Chemico-Biological Interactions 271 (2017) 1–8

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Copper sulfate pretreatment prevents mitochondrial electron transport chain damage and apoptosis against MPP^+-induced neurotoxicity

Moisés Rubio-Osornio [a], Marisol Orozco-Ibarra [b], Araceli Díaz-Ruiz [c], Eduardo Brambila [d], Marie-Catherine Boll [c], Antonio Monroy-Noyola [e], Jorge Guevara [f], Sergio Montes [c], Camilo Ríos [c, g, *]

[a] Laboratorio Experimental de Enfermedades Neurodegenerativas, Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez S.S.A., Mexico
[b] Departamento de Neurobiología Celular y Molecular, Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez S.S.A., Mexico
[c] Departamento de Neuroquímica, Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez S.S.A., Mexico
[d] Laboratorio de Investigaciones Químico-Clínicas, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Puebla, Mexico
[e] Laboratorio de Neuroprotección, Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Morelos, Mexico
[f] Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico
[g] Departamento de Sistemas Biológicos de la Universidad Autónoma Metropolitana, Unidad Xochimilco, Mexico

ARTICLE INFO

Article history:
Received 8 February 2017
Received in revised form
14 April 2017
Accepted 20 April 2017
Available online 22 April 2017

Keywords:
Parkinson's disease
MPP+
Copper sulfate
Cytochrome *c* oxidase
Cu/Zn-superoxide dismutase
Metallothionein

ABSTRACT

Intrastriatal injection of 1-methyl-4-phenylpyridinium (MPP^+) is considered a model to reproduce some biochemical alterations observed in Parkinson's disease (PD) patients. Among those alterations, inhibition of mitochondrial complex I activity, increased free radical production and reduced antioxidant responses have been reported. Copper (Cu) plays an important role in the metabolism and antioxidative responses through its participation as a cofactor in the cytochrome *c* oxidase enzyme (COX), Cu/Zn-superoxide dismutase (Cu/Zn-SOD), and metallothioneins. We tested the effect of copper sulfate ($CuSO_4$) pretreatment on the mitochondrial electron transport chain (METC) in the striatum after MPP^+ toxicity in rats. The results showed that the MPP^+ intrastriatal injection reduced mitochondrial complex I, II, IV and V activities; while 10 μmol of $CuSO_4$ pretreatment counteracted this damage. Activities of complexes I, II and IV, were coincident with ATP recovery. Moreover, Cu/Zn-SOD activity was reduced as a consequence of MPP^+ damage; however, copper pre-treatment kept the striatal Cu/Zn-SOD activity unchanged in MPP^+-damaged animals. We observed that MPP^+ also reduced the metallothionein (MT) content and that $CuSO_4$ pretreatment maintained baseline values. $CuSO_4$ pretreatment also reduced the striatal caspase-3 and caspase-9 activities that were increased three days after MPP^+-induced damage. The present study provided evidence that copper pretreatment reduced MPP^+-induced apoptotic damage, probably through direct action on copper-dependent proteins or indirectly on proteins in the apoptotic pathway.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder that is clinically characterized by resting tremor, rigidity, bradykinesia, and postural instability. Despite advances in understanding the neurochemical processes underlying behavioral alterations in PD, the cause that initiates neuronal death is still unknown. The main factors involved in neuronal death are mitochondrial dysfunction, free radicals' over-production, and diminished antioxidant response, among others [1]. PD patients exhibit decreased complex I activity (NADH ubiquinone oxidoreductase) in the mitochondrial electron transport chain (METC) [2]. Similarly, studies using substantia nigra pars compacta (SNpc) postmortem tissue from Parkinson's patients showed inhibition in METC complex I activity [3]. This alteration is considered an important factor in oxidative damage, leading to electron uncoupling in the METC and causing

* Corresponding author. Departamento de Neuroquímica, Instituto Nacional de Neurología y Neurocirugía, Ave. Insurgentes Sur No. 3877, C.P. 14269, D.F., Mexico.
E-mail address: crios@correo.xoc.uam.mx (C. Ríos).

http://dx.doi.org/10.1016/j.cbi.2017.04.016
0009-2797/

increased reactive oxygen species (ROS) formation such as superoxide radical ($^{\bullet}O_2^-$) and peroxynitrite ($ONOO^-$) [3]. Administration of 1-methyl-4-phenylpyridinium (MPP^+) produces an adequate model of PD in laboratory animals. The MPP^+ neurotoxic potential is based on its ability to inhibit complex I of the METC, and it is thought that this alteration results in the over-production of free radicals, oxidative stress, metal dyshomeostasis and neuronal death [4].

Copper is a trace metal that acts as a prosthetic group in several proteins involved in the metabolism and antioxidant responses, such as cytochrome *c* oxidase (COX), Cu/Zn-superoxide dismutase (Cu/Zn-SOD), and metallothioneins (MTs), among others [5]. Copper is imported by the plasma membrane transporters protein (Ctr1), and it rapidly binds to intracellular copper chaperone proteins or MTs. Copper homeostasis is tightly regulated, and the unbound copper concentration is extremely low [6]. Recently, our group found that $CuSO_4$ pretreatment increased ferroxidase activity and prevented MPP^+-induced lipid peroxidation [7].

The aim of the present study was to evaluate the $CuSO_4$ pretreatment effects on some copper-dependent proteins. We tested the hypothesis that the increase of copper in the brain might activate proteins that are copper-dependent, reducing metabolic and apoptotic damage induced by MPP^+ administration.

2. Methods

2.1. Animals and treatments

All the experimental procedures involving the use of animals were performed according to regulatory official guides regarding laboratory animal use and care (NOM-62-ZOO-2001). In this study, male Wistar rats (280–300 g) were housed in acrylic box cages and placed under constant conditions of temperature, humidity and light (12 h light/dark cycles) and were provided with a standard commercial rat chow diet and water ad libitum. The experimental groups were dosed sixteen h previous to MPP^+ damage, with a single intraperitoneal (i.p.) injection of 10 μmol/kg of $CuSO_4$ or 10 μmol/kg of Na_2SO_4 in the control group. $CuSO_4$ was administered as a pretreatment in order to prevent damage produced by MPP^+, as reported previously [7].

2.2. MPP^+ intra-striatal injection

Sixteen hours after $CuSO_4$ or sodium sulfate (Na_2SO_4) pretreatment, animals were infused in the right striatum with 10 μg of MPP^+ iodide (Sigma-RBI, St. Louis, MO, U.S.A.) dissolved in 8 μL sterile saline solution (s.s.) under ketamine/xylazine anesthesia (70/10 mg/kg). The stereotaxic coordinates were 0.5 mm anterior to the bregma, −3.0 mm lateral to the bregma and −4.5 mm ventral to the dura in the right striatum, according to the stereotaxic Paxinos and Watson atlas [8]. Control animals received 8 μL intrastriatal s.s. Animals from all groups were sacrificed by decapitation 6 h after MPP^+ or s.s. and were immediately processed. In order to observe changes in the activity of caspase 3 and 9, we sacrificed the animals by decapitation 72 h after of damage induced by MPP^+-injection.

2.3. Mitochondrial activity and isolation of mitochondria

Mitochondria were isolated as described by Mirandola et al. [9], with minor modifications. The striatum was dissected and extensively washed with ice-cold isolation buffer containing 225 mM mannitol, 75 mM sucrose, 1 mM EGTA, 0.05% bovine serum albumin and 10 mM HEPES pH 7.2. Then, the tissue was homogenized, and the homogenate was centrifuged for 5 min at 2000 × g; the resulting supernatant was centrifuged for 10 min at 12,000 × g. The pellet was re-suspended in 2 mL of isolation buffer and centrifuged for 10 min at 12,000 × g. The supernatant was discarded, and the final dark pellet was gently washed and re-suspended in isolation buffer devoid of EGTA at an approximate protein concentration of 3–4 mg/mL.

2.4. Mitochondrial complex I (NADH-ubiquinone oxidoreductase) activity

Mitochondrial complex I (NADH-ubiquinone oxidoreductase) activity was evaluated with a spectrophotometric assay previously described by Molina-Jijón et al., [10]. The assay was carried out at a final volume of 110 μL. Briefly, 30 μg of mitochondrial protein contained in 10 μL was added to a 90 μL of reaction medium containing 60 μM decylubiquinone, 0.1 μg/mL antimycin A and 1 mM KCN in standard reaction medium (125 mM KCl, 50 mM Tris-HCl and 3 mM Pi, pH 7.3). The reaction was initiated by adding 1 mM NADH (10 μL), and the NADH absorbance decrease was followed at 340 nm. The rotenone-insensitive activity was determined as the difference in activity in the absence and in the presence of rotenone (2 μM).

2.5. Mitochondrial complex II (succinate dehydrogenase) activity

Mitochondrial complex II (Succinate dehydrogenase) activity was evaluated with a spectrophotometric assay previously described by Mao et al. [11], with slight modifications. Isolated mitochondria were subjected to freeze-thaw cycles in liquid nitrogen to increase the exposure of the enzyme to substrates. The assay was carried out at a final volume of 120 μL. The reaction medium (90 μL) consisted of 20 mM succinate, 2 μg/mL of antimycin A, 2 μg/mL rotenone, 2 mM KCN and 50 μM 2,6-dichlorophenolindophenol (DCPIP) in 10 mM potassium phosphate, pH 7.4. Thirty micrograms (10 μL) of mitochondrial protein were added, and the reaction was started by addition of 50 μM decylubiquinone (20 μL). The reduction of DCPIP was monitored at 600 nm for 3 min. Calculations of activity were obtained using the extinction coefficient of 19.1 mM-1 cm-1 for DCPIP.

2.6. Mitochondrial complex IV cytochrome c oxidase (COX) activity

The activity of complex IV was evaluated with a spectrophotometric assay previously described by Spinazzi et al. [12], modified to be measured at a final volume of 100 μL. The reaction medium consisted in 90 μL of 60 μM reduced cytochrome *c* in potassium phosphate buffer 25 mM (pH 7.0). The reaction was started by adding 10 μL of sample containing 2.5 μg of mitochondrial protein, and the decrease in absorbance was monitored at 550 nm for 3 min. The specific activity of complex IV was estimated by subtracting the residual activity after inhibition with 300 μM KCN. Calculations were obtained using the extinction coefficient for reduced cytochrome *c* (18.5 mM-1 cm-1). Activity was expressed as nmol of cyt *c* min^{-1} mg of $protein^{-1}$.

2.7. Mitochondrial complex V (ATP synthase) activity

The rate of ATP synthesis was measured using an enzyme-linked assay following the reduction of $NADP^+$ at 340 nm [13]. Fifty μg of mitochondrial protein (15 μL) was mixed in a medium containing the respiratory substrate (5 mM glutamate/5 mM malate to feed complex I or 15 mM succinate to feed complex II), the enzyme-linked assay was 4 U/mL hexokinase, 2 U/mL glucose-6-phosphate dehydrogenase, 20 mM glucose and 1.4 mM $NADP^+$ in a standard medium with 10 mM $MgCl_2$ at a final volume of 270 μL. The reaction was started by adding 30 μL of 1 mM ADP. The specific