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Research article

MPTP-induced parkinsonism in mice alters striatal and nigral xCT expression but is unaffected by the genetic loss of xCT

Eduard Bentea[a,1], Michelle D. Sconce[b,1], Madeline J. Churchill[b,1], Joeri Van Liefferinge[d], Hideyo Sato[e], Charles K. Meshul[b,c], Ann Massie[a,*]

[a] Department of Pharmaceutical Biotechnology and Molecular Biology, Center for Neurosciences, Vrije Universiteit Brussel, Brussels, Belgium
[b] Research Services, Neurocytology Laboratory, Veterans Affairs Medical Center, Portland, OR, USA
[c] Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR, USA
[d] Department of Pharmaceutical Chemistry and Drug Analysis, Center for Neurosciences, Vrije Universiteit Brussel, Brussels, Belgium
[e] Laboratory of Biochemistry and Molecular Biology, Department of Medical Technology, Faculty of Medicine, Niigata University, Niigata, Japan

HIGHLIGHTS

- MPTP treatment increases levels of xCT in the striatum.
- MPTP treatment decreases levels of xCT in the substantia nigra.
- Mice lacking xCT are equally sensitive to MPTP-induced neurodegeneration as WT mice.

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ABSTRACT

Nigral cell loss in Parkinson's disease (PD) is associated with disturbed glutathione (GSH) and glutamate levels, leading to oxidative stress and excitotoxicity, respectively. System xc- is a plasma membrane antiporter that couples cystine import (amino acid that can be further used for the synthesis of GSH) with glutamate export to the extracellular environment, and can thus affect both oxidative stress and glutamate excitotoxicity. In the current study, we evaluated the involvement of system xc- in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. Our results indicate that the expression of xCT (the specific subunit of system xc-) undergoes region-specific changes in MPTP-treated mice, with increased expression in the striatum, and decreased expression in the substantia nigra. Furthermore, mice lacking xCT were equally sensitive to the neurotoxic effects of MPTP compared to wild-type (WT) mice, as they demonstrate similar decreases in striatal dopamine content, striatal tyrosine hydroxylase (TH) expression, nigral TH immunopositive neurons and forelimb grip strength, five weeks after commencing MPTP treatment. Altogether, our data indicate that progressive lesioning with MPTP induces striatal and nigral dysregulation of system xc-. However, loss of system xc- does not affect MPTP-induced nigral dopaminergic neurodegeneration and motor impairment in mice.

1. Introduction

System xc- is a plasma membrane antiporter, exchanging cystine for glutamate in a 1:1 ratio [14]. Structurally, system xc- is composed of two chains linked by a disulfide bridge, xCT and 4F2. The light-chain xCT is the specific subunit of system xc- and mediates the transport function, while the heavy chain 4F2 is shared by other amino acid transporters and acts as a chaperone in guiding xCT to the plasma membrane [5]. Expression of xCT is enriched in, but not restricted to, glial cells in the brain [4,13,21,26]. Due to the concentration gradient established in vivo, system xc- mediates cystine import into the cell, and glutamate export to the extracellular environment [5]. Imported cystine is reduced to cysteine, which contributes to glutathione (GSH) synthesis, as well as in the maintenance of the extracellular cysteine/cystine redox couple after being shuttled via system alanine-serine-cysteine (ASC) or system L [14]. In the brain, system xc- has been indicated as a major source of extracellular glutamate in various regions, including

* Corresponding author at: Vrije Universiteit Brussel, Center for Neurosciences, Department of Pharmaceutical Biotechnology and Molecular Biology, Laarbeeklaan 103, Brussels, Belgium. Tel.: +32 2477 45 02.
E-mail address: Ann.Massie@vub.ac.be (A. Massie).
[1] These authors contributed equally to the work.

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hippocampus [6] and striatum [16]. Glutamate released by system xc- acts on extrasynaptic glutamate receptors, such as ionotropic and metabotropic glutamate receptors [3].

While the exact function of system xc- in vivo remains poorly defined, its transport function intricately links the maintenance of intra- or extracellular redox balance with modulation of extracellular glutamate levels. As both oxidative stress and glutamate excitotoxicity are thought to be involved in the progressive loss of dopamine (DA)-ergic neurons in Parkinson's disease (PD) [7], a potential link between system xc- and DA-ergic neurodegeneration has been hypothesized. 6-Hydroxydopamine (6-OHDA) hemi-Parkinson rats demonstrate time-dependent changes in xCT expression, with increased expression in the ipsilateral striatum 3 weeks post lesion [17]. Furthermore, mice lacking xCT resist 6-OHDA-induced nigral cell loss after an acute unilateral striatal lesion, indicating a toxic contribution of overactive system xc- [16]. Still, studies evaluating system xc- in other PD models, with different mechanisms of toxicity, are currently lacking.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a mitochondrial toxin that induces parkinsonism when administered to humans, primates, and mice [8]. Due to the translation across species of MPTP-associated neurotoxicity and the development of parkinsonian symptoms, chronic systemic MPTP administration is believed to be one of the closest representations of PD in terms of pathology and behavioral outcome. Recently, a progressive MPTP administration regimen in mice was proposed as an alternative protocol leading to stepwise decline in the nigrostriatal DA-ergic system that correlates with gradual behavioral loss-of-function [10]. In this study we investigated the involvement of system xc- in a PD mouse model induced by chronic and progressive administration of MPTP [10].

2. Methods

2.1. Mice

This study was performed on adult (10–14 weeks old) male xCT homozygous knock-out (−/−) mice and wild-type (+/+) littermates. The mice used in this study are high-generation descendants of the strain described previously (C57BL/6 background) [24], and were bred in the animal facilities of the Veterans Affairs Medical Center/Portland, OR. Mice were housed under standardized conditions, on a 12 h light/12 h dark cycle, with food and water available ad libitum. All procedures were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996, and were approved by the Portland VA Medical Center Institutional Animal Care and Use Committee.

2.2. Genotyping

Mice were genotyped by PCR amplification of tail DNA using REDExtract-N-Amp Tissue PCR Kit (Sigma–Aldrich, St. Louis, MO, USA), and the following primers: 5′-GATGCCCTTCAGCTCGATGCGGTTCACCAG-3′ (GFPR3); 5′-CAGAGCAGCCCTAAGGCACTTTCC-3′ [mxCT5′flankF6]; 5′-CCGATGACGCTGCCGATGATGATGG-3′ [mxCT(Dr4)R8]. Genotypes were confirmed post-mortem by xCT Western blotting in midbrain and striatal homogenates.

2.3. MPTP administration protocol

Mice were administered chronic intraperitoneal (i.p.) MPTP in a progressive dosing regimen as described previously [10] with brief modifications. Each MPTP group was injected 5 days a week, during 4 weeks, with a weekly increasing daily dose of MPTP (as the base: 8 mg/kg during the first week, 16 mg/kg during the second week, 24 mg/kg during the third week, and 32 mg/kg during the fourth week; Sigma–Aldrich). Vehicle groups received saline (0.1 ml/0.1 kg). From the 13 xCT−/− mice and 16 xCT+/+ littermates subjected to chronic i.p. MPTP injections, 8 xCT−/− mice (61.53%) and 11 xCT+/+ mice (68.75%) survived. One week following the last MPTP injection (5 weeks after commencing MPTP treatment), mice were subjected to a grip strength test and euthanized. Half of the animals in each group were used for nigral tyrosine hydroxylase (TH) immunohistochemistry, while the other half were used for striatal DA measurement and striatal and nigral immunoblotting for TH and xCT.

2.4. Grip strength test

Mice were tested for grip strength using the Grip Strength Meter (Columbus Instruments, Columbus, Ohio), according to a recent study where this test was used to measure motor deficits following subchronic MPTP administration [2]. Using the Mesh Pull Bar attachment, mice were handled by the tail so that only the forepaws could touch the apparatus. When the forepaws were clearly latched to the Mesh Pull Bar mice were slowly and steadily pulled away from the apparatus, parallel to the counter top until they released their forepaws from the Mesh Pull Bar. Each animal's grip strength was an average of 5 grip strength tests and animals were given a 5 min rest in between each trial. The protocol was repeated additionally, as explained above, for all of the paws latching on the Mesh Pull Bar together. Grip strength was measured in Newtons (N).

2.5. TH immunohistochemistry and cell counting of the substantia nigra (SN) pars compacta

Mice were euthanized with 1% ketamine/0.1% mg xylazine (2.0 ml/0.1 kg, i.p.) followed by transcardial perfusion with 2.5% glutaraldehyde/0.5% paraformaldehyde/0.1% picric acid. The caudal half of the brain was placed into 2% paraformaldehyde/0.1 M phosphate buffer saline for 5 days at 4 °C. Brains were sliced through the entire SN [−2.70 to −3.88 mm relative to bregma] [19] at 40 microns using a vibratome (Leica Microsystems, Buffalo Grove, Il). Every 3rd slice was collected through the rostral/caudal extent of the SN, for a total of 6 slices per animal. Slices were immuno-labeled using the Pelco BioWave® Pro (Ted Pella Inc., Redding CA) as described previously [10], using primary antibody for TH (1:250 dilution, mouse monoclonal, Immunostar, Hudson, WI) and secondary biotinylated goat anti-mouse antibody (1:400 dilution, Jackson ImmunoResearch, West Grove, PA). Mounted slices were counterstained with Cresyl Violet (CV) (0.2% in H_2O) and imaged at 5× using a Zeiss Axioplan (Carl Zeiss, Germany) and a Microbrightfield (MBF) camera and software setup (MBF Bioscience, Williston, VT). TH+ cells were counted using ImagePro Software (Media Cybernetics, Inc., Rockville, MD). Cell numbers for each side were added together and the total number of TH+ cells were added together to get a total sum of cells through the six slices.

2.6. Neurochemical analysis of total DA and DOPAC content in the striatum

Mice were euthanized by cervical dislocation. Striatal and nigral tissue were dissected out, snap-frozen, and stored at −80 °C. Next, one striatum per mouse was weighed and homogenized in 400 μl antioxidant solution (0.05 M HCl, 0.5% $Na_2S_2O_5$, and 0.05% Na_2EDTA) containing 10 ng/100 μl 3,4-dihydroxybenzylamine as internal standard. The homogenate was centrifuged for 20 min at 10,000 × *g* at 4 °C, and the supernatants were immediately diluted 1:5 in 0.5 M acetic acid. Twenty μl of the resulting sample dilution were analyzed for DA and DOPAC content on a narrow-bore (C18