of variance (ANOVA) followed by Fisher's protected

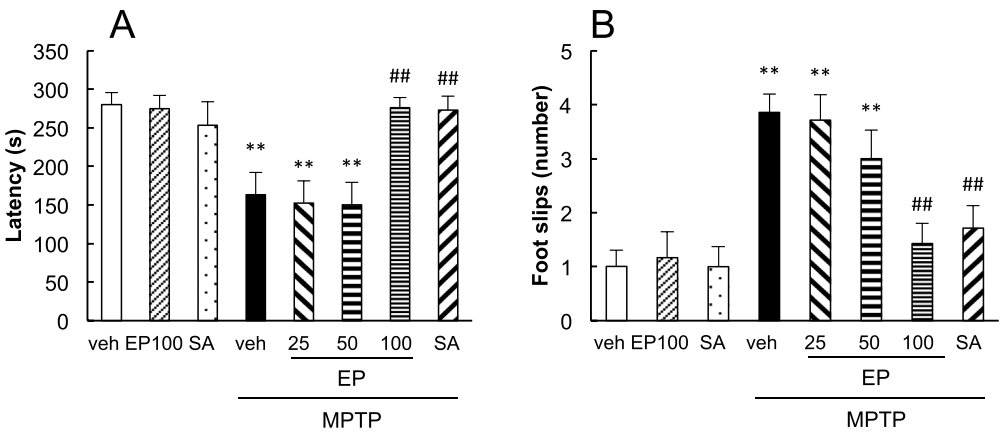

Fig. 2. EP or SA4503 treatment ameliorates motor impairments due to MPTP treatment. (A) MPTP treatment decreased the latency to fall from the drum in the rotarod test, although this effect was attenuated by treatment with EP (100 mg/kg i.p.) or SA4503 (n = 6–7 per group). (B) EP (100 mg/kg i.p.) or SA4503 treatment improved MPTP-induced increases in the number of foot slips (n = 6–7 per group). Error bars represent the standard error of the mean (SEM). **p < 0.01 vs. control group; ##p < 0.01 vs. MPTP-treated mice. veh: vehicle treatment, MPTP: MPTP treatment, EP: EP treatment, EP100: EP (100 mg/kg) treatment, SA: SA4503 treatment. EP: ethyl pyruvate.

least significant difference test was used. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Treatment with EP or SA4503 improves motor and cognitive impairments in MPTP-treated mice

We first investigated whether EP or SA4503 administration prevents motor impairments in MPTP-treated mice. Significant group effects were observed in the rotarod [$F(7, 46) = 6.732$, $p < 0.0001$] and beam walk tests [$F(7, 46) = 8.544$, $p < 0.0001$]. Consistent with previous findings (Moriguchi et al., 2012; Yabuki et al., 2014), MPTP-treated mice exhibited decreased latency to falling in the rotarod test (163.3 ± 28.8 s, $p < 0.01$ vs. control; Fig. 2A) and a greater number of missteps in the beam walk test (3.9 ± 0.3, $p < 0.01$ vs. control; Fig. 2B) 4 weeks after the final MPTP administration, suggestive of motor impairments (Fig. 2). Both EP (100 mg/kg, i.p.) and SA4503 (1 mg/kg, i.p.) treatment significantly attenuated motor impairments in MPTP-treated mice (rotarod test: EP100; 276.1 ± 13.3 s, $p > 0.05$ vs. control, $p < 0.01$ vs. MPTP-treated mice; SA4503, 273.0 ± 18.0 s, $p > 0.05$ vs. control, $p < 0.01$ vs. MPTP-treated mice; beam walk test: EP100; 1.4 ± 0.4, $p > 0.05$ vs. control, $p < 0.01$ vs. MPTP-treated mice; SA4503, 1.7 ± 0.4, $p > 0.05$ vs. control, $p < 0.01$ vs. MPTP-treated mice; Fig. 2). Treatment with EP (100 mg/kg) or SA4503 alone did not alter motor functions in control mice (Fig. 2). The observed improvements in motor function following EP treatment were consistent with those reported in a previous study (Satpute et al., 2013).

We then evaluated the effect of EP and SA4503 on cognitive impairments following sub-chronic MPTP treatment. No significant differences were observed in the trial sessions of the novel object recognition test (Fig. 3A); however, MPTP-treated mice failed to discriminate between familiar and novel objects in the test session (Fig. 3B). MPTP-treated mice that had received EP (100 mg/kg i.p.) or SA4503 (1 mg/kg, i.p.) exhibited normal discrimination index values, similar to control mice (Fig. 3B). Significant group effects were observed in the step-through passive avoidance test at day 3 [$F(7, 36) = 2.950$, $p = 0.0150$] and day 4 [$F(7, 36) = 3.676$, $p = 0.0043$]. Although no significant differences were observed in the trial session (Fig. 3C), MPTP-treated mice exhibited reduced entrance latency in the step-through passive avoidance test at day 3 (188.8 ± 49.7 s, $p < 0.05$ vs. control; Fig. 3D) and day 4 (181.8 ± 43.6 s, $p < 0.05$ vs. control; Fig. 3D). In MPTP-treated mice, treatment with EP (100 mg/kg, i.p.) or SA4503 (1 mg/kg, i.p.) prevented decreases in latency at both day 3 (EP100: 291.7 ± 8.3 s, $p > 0.05$ vs. control, $p < 0.05$ vs. MPTP-treated mice; SA4503: 269.2 ± 30.8 s, $p > 0.05$ vs. control, $p > 0.05$ vs. MPTP-treated mice; Fig. 3D) and day 4 (EP100: 300.0 ± 0 s, $p > 0.05$ vs. control, $p < 0.05$ vs. MPTP-treated mice; SA4503: 271.8 ± 19.6 s, $p > 0.05$ vs. control, $p < 0.05$ vs. MPTP-treated mice; Fig. 3D), suggesting that EP and SA4503 may prevent both motor and cognitive impairments following sub-chronic MPTP treatment. Treatment with EP (100 mg/kg) or SA4503 alone did not alter cognitive functions in control mice (Fig. 3).

3.2. EP and SA4503 attenuate reductions in ATP levels in several brain areas in MPTP-treated mice

Since previous studies have reported that EP treatment improves mitochondrial complex I activity (Satpute et al., 2013), while SA4503 improves mitochondrial function via the TCA cycle (Reisch and Elpeleg, 2007; Tagashira et al., 2013), we next evaluated ATP content in the SNpc including the VTA (SNpc-VTA), striatum, and hippocampal CA1 region, which mediate dopaminergic neuronal activity, motor coordination, and memory, respectively (Brichta et al., 2013; Hornykiewicz, 1966; Eichenbaum et al., 1992). Significant group effects were observed with regard to ATP levels in the SNpc-VTA [$F(7, 47) = 5.739$, $p < 0.0001$], striatum [$F(7, 54) = 5.752$, $p < 0.0001$], and hippocampal CA1 region [$F(7, 51) = 5.614$, $p < 0.0001$]. Following MPTP treatment, ATP levels were significantly decreased in all three brain areas, relative to those observed in control mice (SNpc-VTA: 74.8 ± 3.7%, $p < 0.01$ vs. control; striatum: 64.0 ± 3.6%, $p < 0.01$ vs. control; hippocampal CA1: 55.9 ± 11.0%, $p < 0.01$ vs. control; Fig. 4). Notably, treatment with EP (100 mg/kg, i.p.) or SA4503 (1 mg/kg, i.p.) significantly restored tissue ATP levels in each region in MPTP-treated mice (SNpc-VTA: EP100; 94.9 ± 3.3%, $p > 0.05$ vs. control, $p < 0.01$ vs. MPTP-treated mice; SA4503; 88.8 ± 3.6%, $p > 0.05$ vs. control, $p < 0.05$ vs. MPTP-treated mice; striatum: EP100; 90.4 ± 4.8%, $p > 0.05$ vs. control, $p < 0.01$ vs. MPTP-treated mice; SA4503; 93.5 ± 6.9%, $p > 0.05$ vs. control, $p < 0.01$ vs. MPTP-treated mice; hippocampal CA1: EP100; 93.2 ± 3.5%, $p > 0.05$ vs. control, $p < 0.01$ vs. MPTP-treated mice; SA4503; 97.6 ± 8.4%, $p > 0.05$ vs. control, $p < 0.01$ vs. MPTP-treated mice; Fig. 4). However, EP (100 mg/kg i.p.) or SA4503 treatment alone did not affect ATP levels in naive mice (Fig. 4).

3.3. EP and SA4503 protect dopaminergic neurons from MPTP toxicity

Because EP (100 mg/kg, i.p.) and SA4503 (1 mg/kg, i.p.) significantly improved motor and cognitive impairments by attenuating reductions in ATP levels in MPTP-treated mice, we aimed to verify whether EP or SA4503 prevents loss of dopaminergic neurons due to MPTP neurotoxicity. Significant group effects were observed with regard to the number of tyrosine hydroxylase (TH)-positive cells in the VTA [$F(3, 20) = 3.834$, $p = 0.0256$] and SNpc [$F(3, 20) = 12.011$, $p = 0.0001$]. Mice treated with MPTP exhibited a lower number of TH-positive cells than the control group (VTA, 72.4 ± 5.9% of control, $p < 0.01$ vs. control; SNpc, 63.9 ± 4.5% of control, $p < 0.05$ vs. control; Fig. 5). As expected, reductions in the number of TH-positive

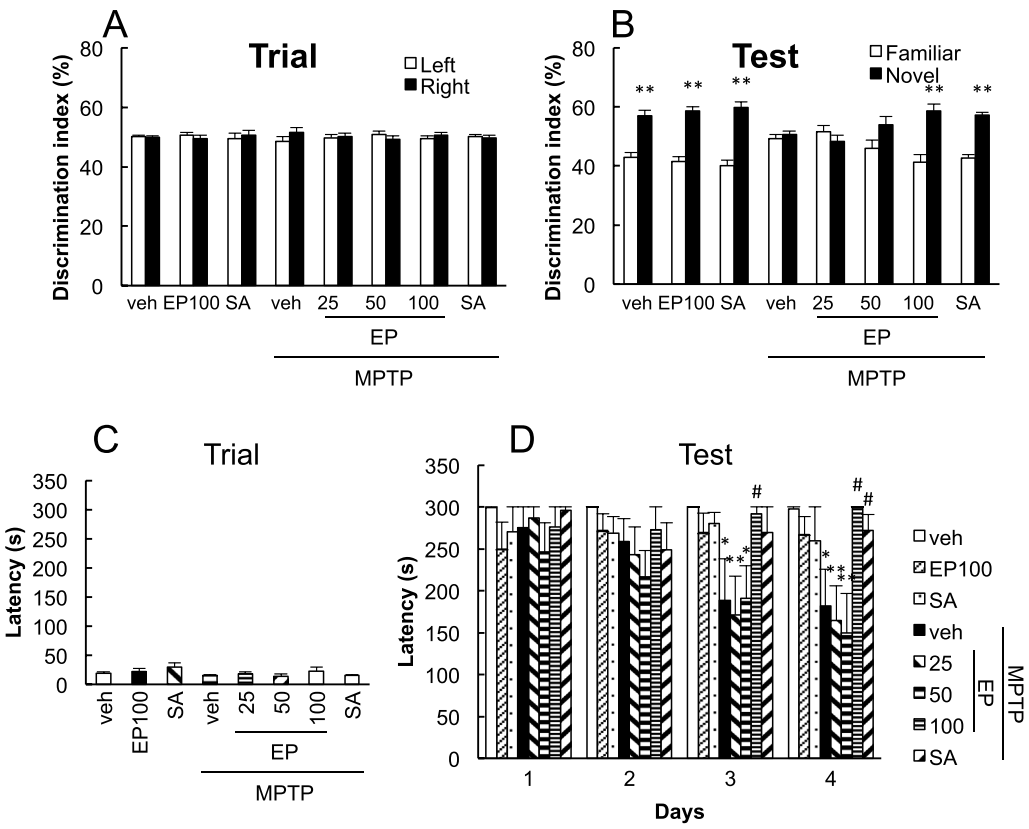

Fig. 3. EP or SA4503 treatment ameliorates cognitive impairments due to MPTP treatment. (A) No significant differences were observed between the groups in the trial sessions of the novel object recognition test. Error bars represent the standard error of the mean (SEM). (B) Treatment with EP (100 mg/kg i.p.) or SA4503 significantly improved the discrimination index between novel and familiar objects in MPTP-treated mice (n = 6–9 per group). Error bars represent the SEM. **p < 0.01 vs. the familiar group. (C) Latencies were comparable in all groups during trial sessions of the step-through passive avoidance test. Error bars represent the SEM. (D) EP (100 mg/kg i.p.) or SA4503 treatment significantly attenuated decreases in latency time relative to MPTP-treated mice (n = 5–6 per group). Error bars represent the SEM. *p < 0.05: **p < 0.01 vs. control mice; #p < 0.05 vs. MPTP-treated mice. veh: vehicle treatment, MPTP: MPTP treatment, EP: EP treatment, EP100: EP (100 mg/kg) treatment, SA: SA4503 treatment. EP: ethyl pyruvate.

neurons in both brain areas were blocked by treatment with EP (100 mg/kg, i.p.) (VTA: 99.7 ± 8.2% of control, p > 0.05 vs. control, p < 0.05 vs. MPTP-treated mice; SNpc: 99.1 ± 6.1% of control, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; Fig. 5) and SA4503 (1 mg/kg, i.p.) (VTA: 100.3 ± 7.6% of control, p > 0.05 vs. control, p < 0.05 vs. MPTP-treated mice; SNpc: 96.8 ± 5.4% of control, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; Fig. 5).

Next, we sought to confirm TH protein levels in the SNpc-VTA, striatum, and hippocampal CA1 region via western blotting. Significant group effects were observed with regard to TH protein levels in the SNpc-VTA [F(5, 30) = 5.623, p = 0.0009], striatum [F(5, 30) = 5.252, p = 0.0014], and hippocampal CA1 region [F(5, 30) = 6.139, p = 0.0005]. In MPTP-treated mice, TH protein levels were significantly reduced in the SNpc-VTA, striatum, and hippocampal CA1 region (SNpc-VTA: 54.0 ± 4.3%, p < 0.01 vs. control; striatum: 63.3 ± 7.2%, p < 0.01 vs. control; hippocampal CA1: 64.7 ± 9.5%, p < 0.01 vs. control; Fig. 6B). EP or SA4503 treatment rescued TH protein levels in MPTP-treated mice (SNpc-VTA: EP 25 mg/kg, 58.9 ± 6.1%, p < 0.05 vs. control, p > 0.05 vs. MPTP-treated mice; EP 50 mg/kg, 64.4 ± 8.5%, p < 0.01 vs. control, p > 0.05 vs. MPTP-treated mice; EP 100 mg/kg, 90.7 ± 9.8%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; SA4503, 92.4 ± 11.5%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; striatum: EP 25 mg/kg, 54.3 ± 8.2%, p < 0.01 vs. control, p > 0.05 vs. MPTP-treated mice; EP 50 mg/kg, 81.6 ± 10.0%, p > 0.05 vs. control, p > 0.05 vs. MPTP-treated mice; EP 100 mg/kg, 98.3 ± 11.5%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; SA4503, 100.3 ± 9.1%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; hippocampal CA1: EP 25 mg/kg, 64.5 ± 2.2%, p < 0.01 vs. control, p > 0.05 vs. MPTP-treated mice; EP 50 mg/kg, 87.9 ± 10.5%, p > 0.05 vs. control, p < 0.05 vs. MPTP-treated mice; EP 100 mg/kg, 106.3 ± 6.6%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; SA4503, 99.2 ± 2.9%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; Fig. 6B). We also evaluated the effects on cholinergic neuron by measurement of ChAT levels in the striatum. No significant group effects were observed [F(5, 30) = 0.411, p = 0.8374; Fig. 6C], suggests that cholinergic neurons are not affected by treatment with MPTP, EP or SA4503. These findings suggest that EP and SA4503 exert neuroprotective effects on dopaminergic neurons against MPTP toxicity.

3.4. EP and SA4503 restore the decrease in dopamine levels

Since dopaminergic neurons were rescued by EP or SA4503 treatment (Fig. 6A and B), we measured dopamine level in striatum using HPLC. A significant group effect was observed in all treatment groups on dopamine content in the striatum [F(3, 21) = 18.470, p < 0.0001]. MPTP treatment significantly reduced the dopamine level in the striatum compared with control mice (control: 7507.9 ± 750.6 ng/g tissue; MPTP-treated mice: 2798.1 ± 213.4 ng/g tissue, p < 0.01 vs. control mice; Fig. 6D). As expected, EP (100 mg/kg, i.p.) and SA4503 (1 mg/kg, i.p.) treatments restored the decreased dopamine contents in the striatum (EP: 4763.6 ± 276.3 ng/g tissue, p < 0.01 vs. control mice, p < 0.01 vs. MPTP-treated mice; SA4503: 4025.0 ± 119.5 ng/g tissue, p < 0.01 vs. control mice, p < 0.05 vs. MPTP-treated mice; Fig. 6D). Thus, the dopamine contents in MPTP-treated mice were significantly restored by EP and SA4503 administration.

3.5. EP and SA4503 attenuate MPTP-induced oxidative damage

Since 4-HNE- and nitrotyrosine-protein levels are known to increase following oxidative stress in the brain (Sakul et al., 2013; Zhong et al., 2013), we next confirmed anti-oxidative effects of EP (100 mg/kg, i.p.) and SA4503 (1 mg/kg, i.p.) treatments by western blotting analyses using anti-4-HNE and anti-nitrotyrosine antibodies in SNpc-VTA, striatum, and hippocampal CA1 region homogenates. A significant group effects were observed on 4-HNE immunoreactive protein levels [SNpc-VTA: F(3, 20) = 9.773, p = 0.0004; striatum: F(3, 20) = 8.123, p = 0.0010; hippocampal CA1 region: F(3, 20) = 4.155, p = 0.0193] and nitrotyrosine-immunoreactive protein levels [SNpc-VTA: F(3, 20) = 10.862, p = 0.0002; striatum: F(3, 20) = 4.060, p = 0.0209;

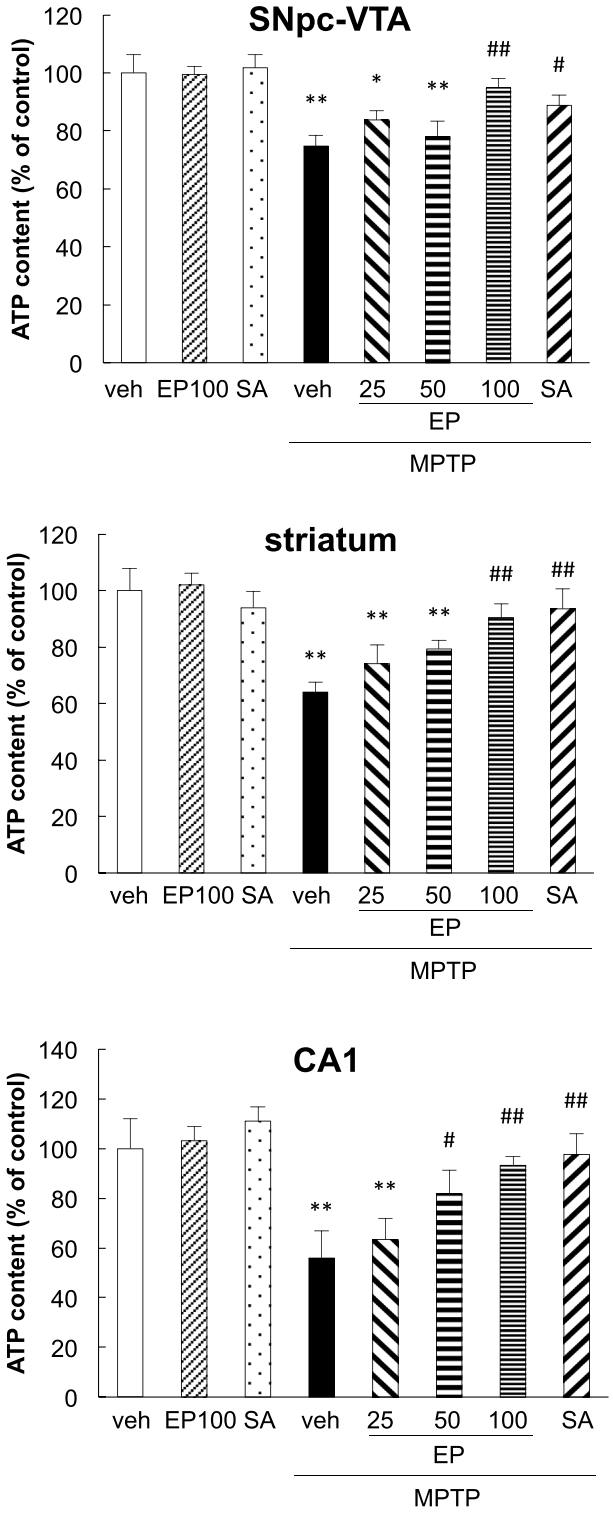

Fig. 4. EP and SA4503 treatment prevents decreases in brain ATP in MPTP-treated mice. MPTP treatment reduced tissue ATP levels in the SNpc-VTA, striatum, and hippocampal CA1 region. However, treatment with EP (100 mg/kg i.p.) or SA4503 attenuated these decreases in each area (n = 5–8 per group). Error bars represent the standard error of the mean (SEM). *p < 0.05; **p < 0.01 vs. control mice; #p < 0.05; ##p < 0.01 vs. MPTP-treated mice. veh: vehicle treatment, MPTP: MPTP treatment, EP: EP treatment, EP100: EP (100 mg/kg) treatment, SA: SA4503 treatment, CA1: hippocampal CA1. EP: ethyl pyruvate.

hippocampal CA1 region: F(3, 20) = 6.844, p = 0.0023]. In MPTP-treated mice, the immunoreactivities of 46 kDa protein against anti-4-HNE and 62 kDa protein against anti-nitrotyrosine antibodies were markedly elevated in the SNpc-VTA, striatum, and hippocampal CA1 region (4-HNE; SNpc-VTA: 196.2 ± 13.8%, p < 0.01 vs. control; striatum: 147.1 ± 16.0%, p < 0.01 vs. control; hippocampal CA1: 191.9 ± 29.7%, p < 0.01 vs. control; nitrotyrosine; SNpc-VTA: 124.0 ± 8.1%, p < 0.05 vs. control; striatum: 138.8 ± 4.2%, p < 0.01 vs. control; hippocampal CA1: 177.8 ± 21.0%, p < 0.01 vs. control; Fig. 7). EP or SA4503 treatment suppressed the increase of 4-HNE- (SNpc-VTA: EP, 130.0 ± 14.0%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; SA4503, 108.1 ± 18.8%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; striatum: EP, 99.2 ± 6.3%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; SA4503, 83.9 ± 2.8%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; hippocampal CA1: EP, 109.4 ± 14.1%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; SA4503, 128.2 ± 21.6%, p > 0.05 vs. control, p < 0.05 vs. MPTP-treated mice; Fig. 7) and nitrotyrosine-reactive protein levels (SNpc-VTA: EP, 71.9 ± 6.4%, p < 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; SA4503, 80.8 ± 9.0%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; striatum: EP, 108.1 ± 8.5%, p > 0.05 vs. control, p < 0.05 vs. MPTP-treated mice; SA4503, 110.3 ± 11.9%, p > 0.05 vs. control, p < 0.05 vs. MPTP-treated mice; hippocampal CA1: EP, 114.9 ± 11.2%, p > 0.05 vs. control, p < 0.01 vs. MPTP-treated mice; SA4503, 131.0 ± 7.5%, p > 0.05 vs. control, p < 0.05 vs. MPTP-treated mice; Fig. 7) induced by MPTP-treatment.

4. Discussion

In the present study, we investigated the improvement effects of EP and SA4503 on cognitive impairments in MPTP-treated mice. Our findings indicated that treatment with EP and SA4503 attenuates MPTP-induced reductions in ATP levels in the SNpc-VTA, striatum, and hippocampal CA1 region. Furthermore, our results suggest that treatment with EP and SA4503 significantly inhibits dopaminergic neuronal loss following MPTP injection.

Previous studies have indicated that EP promotes the production of ATP by generating nicotinamide adenine dinucleotide (NAD) via the conversion of pyruvate into lactate in a murine model of hind-limb ischemia and reperfusion (Crawford et al., 2011). In this previous study, levels of ATP production were higher in the EP treatment group (20 mM, 4 h) than in the no-treatment group following oxidative stress (H_2O_2) in neonatal rats (Zeng et al., 2007). There are two possible mechanisms underlying the facilitation of ATP production by EP. Metabolism of EP increases the supply of pyruvate, which may enhance the TCA cycle, in turn improving the function of the electron transport chain and resulting in oxidative phosphorylation and ATP production (Reisch and Elpeleg, 2007). Alternatively, infused pyruvate is converted to lactate, following which NAD is generated via the reduction of nicotinamide adenine dinucleotide (NADH), thereby inhibiting glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). This process may then lead to ATP production by stimulating anaerobic glycolysis, as previously demonstrated in a dog model of cardiac infarction (Regitz et al., 1981). In the present study, EP (100 mg/kg, i.p.) treatment significantly attenuated decreases in ATP levels in the SNpc-VTA following MPTP treatment, in turn protecting dopaminergic neurons from MPTP toxicity. Treatment with esculetin, which acts as an agonistic ligand of estrogen receptor-related receptors, also increases cellular ATP levels. Previous studies have revealed that increases in ATP prevent cell death following treatment with 75 μM 1-methyl-4-phenylpyridinium (MPP^+) in PC12 cells (Nakano et al., 2017). In addition, chronic esculetin (50 mg/kg, p.o.) treatment has been shown to exert neuroprotective effects on dopaminergic neurons in MPTP-treated mice (Nakano et al., 2017). Taken together, these findings suggest that EP enhances energy production and promotes neuronal survival in MPTP-treated mice.

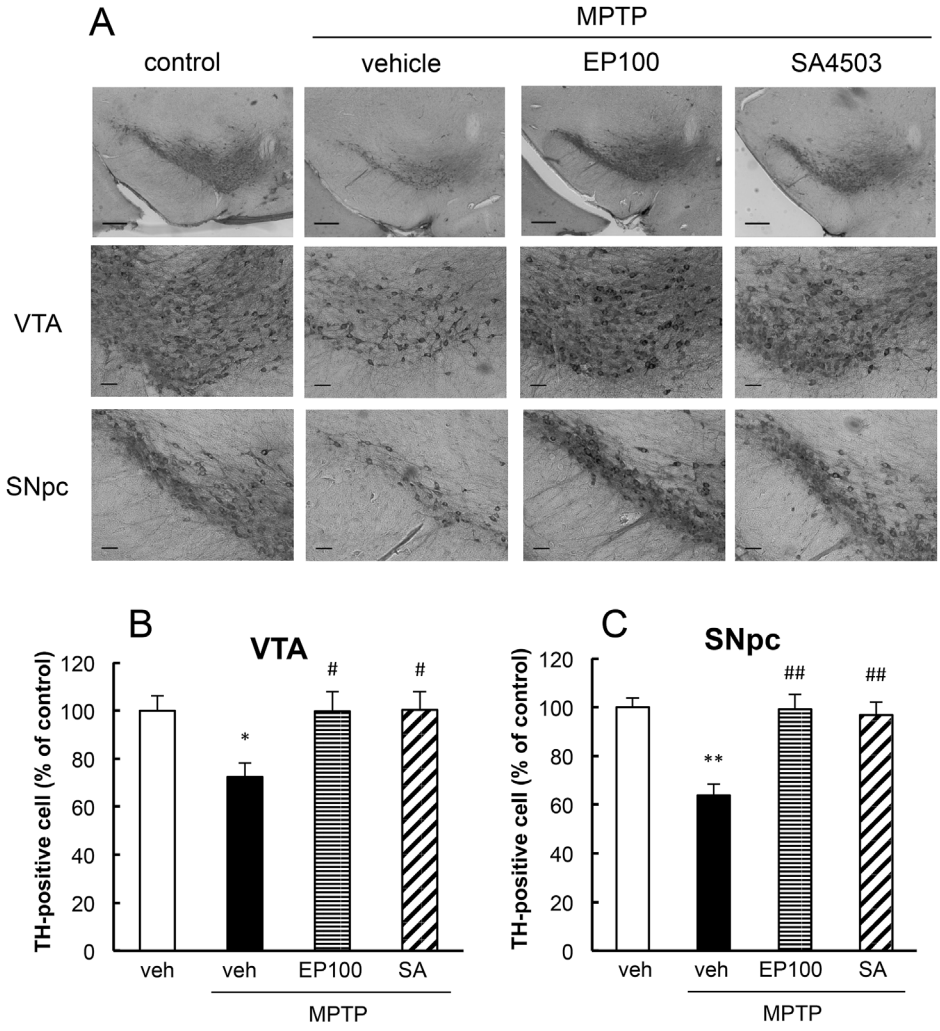

Fig. 5. EP or SA4503 treatment prevents dopaminergic neuronal death due to MPTP toxicity in the VTA and SNpc. (A) Representative histological sections of the VTA and SNpc in control and MPTP-treated mice treated with EP (100 mg/kg i.p.), SA4503, or vehicle. The middle and lower panels show higher magnification images of the VTA and SNpc, respectively. Scale bars: low magnification, 250 μm; high magnification, 50 μm. (B, C) Cell viability was assessed in the VTA (B) and SNpc (C), and is expressed as a percentage of the average number of viable cells in control mice. EP (100 mg/kg i.p.) or SA4503 treatment exerted neuroprotective effects in MPTP-treated mice (n = 6 per group). Error bars represent the standard error of the mean (SEM). *p < 0.05; **p < 0.01 vs. control mice; #p < 0.05; ##p < 0.01 vs. MPTP-treated mice. veh: vehicle treatment, MPTP: MPTP treatment, EP100: EP (100 mg/kg) treatment, SA: SA4503 treatment. EP: ethyl pyruvate; VTA: ventral tegmental area; SNpc: substantia nigra pars compacta.

However, additional studies have reported that EP exerts anti-inflammatory effects by inhibiting high-mobility group box 1 (HMGB1) production (Ulloa et al., 2002; Davé et al., 2009). Ulloa et al. (2002) reported that pretreatment with EP (40 mg/kg, i.p.) decreases serum levels of both tumor necrosis factor and HMGB1 in endotoxemia model mice, and that treatment with 5 mM EP prevents lipopolysaccharide (LPS)-induced activation of nuclear factor-kappa B (NF-κB) and p38 mitogen-activated protein kinase (p38MAPK) pathways in macrophage cultures. Pre-incubation with 2 mM EP for 1 h induces antioxidant effects and scavenges radical oxygen species by modulating the components of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (suppressing $gp91^{phox}$ transcription and Rac1 activity) in BV2 immortalized murine microglial cells (Kim et al., 2008). Likewise, EP has anti-inflammatory effects in BV2 cells. For example, the short-term exposure (10–120 min) to EP (6.5–25 mM) inhibits the production of interleukin-6, tumor necrosis factor and nitric oxide in BV2 cells (Stanisavljević et al., 2015). EP (5 mM) treatment suppresses the nuclear translocations of protein kinase C alpha and calcium/calmodulin-dependent protein kinase IV, HMGB1 phosphorylation, and subsequent secretion of HMGB1 induced by LPS (200 ng/ml) in BV2 cells (Shin et al., 2014). Since sigma-1 receptors are expressed in glial cells, sigma-1 receptor agonist also inhibits the inflammatory reaction in microglia in zebrafish (Moritz et al., 2015). According to these evidences, EP and SA4503 have protective effects not only in neurons but also in glial cells. Taken together, these findings suggest that the mechanism underlying the neuroprotective effect of EP against MPTP toxicity not only promotes energy production but also exerts anti-inflammatory and anti-oxidative effects.

Sodium pyruvate protects cortical and hippocampal structures in rat models of ischemia (Lee et al., 2001). Additional studies have indicated that sodium pyruvate exerts cytoprotective effects against mitochondrial dysfunction following MPP^+ or rotenone treatment *in vitro* (Mazzio and Soliman, 2003; Ramos-Ibeas et al., 2017). While some evidence suggests that sodium pyruvate can be used in the treatment of mitochondrial disorders (Tanaka et al., 2007), it is unstable and easily metabolized in the liver (Vonkorff, 1964; Montgomery and Webb, 1956), indicating that high doses may be necessary to achieve sufficient efficacy. Previous reports have indicated that the effective dose of sodium pyruvate (1000 mg/kg, i.p.) for achieving anti-apoptotic and anti-inflammatory effects in the rat brain is higher than that of EP (40–50 mg/kg, i.p.) (Moro and Sutton, 2010; Pan et al., 2012; Shen et al., 2010), suggesting that EP is superior to sodium pyruvate. Thus, EP may represent a suitable candidate for the treatment of neurodegenerative diseases associated with mitochondrial dysfunction, including PD.

Hayashi and Su revealed that the sigma-1 receptor is localized in the mitochondria-associated ER membrane, where it interacts with IP_3R, which is responsible for calcium transport from the ER to mitochondria in Chinese hamster ovary cells (Hayashi and Su, 2007), thereby contributing to ATP generation and cell survival (Robb-Gaspers et al., 1998; Csordás et al., 2006). We previously reported that overexpression of the sigma-1 receptor promotes IP_3R-dependent calcium transport to the mitochondria as well as ATP production, thereby attenuating ER stress-induced cell death in Neuro2A cells (Tagashira et al., 2014). In

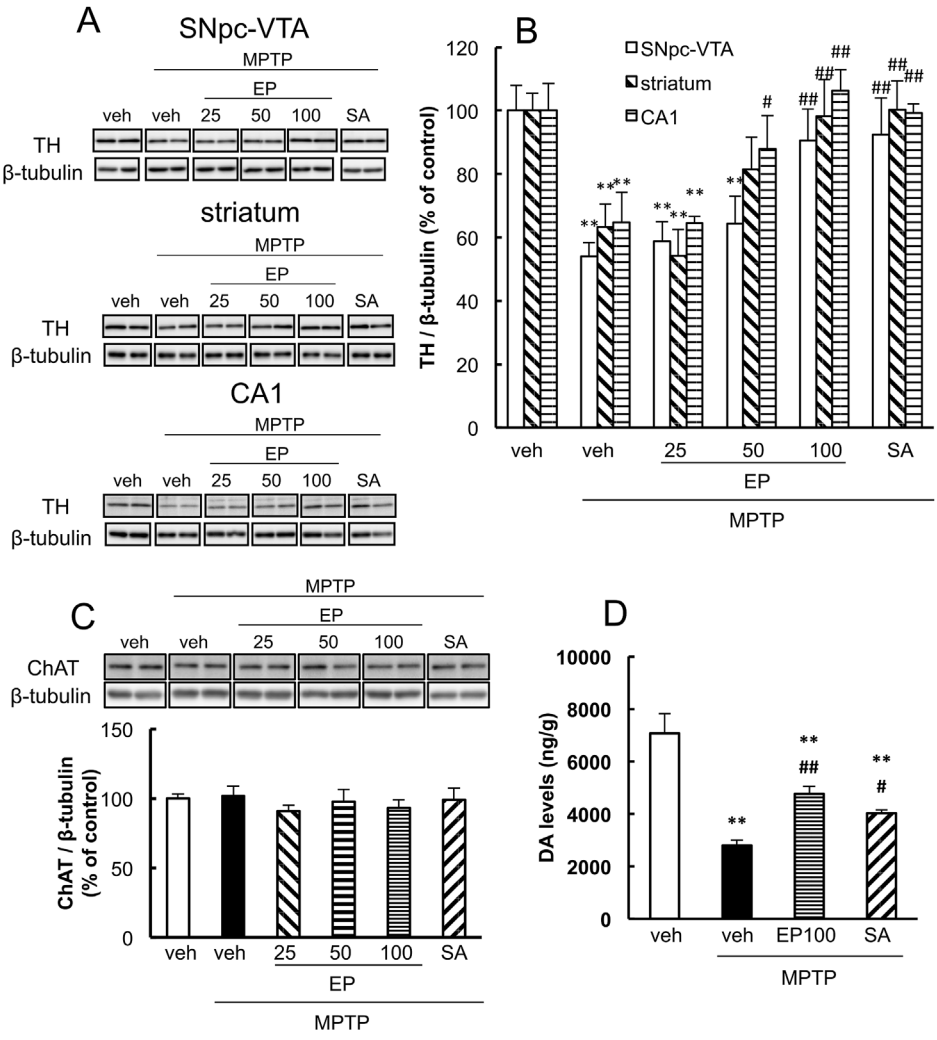

Fig. 6. EP or SA4503 treatment attenuates decreases in protein levels of TH in the SNpc-VTA, striatum, and hippocampal CA1 region of MPTP-treated mice and restores dopamine levels in striatum. (A) Representative western blots of TH and β-tubulin are shown (top, lower). (B) Quantitative analysis of western blotting results. TH protein levels were lower in MPTP-treated mice than in controls for each brain region. EP or SA4503 treatment attenuated decreases in TH levels in MPTP-treated mice (n = 6 per group). (C) Representative western blots of ChAT (top) and β-tubulin (lower) and the quantitative analysis are shown. There were no significant differences in each group (n = 6 per group). (D) Dopamine contents were measured in striatum by HPLC. Dopamine level was reduced in MPTP-treated group. EP or SA4503 treatment significantly restored dopamine levels (n = 6–7 per group). Error bars represent the standard error of the mean (SEM). **p < 0.01 vs. control mice; #p < 0.05; ##p < 0.01 vs. MPTP-treated mice. veh: vehicle treatment, MPTP: MPTP treatment, EP: EP treatment, SA: SA4503 treatment, CA1: hippocampal CA1. EP: ethyl pyruvate; TH: tyrosine hydroxylase; SNpc: substantia nigra pars compacta; VTA: ventral tegmental area.

addition, the sigma-1 receptor agonist dehydroepiandrosterone has been shown to prevent hippocampal neuronal injury due to transient brain ischemia by attenuating decreases in ATP levels via stimulation of the sigma-1 receptor (Yabuki et al., 2015).

In the present study, we used SA4503, which acts to enhance cognition by promoting mitochondrial ATP production. Indeed, we observed that SA4503 promoted ATP production and dopaminergic cell survival, thereby attenuating cognitive impairments in MPTP-treated mice. EP has been reported to exert neuroprotective effects against MPTP neurotoxicity in the midbrain, thus resulting in improvements in motor dysfunction (Satpute et al., 2013; Huh et al., 2011). In addition to these improvements in motor dysfunction, we confirmed that the mitochondrial enhancers EP and SA4503 attenuated cognitive dysfunction in MPTP-treated mice. However, further studies are required to determine whether enhancement of ATP levels in the hippocampus mediates hippocampal-dependent memory and whether restoration of the dopaminergic system via EP treatment accounts for improvements in memory.

Considering that treatment with EP or SA4503 alone has no effects (Figs. 2–4) and that MPTP treatment did not affect the cholinergic neurons (Fig. 6C), EP or SA4503 treatment improved both motor and cognitive impairments by preventing dopaminergic loss in MPTP-treated mice. Previous reports have indicated that dopaminergic pathways play an important role in various cognitive functions (Luciana et al., 1998; Arnsten et al., 1995). D1/D5 receptor antagonists impair long-term potentiation (LTP) and spatial memory, which can be recovered via treatment with D1/D5 receptor agonists (da Silva et al., 2012; Lemon and Manahan-Vaughan, 2006; de Lima et al., 2011). The D1 receptor is especially critical for hippocampal LTP (Granado et al., 2008). Furthermore, KO or downregulation of the D1 receptor via intrahippocampal injections of siRNA markedly reduce spatial learning and hippocampal LTP in mice (Ortiz et al., 2010). In addition, treatment with D1 receptor agonists promotes acetylcholine release in the hippocampus and improves scopolamine-induced memory impairments in rats (Zarrindast et al., 2012; Hersi et al., 1995). These findings indicate that the cognition improving effects of EP and SA4503 in DA neurons in part mediate improvements in cognitive impairments in MPTP-treated mice.

Treatment with EP or SA4503 did not affect tissue ATP levels in naive mice. High concentrations of ATP are generally regarded as cytotoxic (Filippini et al., 1990; Zanovello et al., 1990; Pizzo et al., 1991), suggesting that safe doses of EP and SA4503 were used in the present study. Previous studies have reported that increases in ATP and citrate levels inhibit the activity of phosphofructokinase-1, a key enzyme involved in glycolysis (Webb et al., 2015; Kemp and Foe, 1983). Due to negative feedback mechanisms, excessive ATP production following treatment with EP or SA4503 may inhibit glycolytic activity under physiological conditions, thereby maintaining normal ATP levels. In contrast, the ATP enhancer esculetin increases cellular ATP levels in both MPP^+-treated and control cells (Nakano et al., 2017). These findings suggest that EP is superior to esculetin, as EP exerts additional anti-inflammatory effects without producing excessive ATP.

In summary, the findings of the present study indicate that treatment with EP or SA4503 promotes ATP production and dopaminergic

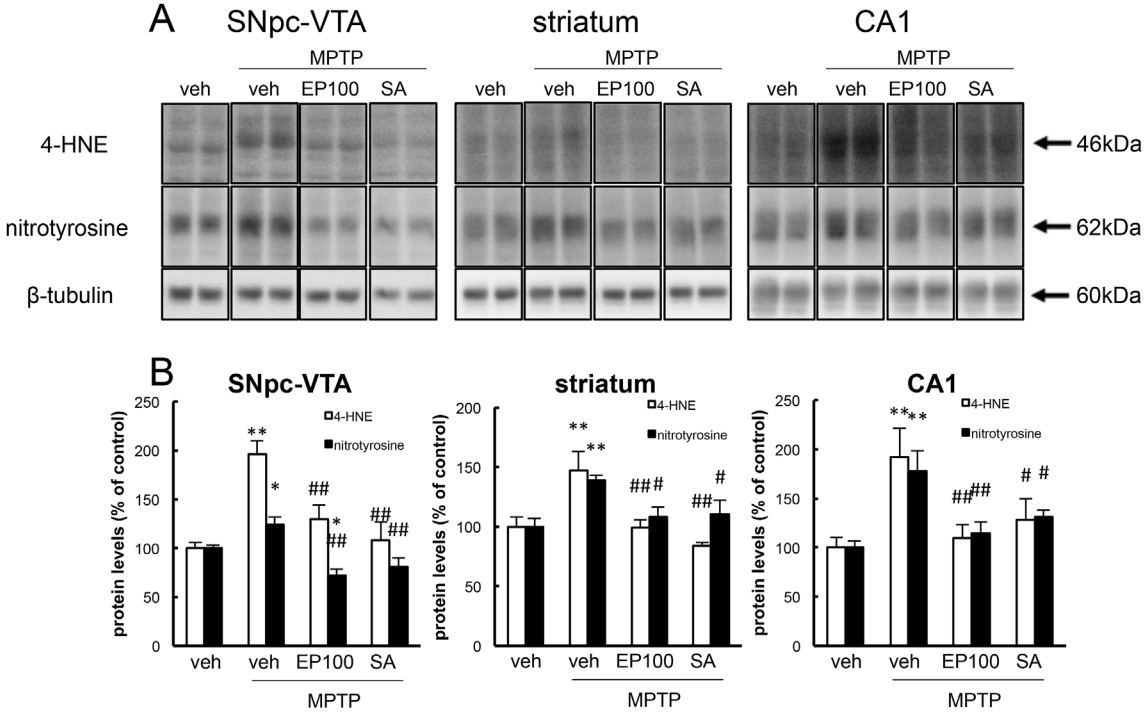

Fig. 7. EP and SA4503 treatment attenuates MPTP-induced oxidative stress. (A) Representative western blots of 4-HNE- and nitrotyrosine-reactive proteins and β-tubulin are shown (top, middle, lower) (B) Quantitative analysis of western blotting results. MPTP-induced increases of 4-HNE- and nitrotyrosine-immunoreactive protein levels are inhibited by EP and SA4503 administration in SNpc-VTA, striatum, and hippocampal CA1 region. (n = 6 per group). Error bars represent the standard error of the mean (SEM). *p < 0.05; **p < 0.01 vs. control mice; #p < 0.05; ##p < 0.01 vs. MPTP-treated mice. veh: vehicle treatment, MPTP: MPTP treatment, EP100: EP (100 mg/kg, i.p.) treatment, SA: SA4503 treatment, CA1: hippocampal CA1. EP: ethyl pyruvate, 4-HNE: 4-hydroxy-2-nonenal; SNpc: substantia nigra pars compacta; VTA: ventral tegmental area.

cell survival in MPTP-treated mice. Treatment with both EP and SA4503 improved cognitive impairments as well as motor dysfunction in MPTP-treated PD mice, suggesting that enhancers of mitochondrial function such as EP and SA4503 can prevent cognitive impairment in patients with early- or late-phase PD. Given this context, future studies should evaluate the cognition-enhancing effects of these agents following the appearance of motor dysfunction in MPTP-treated mice.

Conflicts of interest

The authors have no conflicts of interest to declare.

Funding

This work was supported in part by grants-in-aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (Kakenhi 15H06036 to Y.Y.) and the Strategic Research Program for Brain Sciences from AMED (JP17dm0107071) (to K.F.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.104492.

References

Arnsten, A.F., Cai, J.X., Steere, J.C., Goldman-Rakic, P.S., 1995. Dopamine D2 receptor mechanisms contribute to age-related cognitive decline: the effects of quinpirole on memory and motor performance in monkeys. J. Neurosci. 15 (5 Pt 1), 3429–3439.

Barazzoni, R., Short, K.R., Nair, K.S., 2000. Effects of aging on mitochondrial DNA copy number and cytochrome c oxidase gene expression in rat skeletal muscle, liver, and heart. J. Biol. Chem. 275 (5), 3343–3347.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brichta, L., Greengard, P., Flajolet, M., 2013. Advances in the pharmacological treatment of Parkinson's disease: targeting neurotransmitter systems. Trends Neurosci. 36 (9), 543–554.

Chu, C.T., 2018. Multiple pathways for mitophagy: a neurodegenerative conundrum for Parkinson's disease. Neurosci. Lett. S0304–3940 (18) 30259-3.

Conley, K.E., Jubrias, S.A., Esselman, P.C., 2000. Oxidative capacity and ageing in human muscle. J. Physiol. 526 (Pt 1), 203–210.

Crane, J.D., Devries, M.C., Safdar, A., Hamadeh, M.J., Tarnopolsky, M.A., 2010. The effect of aging on human skeletal muscle mitochondrial and intramyocellular lipid ultrastructure. J. Gerontol. A. Biol. Sci. Med. Sci. 65 (2), 119–128.

Crawford, R.S., Albadawi, H., Atkins, M.D., Jones, J.J., Conrad, M.F., Austen Jr., W.G., Fink, M.P., Watkins, M.T., 2011. Postischemic treatment with ethyl pyruvate prevents adenosine triphosphate depletion, ameliorates inflammation, and decreases thrombosis in a murine model of hind-limb ischemia and reperfusion. J. Trauma 70 (1), 103–110.

Csordás, G., Renken, C., Várnai, P., Walter, L., Weaver, D., Buttle, K.F., Balla, T., Mannella, C.A., Hajnóczky, G., 2006. Structural and functional features and significance of the physical linkage between ER and mitochondria. J. Cell Biol. 174 (7), 915–921.

da Silva, W.C., Köhler, C.C., Radiske, A., Cammarota, M., 2012. D1/D5 dopamine receptors modulate spatial memory formation. Neurobiol. Learn. Mem. 97 (2), 271–275.

Dang, C.V., 2010. Rethinking the Warburg effect with Myc micromanaging glutamine metabolism. Cancer Res. 70 (3), 859–862.

Dauer, W., Przedborski, S., 2003. Parkinson's disease: mechanisms and models. Neuron 39 (6), 889–909.

Davé, S.H., Tilstra, J.S., Matsuoka, K., Li, F., DeMarco, R.A., Beer-Stolz, D., Sepulveda, A.R., Fink, M.P., Lotze, M.T., Plevy, S.E., 2009. Ethyl pyruvate decreases HMGB1 release and ameliorates murine colitis. J. Leukoc. Biol. 86 (3), 633–643.

de Lima, M.N., Presti-Torres, J., Dornelles, A., Scalco, F.S., Roesler, R., Garcia, V.A., Schröder, N., 2011. Modulatory influence of dopamine receptors on consolidation of object recognition memory. Neurobiol. Learn. Mem. 95 (3), 305–310.

Edgar, D., Trifunovic, A., 2009. The mtDNA mutator mouse: dissecting mitochondrial involvement in aging. Aging (Albany NY) 1 (12), 1028–1032.

Eichenbaum, H., Otto, T., Cohen, N.J., 1992. The hippocampus–what does it do? Behav. Neural. Biol. 57 (1), 2–36.

Filippini, A., Taffs, R.E., Agui, T., Sitkovsky, M.V., 1990. Ecto-ATPase activity in cytolytic T-lymphocytes. Protection from the cytolytic effects of extracellular ATP. J. Biol. Chem. 265 (1), 334–340.

Fujita, K., Seike, T., Yutsudo, N., Ohno, M., Yamada, H., Yamaguchi, H., Sakumi, K., Yamakawa, Y., Kido, M.A., Takaki, A., Katafuchi, T., Tanaka, Y., Nakabeppu, Y., Noda, M., 2009. Hydrogen in drinking water reduces dopaminergic neuronal loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's

disease. PLoS One 4 (9), e7247.
Fukae, J., Higuchi, M.A., Yanamoto, S., Fukuhara, K., Tsugawa, J., Ouma, S., Hatano, T., Yoritaka, A., Okuma, Y., Kashihara, K., Hattori, N., Tsuboi, Y., 2015. Utility of the Japanese version of the 9-item wearing-off questionnaire. Clin. Neurol. Neurosurg. 134, 110–115.
Giguere, N., Pacelli, C., Saumure, C., Bourque, M.J., Matheoud, D., Levesque, D., Slack, R.S., Park, D.S., Trudeau, L.E., 2018. Comparative analysis of Parkinson's disease-associated genes reveals altered survival and bioenergetics of parkin-deficient dopamine neurons in mice. J. Biol. Chem. 293 (25), 9580–9593.
Granado, N., Ortiz, O., Suárez, L.M., Martín, E.D., Ceña, V., Solís, J.M., Moratalla, R., 2008. D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-induced arc and zif268 expression in the Hippocampus. Cerebr. Cortex 18 (1), 1–12.
Hashiguchi, A., Kawano, T., Yano, S., Morioka, M., Hamada, J., Sato, T., Shirasaki, Y., Ushio, Y., Fukunaga, K., 2003. The neuroprotective effect of a novel calmodulin antagonist, 3-[2-[4-(3-chloro-2-methylphenyl)-1-piperazinyl]ethyl]-5,6-dimethoxy-1-(4-imidazolylmethyl)-1H-indazole dihydrochloride 3.5 hydrate, in transient forebrain ischemia. Neuroscience 121 (2), 379–386.
Hayashi, T., Su, T.P., 2007. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. Cell 131 (3), 596–610.
Hersi, A.I., Richard, J.W., Gaudreau, P., Quirion, R., 1995. Local modulation of hippocampal acetylcholine release by dopamine D1 receptors: a combined receptor autoradiography and in vivo dialysis study. J. Neurosci. 115 (11), 7150–7157.
Hornykiewicz, O., 1966. Dopamine (3-hydroxytyramine) and brain function. Pharmacol. Rev. 18 (2), 925–964.
Huh, S.H., Chung, Y.C., Piao, Y., Jin, M.Y., Son, H.J., Yoon, N.S., Hong, J.Y., Pak, Y.K., Kim, Y.S., Hong, J.K., Hwang, O., Jin, B.K., 2011. Ethyl pyruvate rescues nigrostriatal dopaminergic neurons by regulating glial activation in a mouse model of Parkinson's disease. J. Immunol. 187 (2), 960–969.
Kao, K.K., Fink, M.P., 2010. The biochemical basis for the anti-inflammatory and cytoprotective actions of ethyl pyruvate and related compounds. Biochem. Pharmacol. 80 (2), 151–159.
Kemp, R.G., Foe, L.G., 1983. Allosteric regulatory properties of muscle phosphofructokinase. Mol. Cell. Biochem. 57 (2), 147–154.
Kim, H.S., Cho, I.H., Kim, J.E., Shin, Y.J., Jeon, J.H., Kim, Y., Yang, Y.M., Lee, K.H., Lee, J.W., Lee, W.J., Ye, S.K., Chung, M.H., 2008. Ethyl pyruvate has an anti-inflammatory effect by inhibiting ROS-dependent STAT signaling in activated microglia. Free Radic. Biol. Med. 45 (7), 950–963.
Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (5259), 680–685.
Lee, J.Y., Kim, Y.H., Koh, J.Y., 2001. Protection by pyruvate against transient forebrain ischemia in rats. J. Neurosci. 21 (20), RC171.
Lemon, N., Manahan-Vaughan, D., 2006. Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. J. Neurosci. 26 (29), 7723–7729.
Luciana, M., Collins, P.F., Depue, R.A., 1998. Opposing roles for dopamine and serotonin in the modulation of human spatial working memory functions. Cerebr. Cortex 8 (3), 218–226.
Mazzio, E., Soliman, K.F., 2003. Pyruvic acid cytoprotection against 1-methyl-4-phenylpyridinium, 6-hydroxydopamine and hydrogen peroxide toxicities in vitro. Neurosci. Lett. 337 (2), 77–80.
Montgomery, C.M., Webb, J.L., 1956. Metabolic studies on heart mitochondria. II. The inhibitory action of parapyruvate on the tricarboxylic acid cycle. J. Biol. Chem. 221 (1), 359–368.
Moriguchi, S., Yabuki, Y., Fukunaga, K., 2012. Reduced calcium/calmodulin-dependent protein kinase II activity in the hippocampus is associated with impaired cognitive function in MPTP-treated mice. J. Neurochem. 120 (4), 541–551.
Moritz, C., Berardi, F., Abate, C., Peri Live, F., 2015. Imaging reveals a new role for the sigma-1 (σ1) receptor in allowing microglia to leave brain injuries. Neurosci. Lett. 591, 13–18.
Moro, N., Sutton, R.L., 2010. Beneficial effects of sodium or ethyl pyruvate after traumatic brain injury in the rat. Exp. Neurol. 225 (2), 391–401.
Nakano, M., Imamura, H., Sasaoka, N., Yamamoto, M., Uemura, N., Shudo, T., Fuchigami, T., Takahashi, R., Kakizuka, A., 2017. ATP maintenance via two types of ATP regulators mitigates pathological phenotypes in mouse models of Parkinson's disease. EBioMedicine 22, 225–241.
Nakazawa, M., Matsuno, K., Mita, S., 1998. Activation of sigma1 receptor subtype leads to neuroprotection in the rat primary neuronal cultures. Neurochem. Int. 32 (4), 337–343.
Olanow, C.W., Tatton, W.G., 1999. Etiology and pathogenesis of Parkinson's disease. Annu. Rev. Neurosci. 22, 123–144.
Ordureau, A., Paulo, J.A., Zhang, W., Ahfeldt, T., Zhang, J., Cohn, E.F., Hou, Z., Heo, J.M., Rubin, L.L., Sidhu, S.S., Gygi, S.P., Harper, J.W., 2018. Dynamics of PARKIN-dependent mitochondrial ubiquitylation in induced neurons and model systems revealed by digital snapshot proteomics. Mol. Cell 70 (2), 211–227.
Ortiz, O., Delgado-García, J.M., Espadas, I., Bahí, A., Trullas, R., Dreyer, J.L., Gruart, A., Moratalla, R., 2010. Associative learning and CA3-CA1 synaptic plasticity are impaired in D1R null, Drd1a-/- mice and in hippocampal siRNA silenced Drd1a mice. J. Neurosci. 30 (37), 12288–12300.
Pan, R., Rong, Z., She, Y., Cao, Y., Chang, L.W., Lee, W.H., 2012. Sodium pyruvate reduces hypoxic-ischemic injury to neonatal rat brain. Pediatr. Res. 72 (5), 479–489.
Paxinos, G., Franklin, K., 2001. The Mouse Brain in Stereotaxic Coordinates. Academic, San Diego.
Pickrell, A.M., Youle, R.J., 2015. The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. Neuron 85 (2), 257–273.
Pizzo, P., Zanovello, P., Bronte, V., Di Virgilio, F., 1991. Extracellular ATP causes lysis of mouse thymocytes and activates a plasma membrane ion channel. Biochem. J. 274 (Pt 1), 139–144.
Ramos-Ibeas, P., Barandalla, M., Colleoni, S., Lazzari, G., 2017. Pyruvate antioxidant roles in human fibroblasts and embryonic stem cells. Mol. Cell. Biochem. 429 (1–2), 137–150.
Regitz, V., Azumi, T., Stephan, H., Naujocks, S., Schaper, W., 1981. Biochemical mechanism of infarct size reduction by pyruvate. Cardiovasc. Res. 15 (11), 652–658.
Reisch, A.S., Elpeleg, O., 2007. Biochemical assays for mitochondrial activity: assays of TCA cycle enzymes and. PDHc Methods Cell Biol 80, 199–222.
Robb-Gaspers, L.D., Burnett, P., Rutter, G.A., Denton, R.M., Rizzuto, R., Thomas, A.P., 1998. Integrating cytosolic calcium signals into mitochondrial metabolic responses. EMBO J. 17 (17), 4987–5000.
Ruscher, K., Shamloo, M., Rickhag, M., Ladunga, I., Soriano, L., Gisselsson, L., Toresson, H., Ruslim-Litrus, L., Oksenberg, D., Urfer, R., Johansson, B.B., Nikolich, K., Wieloch, T., 2011. The sigma-1 receptor enhances brain plasticity and functional recovery after experimental stroke. Brain 134 (Pt 3), 732–746.
Sakul, A., Cumaoğlu, A., Aydin, E., Ari, N., Dilsiz, N., Karasu, C., 2013. Age- and diabetes-induced regulation of oxidative protein modification in rat brain and peripheral tissues: consequences of treatment with antioxidant pyridoindole. Exp. Gerontol. 48, 476–484.
Satpute, R., Lomash, V., Kaushal, M., Bhattacharya, R., 2013. Neuroprotective effects of α-ketoglutarate and ethyl pyruvate against motor dysfunction and oxidative changes caused by repeated 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine exposure in mice. Hum. Exp. Toxicol. 32 (7), 747–758.
Savitt, J.M., Dawson, V.L., Dawson, T.M., 2006. Diagnosis and treatment of Parkinson disease: molecules to medicine. J. Clin. Investig. 116 (7), 1744–1754.
Schapira, A.H., 1993. Mitochondrial complex I deficiency in Parkinson's disease. Adv. Neurol. 60, 288–291.
Shamir, R., Klein, C., Amar, D., Vollstedt, E.J., Bonin, M., Usenovic, M., Wong, Y.C., Maver, A., Poths, S., Safer, H., Corvol, J.C., Lesage, S., Lavi, O., Deuschl, G., Kuhlenbaeumer, G., Pawlack, H., Ulitsky, I., Kasten, M., Riess, O., Brice, A., Peterlin, B., Krainc, D., 2017. Analysis of blood-based gene expression in idiopathic Parkinson disease. Neurology 89 (16), 1676–1683.
Shen, H., Hu, X., Liu, C., Wang, S., Zhang, W., Gao, H., Stetler, R.A., Gao, Y., Chen, J., 2010. Ethyl pyruvate protects against hypoxic-ischemic brain injury via anti-cell death and anti-inflammatory mechanisms. Neurobiol. Dis. 37 (3), 711–722.
Shin, J.H., Lee, H.K., Lee, H.B., Jin, Y., Lee, J.K., 2014. Ethyl pyruvate inhibits HMGB1 phosphorylation and secretion in activated microglia and in the postischemic brain. Neurosci. Lett 558, 159–163.
Short, K.R., Bigelow, M.L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S., Nair, K.S., 2005. Decline in skeletal muscle mitochondrial function with aging in humans. Proc. Natl. Acad. Sci. U.S.A. 102 (15), 5618–5623.
Shults, C.W., Haas, R.H., Passov, D., Beal Coenzyme, M.F., 1997. Q10 levels correlate with the activities of complexes I and II/III in mitochondria from parkinsonian and nonparkinsonian subjects. Ann. Neurol. 42 (2), 261–264.
Stanisavljević, S., Jevtić, B., Djedović, N., Miljković, D., 2015. Short term exposure to ethyl pyruvate has long term anti-inflammatory effects on microglial cells. Biomed. Pharmacother. 72, 11–16.
Stocchi, F., Antonini, A., Barone, P., Tinazzi, M., Zappia, M., Onofrj, M., Ruggieri, S., Morgante, L., Bonuccelli, U., Lopiano, L., Pramstaller, P., Albanese, A., Attar, M., Posocco, V., Colombo, D., Abbruzzese, G., 2013. Early DEtection of wEaring off in Parkinson disease: the DEEP study. Park. Relat. Disord. 20 (2), 204–211.
Tagashira, H., Zhang, C., Lu, Y.M., Hasegawa, H., Kanai, H., Han, F., Fukunaga, K., 2013. Stimulation of σ1-receptor restores abnormal mitochondrial Ca^{2+} mobilization and ATP production following cardiac hypertrophy. Biochim. Biophys. Acta 1830 (4), 3082–3094.
Tagashira, H., Shinoda, Y., Shioda, N., Fukunaga, K., 2014. Methyl pyruvate rescues mitochondrial damage caused by SIGMAR1 mutation related to amyotrophic lateral sclerosis. Biochim. Biophys. Acta 1840 (12), 3320–3334.
Tanaka, M., Nishigaki, Y., Fuku, N., Ibi, T., Sahashi, K., Koga, Y., 2007. Therapeutic potential of pyruvate therapy for mitochondrial diseases. Mitochondrion 7, 399–401.
Ulloa, L., Ochani, M., Yang, H., Tanovic, M., Halperin, D., Yang, R., Czura, C.J., Fink, M.P., Tracey, K.J., 2002. Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. Proc. Natl. Acad. Sci. U.S.A. 99 (19), 12351–12356.
Vander Heiden, M.G., Cantley, L.C., Thompson, C.B., 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324 (5930), 1029–1033.
Vonkorff, R.W., 1964. Pyruvate-C14, purity and stability. Anal. Biochem. 8, 171–178.
Webb, B.A., Forouhar, F., Szu, F.E., Seetharaman, J., Tong, L., Barber, D.L., 2015. Structures of human phosphofructokinase-1 and atomic basis of cancer-associated mutations. Nature 523 (7558), 111–114.
Woo, Y.J., Taylor, M.D., Cohen, J.E., Jayasankar, V., Bish, L.T., Burdick, J., Pirolli, T.J., Berry, M.F., Hsu, V., Grand, T., 2004. Ethyl pyruvate preserves cardiac function and attenuates oxidative injury after prolonged myocardial ischemia. J. Thorac. Cardiovasc. Surg. 127 (5), 1262–1269.
Yabuki, Y., Fukunaga, K., 2013. Oral administration of glutathione improves memory deficits following transient brain ischemia by reducing brain oxidative stress. Neuroscience 250, 394–407.
Yabuki, Y., Ohizumi, Y., Yokosuka, A., Mimaki, Y., Fukunaga, K., 2014. Nobiletin treatment improves motor and cognitive deficits seen in MPTP-induced Parkinson model mice. Neuroscience 259, 126–141.
Yabuki, Y., Shinoda, Y., Izumi, H., Ikuno, T., Shioda, N., Fukunaga, K., 2015. Dehydroepiandrosterone administration improves memory deficits following transient brain ischemia through sigma-1 receptor stimulation. Brain Res. 1622, 102–113.
Zanovello, P., Bronte, V., Rosato, A., Pizzo, P., Di Virgilio, F., 1990. Responses of mouse

lymphocytes to extracellular ATP. II. Extracellular ATP causes cell type-dependent lysis and DNA fragmentation. J. Immunol. 145 (5), 1545–1550.
Zarrindast, M.R., Ardjmand, A., Ahmadi, S., Rezayof, A., 2012. Activation of dopamine D1 receptors in the medial septum improves scopolamine-induced amnesia in the dorsal hippocampus. Behav. Brain Res. 229 (1), 68–73.
Zeng, J., Liu, J., Yang, G.Y., Kelly, M.J., James L., T.L., 2007. Litt Exogenous ethyl pyruvate versus pyruvate during metabolic recovery after oxidative stress in neonatal rat cerebrocortical slices. Anesthesiology 107 (4), 630–640.
Zhong, Z., Zeng, T., Xie, K., Zhang, C., Chen, J., Bi, Y., Zhao, X., 2013. Elevation of 4-hydroxynonenal and malondialdehyde modified protein levels in cerebral cortex with cognitive dysfunction in rats exposed to 1-bromopropane. Toxicology 306, 16–23.