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Research report

Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures

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Abstract

Rotenone, a pesticide and complex I inhibitor, causes nigrostriatal degeneration similar to Parkinson disease pathology in a chronic, systemic, in vivo rodent model [M. Alam, W.J. Schmidt, Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats, Behav. Brain Res. 136 (2002) 317–324; R. Betarbet, T.B. Sherer, G. MacKenzie, M. Garcia-Osuna, A.V. Panov, J.T. Greenamyre, Chronic systemic pesticide exposure reproduces features of Parkinson's disease, Nat. Neurosci. 3 (2000) 1301–1306; S.M. Fleming, C. Zhu, P.O. Fernagut, A. Mehta, C.D. DiCarlo, R.L. Seaman, M.F. Chesselet, Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone, Exp. Neurol. 187 (2004) 418–429; T.B. Sherer, J.H. Kim, R. Betarbet, J.T. Greenamyre, Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation, Exp. Neurol. 179 (2003) 9–16.]. To better investigate the role of mitochondria and complex I inhibition in chronic, progressive neurodegenerative disease, we developed methods for long-term culture of rodent postnatal midbrain organotypic slices. Chronic complex I inhibition over weeks by low dose (10–50 nM) rotenone in this system lead to dose- and time-dependent destruction of substantia nigra pars compacta neuron processes, morphologic changes, some neuronal loss, and decreased tyrosine hydroxylase (TH) protein levels. Chronic complex I inhibition also caused oxidative damage to proteins, measured by protein carbonyl levels. This oxidative damage was blocked by the antioxidant α-tocopherol (vitamin E). At the same time, α-tocopherol also blocked rotenone-induced reductions in TH protein and TH immunohistochemical changes. Thus, oxidative damage is a primary mechanism of mitochondrial toxicity in intact dopaminergic neurons. The organotypic culture system allows close study of this and other interacting mechanisms over a prolonged time period in mature dopaminergic neurons with intact processes, surrounding glia, and synaptic connections.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Parkinson's

Keywords: Mitochondria; Complex I; Organotypic; α-Tocopherol; Parkinson disease; Antioxidant

1. Introduction

Parkinson disease (PD) is a slowly progressive neurodegenerative disorder marked by relatively selective loss of substantia nigra pars compacta (SNpc) dopaminergic neurons [26], along with the appearance of Lewy body intraneuronal inclusions and dystrophic neurites in SNpc and other affected areas [9,41]. Less than 10% of PD cases are clearly familial [13,18]. The cause of sporadic PD is unknown, but it likely involves a combination of genetic predisposition and long-term environmental exposures [22,58]. Epidemiological studies suggest that pesticide exposure can increase PD risk [21,42,59]. Many pesticides inhibit mitochondrial function. There is increasing evidence

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that mitochondrial dysfunction may be a key factor underlying specific neuronal loss in neurodegenerative disorders, particularly PD [5,22].

Investigation of mitochondrial dysfunction in PD gained momentum with the finding that a synthetic opiate contaminant, MPTP (*N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), induces acute, permanent parkinsonism via its active metabolite, MPP+ (1-methyl-4-phenyl-2,3,-dihydropyridinium), a mitochondrial complex I inhibitor [36,38]. The specific effect of MPTP on dopaminergic neurons, however, stems from its selective uptake into dopaminergic cells via the dopamine transporter [31]. In contrast, studies of idiopathic PD point to a systemic complex I impairment, with defects seen in platelets, muscle, and brain [10,24,37,39,45], implying that a systemic mitochondrial defect can lead to relatively selective neuronal damage. Indeed, chronic systemic exposure of rats to the pesticide rotenone, a classic mitochondrial complex I inhibitor, reproduces many features of PD, including levodopa-responsive motor deficits, nigrostriatal dopaminergic pathway degeneration, and intraneuronal inclusions [1,2,6,15,27,48]. This in vivo model therefore ties together systemic pesticide exposure, widespread complex I inhibition, and PD-like pathology.

Rotenone models are now being used to investigate the mechanisms whereby dopaminergic neurons are injured in this chronic, systemic process. Complex I inhibition has several potentially damaging consequences. One possible result of complex I inhibition is increased formation of reactive oxygen species (ROS), creating oxidative damage within the cell. Oxidative stress has been implicated in PD [63]. Increased oxidative damage to lipids [14,32], DNA [44,62], and proteins [16] has been observed in PD SNpc, along with decreased levels of reduced glutathione [49]. Oxidative damage, rather than a bioenergetic defect, is also seen in the in vivo rotenone model [4,47].

The in vivo rotenone model is relevant to human PD pathophysiology as it reproduces key features of PD in a mature, intact adult mammalian brain with all of its inherent connections and cell–cell interactions. On the other hand, it is labor-intensive, expensive, and variable [15,27,48,65]. Dissociated cell culture systems are more easily manipulated, but these systems employ isolated, often non-neuronal cell types to investigate changes over a limited (hours to few days) time frame. While some relevant studies of complex I inhibition in dissociated cell culture employ mature dopaminergic neurons [52], the majority use immature cells. Organotypic 'slice' culture models represent a useful intermediate tool for studying chronic, progressive cell damage [17]. Slice cultures are simplified and flexible compared to in vivo models, yet still make use of mature neurons, remain viable in cultures for weeks to months, and maintain substantial neuron–neuron and neuronal–glial interactions [30,43]. Here, we use an organotypic slice culture system to investigate how mitochondrial dysfunction can lead to SNpc cell damage in pathologic conditions such as PD. Rotenone was tested on intact postnatal neurons in this system for its chronic injury, rather than acute toxicity, effects. The system was then used to examine oxidative damage as a potential mechanism of chronic dopaminergic neuronal injury. Low concentrations of rotenone over weeks resulted in slow loss of dopaminergic cell processes, changes in cell morphology, and decreased tyrosine hydroxylase (TH) protein, along with increased oxidative damage to proteins. The antioxidant α-tocopherol protected slices from oxidative damage, while simultaneously preventing SNpc morphologic damage and TH protein loss. Thus, oxidative damage is an important mechanism underlying dopaminergic cell damage. Chronic organotypic slice cultures provide a useful model system for investigating mechanisms of neurodegenerative disease.

2. Materials and methods

2.1. Organotypic slice cultures

The protocol was modified from Stoppini et al. [57]. All animal use was in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*, and was approved by Emory University Institutional Animal Care and Use Committee. All care was taken to minimize pain or discomfort. Postnatal day 10 (P10) Lewis rat pups were fully anesthetized with isoflurane. Brains were rapidly removed and transferred to cold sterile chopping buffer (in mM, 110 sucrose, 60 NaCl, 3 KCl, 1.25 NaH_2PO_4, 28 $NaHCO_3$, 5 D-glucose, 0.5 $CaCl_2$, 7 $MgCl_2$, 0.6 ascorbate) in a sterile hood work area. Brains were rapidly blocked, glued to the slicer chuck (OTS-4000 tissue slicer, FHC Inc, Maine), and transferred to the slicer basin filled with cold, oxygenated chopping buffer. Sections were cut to 300 μm and transferred to a sterile petri dish of cold dissection medium [Gey's balanced salt solution (Sigma, Saint Louis, Missouri) with 0.5% glucose and 3 mM KCl]. Coronal sections at the level of the substantia nigra were chosen. Sections were inspected and cut into slices under a dissection microscope. During this procedure, the midbrain was isolated and then cut into two hemispheres, as detailed below. Slices therefore included the SNpc, surrounding cells, other midbrain nuclei, and local connections, not a full nigrostriatal pathway. After inspection, each slice was transferred onto a Millicell-CM membrane insert (Millipore, Billreka, Massachusetts) set in a 6-well plate on 1 ml of OPTI-MEM (GibcoBRL, Carlsbad, California)-based serum-containing medium. Plates were kept in a 37 °C tissue culture incubator. After 2–4 days, medium was changed to Neurobasal (GibcoBRL)-based serum-free medium with B-27 (GibcoBRL) and L-glutamine without antibiotics or antimitotics. Medium was then changed three times a week. Slices were grown for 10 days prior to drug treatments.