ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 20 (2005) 701 – 708

Promethazine protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity

Carine Cleren,*[,1] Anatoly A. Starkov,[1] Noel Y. Calingasan, Beverly J. Lorenzo, Junya Chen, and M. Flint Beal

Department of Neurology and Neuroscience, Weill Medical College of Cornell University, 525 East 68th Street, Room A-501, New York, NY 10021, USA

Received 28 February 2005; revised 26 April 2005; accepted 2 May 2005
Available online 26 August 2005

Promethazine (PMZ) is an FDA-approved antihistaminergic drug that was identified as a potentially neuroprotective compound in the NINDS screening program. PMZ accumulates in brain mitochondria in vivo and inhibits Ca^{2+}-induced mitochondrial permeability transition pore (PTP) in rat liver mitochondria in vitro. We hypothesized that PMZ may have a protective effect in a mitochondrial toxin model of Parkinson's disease (PD). Mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) sustained a significant loss of dopaminergic neurons within the SNpc that was strongly attenuated by PMZ treatment. However, neither striatal MPP^+ concentrations nor MPTP-induced inhibition of mitochondrial complex I were affected by PMZ treatment. In isolated mouse brain mitochondria, PMZ partially prevented and reversed MPP^+-induced depolarization of membrane potential and inhibited the Ca^{2+}-induced PTP in brain mitochondria. The sum of data indicates that PMZ is a strong neuroprotective agent capable of protecting dopaminergic neurons against MPTP toxicity in vivo.

Keywords: Promethazine; MPTP; MPP^+; Parkinson; Neuroprotection; Mitochondria; Permeability transition pore; Membrane potential; Complex I; Mice

Introduction

Parkinson's disease (PD) is a progressive disorder leading to degeneration of a large number of dopaminergic neurons in the substantia nigra pars compacta (SNpc), which results in depletion of dopamine (DA) in the striatum (Hornykiewicz, 1966). The causes of PD remain unidentified but substantial evidence suggests an etiology involving both environmental and genetic factors (Moore et al., 2004; Greenamyre and Hastings, 2004). Administration of the mitochondrial complex I inhibitor MPTP, in primates and rodents, reproduces the characteristic degeneration of nigrostriatal dopaminergic neurons with a depletion of striatal dopamine levels (Heikkila et al., 1984). Both mitochondrial dysfunction and oxidative stress appear to play a role in the pathogenesis of Parkinson's disease (Beal, 2003; Blum et al., 2001).

PMZ is a clinically approved drug that could readily be moved to late-stage preclinical trials and then into clinical trials. It was identified as a neuroprotective compound as part of the NINDS program to screen 1040 FDA-approved compounds in a variety of high-throughput assays (Stavrovskaya et al., 2004). Promethazine protected primary neuronal cultures subjected to oxygen/glucose deprivation and reduced infarct size and neurological impairments in mice subjected to middle cerebral artery occlusion/reperfusion (Stavrovskaya et al., 2004). In experiments with isolated rat liver mitochondria, PMZ delayed the Ca^{2+}-induced mitochondrial permeability transition pore (PTP) without impairing mitochondrial physiology (Stavrovskaya et al., 2004). In brain cells, in vivo, PMZ has been shown to accumulate into mitochondria and vesicles (Muller, 1996). We examine the neuroprotective effects of PMZ treatment in the MPTP model of Parkinson's disease. We found a strong neuroprotective effect and in vitro experiments led us to hypothesize that PMZ may be neuroprotective due to its stabilizing effects on mitochondria.

Material and methods

Reagents

All reagents were purchased from Sigma (St. Louis, USA). Promethazine and MPTP were dissolved in phosphate-buffered saline (PBS).

Animals and procedures

The experiments were carried out on mice, in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All

* Corresponding author. Fax: +1 212 746 8276.
E-mail address: cac2005@med.cornell.edu (C. Cleren).
[1] Authors contributed equally to this study.
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2005.05.022

procedures were approved by the local Animal Care and Use Committee. Male Swiss Webster mice (12 weeks old, 4 per cage) were maintained in a temperature/humidity-controlled environment under a 12-h light/dark cycle with free access to food and water.

MPTP treatments

Protocol 1

Experiments involving measurements of neurodegeneration (TH, Nissl, DA) required sacrificing mice 7 days after treatment in order to observe the total cell death and to avoid later compensatory effects. A preliminary dose effect showed that an injection of a 5-mg/kg/dose of PMZ, twice a day, was insufficient to protect against MPTP-induced decrease in DA levels, while the 10-, 20-, and 40-mg/kg/dose were protective (Fig. 1A). Consequently, mice (9–12 per group) were injected with the lowest dose of PMZ that showed protection (10 mg/kg ip) or with PBS (10 ml/kg ip) 1 h before and 12 h after the first MPTP injection. Mice were treated with MPTP (10 mg/kg ip q 2 h × 4 doses) according to a standard dosage protocol (Schmidt and Ferger, 2001) or with PBS (10 ml/kg). Mice were sacrificed 7 days later by cervical dislocation.

Protocol 2

In order to determine if PMZ disturbed MPTP metabolism, we examined the time course of changes in MPP^+ striatal concentrations following PMZ treatment. We used the highest concentration of PMZ (40 mg/kg ip) that protected against MPTP-induced DA depletion in our preliminary experiment. Mice (9–12 per group) were injected with PMZ (40 mg/kg ip) 1 h before a

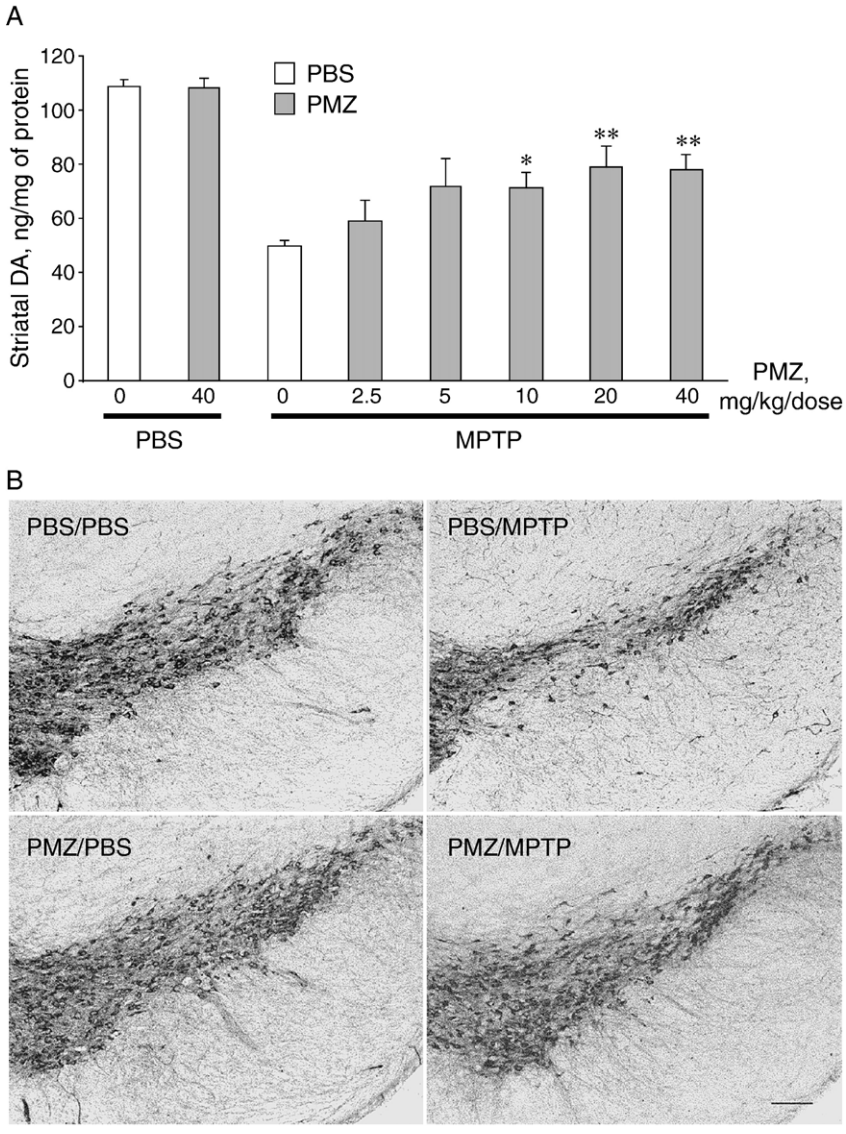

Fig. 1. Effects of PMZ treatment on striatal DA and nigral TH levels induced by MPTP treatment. (A) Injection of a 5 mg/kg/dose of PMZ, twice a day, was insufficient to protect against MPTP-induced decrease in DA levels, while the 10, 20, and 40 mg/kg/dose were protective. (B) TH immunoreactivity in the substantia nigra pars compacta of control or MPTP-lesioned mice treated with either PBS or PMZ. MPTP treatment (10 mg/kg/dose) produced a significant loss of TH-immunoreactive neurons in the substantia nigra pars compacta, while PMZ significantly mitigated the MPTP-induced loss of TH-positive neurons. Scale bar, 100 μm.