Available online at www.sciencedirect.com

SCIENCE DIRECT

Free Radical Biology & Medicine 39 (2005) 133 – 142

www.elsevier.com/locate/freeradbiomed

Original Contribution

Increased sensitivity of striatal dopamine release to H_2O_2 upon chronic rotenone treatment

Elisaveta Milusheva[a,b], Mária Baranyi[a], Ágnes Kittel[a], Beáta Sperlágh[a,*], E. Sylvester Vizi[a]

[a]*Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1083 Budapest, Szigony u. 43, Hungary*
[b]*Institute of Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, BL. 23, 1113 Sofia, Bulgaria*

Received 7 October 2004; revised 2 February 2005; accepted 28 February 2005
Available online 25 March 2005

Abstract

It is believed that both mitochondrial dysfunction and oxidative stress play important roles in the pathogenesis of Parkinson's disease (PD). We studied the effect of chronic systemic exposure to the mitochondrial inhibitor rotenone on the uptake, content, and release of striatal neurotransmitters upon neuronal activity and oxidative stress, the latter simulated by H_2O_2 perfusion. The dopamine content in the rat striatum is decreased simultaneously with the progressive loss of tyrosine hydroxylase (TH) immunoreactivity in response to chronic intravenous rotenone infusion. However, surviving dopaminergic neurons take up and release only a slightly lower amount of dopamine (DA) in response to electrical stimulation. Striatal dopaminergic neurons showed increased susceptibility to oxidative stress by H_2O_2, responding with enhanced release of DA and with formation of an unidentified metabolite, which is most likely the toxic dopamine quinone (DAQ). In contrast, the uptake of [^{3}H]choline and the electrically induced release of acetylcholine increased, in coincidence with a decline in its D_2 receptor-mediated dopaminergic control. Thus, oxidative stress-induced dysregulation of DA release/uptake based on a mitochondrial deficit might underlie the selective vulnerability of dopaminergic transmission in PD, causing a self-amplifying production of reactive oxygen species, and thereby contributing to the progressive degeneration of dopaminergic neurons.

Keywords: Oxidative stress; Parkinson's disease; Dopamine; Striatum; Release; H_2O_2; Acetylcholine

Introduction

Chronic neurodegenerative diseases, which are characterized by a progressive loss of distinct groups of neurons, have a common pathomechanism, since oxidative damage and dysregulation of transmitter release play a central, but not initiative, role in the development of the disease [1]. Oxidative damage is caused by the overproduction of highly reactive oxygen species, which compromise cellular function on multiple target sites [2]. Oxidative stress also contributes to the dysregulation of transmitter release by increasing the extracellular level of glutamate and monoamine transmitters, which by themselves act as triggers of deleterious cellular events leading to neurodegeneration. Whereas glutamate stimulates nitric oxide (NO) production and might initiate peroxynitrite production, monoamines provide an additional source of highly reactive free radicals during their breakdown by monoamine oxidase (MAO) or autooxidation [3], whereby they could reinforce the harmful effect of oxidative stress. In our previous study we showed that H_2O_2 elicits concentration-dependent [^{3}H]noradrenaline release from hippocampal slices, and that this effect is greatly exacerbated if mitochondrial inhibitors such as

Abbreviations: ACh, acetylcholine; [^{3}H]ACh, [^{3}H]acetylcholine; ANOVA, one-way analysis of variance; DA, dopamine; DAQ, dopamine quinone; [^{3}H]DA, [^{3}H]dopamine; DMSO, dimethyl sulfoxide; DOPAC, 3,4-dihydroxyphenylacetic acid; EFS, electrical field stimulation; HVA, homovanillic acid; HPLC, high performance liquid chromatography; L-DOPA, L-(3,4-dihydroxyphenyl)alanine; 6-OHDA, 6-hydroxydopamine; MAO, monoamine oxidase; NA, noradrenaline; PD, Parkinson's disease; PB, phosphate buffer; PEG, polyethylene glycol; PCA, perchloric acid; ROS, reactive oxygen species; TH, tyrosine hydroxylase.

* Corresponding author. Fax: +36 1 210 9423.
E-mail address: sperlagh@koki.hu (B. Sperlágh).

0891-5849/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2005.02.034

rotenone or oligomycin are applied at the same time [4]. In line with this observation, dopamine-mediated neurotoxicity in striatal neurons is markedly enhanced if it is combined with minimal mitochondrial inhibition [5].

The major feature of PD is a relatively selective degeneration of the nigrostriatal dopaminergic pathway, leading to progressive motor dysfunction. According to the current hypothesis, it is a multifactorial disease, and both genetic and environmental factors play a role in its aetiology. Among environmental factors, the crucial importance of exposure to pesticides, such as the selective mitochondrial complex I inhibitor rotenone, is emerging. The occurrence of PD in the rural population involved in gardening and agriculture is higher [6,7]. As well, PD patients show a systemic deficit in mitochondrial complex I function [8], which is not restricted to brain dopaminergic neurons but is also manifested in other cells, such as platelets and muscle cells. However, the mechanism whereby systemic complex I dysfunction leads to selective dopaminergic neurotoxicity is unknown.

Recently, Betarbet and co-workers introduced a new model of PD based on subchronic, continuous infusion of rotenone. It reproduced the following features of PD in rats: (1) systemic complex I deficit, (2) motor and behavioural symptoms, (3) progressive degeneration of SN dopaminergic neurones, and (4) formation of ubiquitin and α-synuclein-positive cytoplasmic inclusions in neurons [9]. Therefore, the chronic rotenone-induced Parkinson model may represent an animal model, which more adequately mimics the pathogenesis and progress of the disease than previously used models [10]. Nevertheless, it is not yet determined how the neurochemical parameters of striatal dopaminergic and nondopaminergic transmission are altered in the rotenone-induced Parkinson model. It was reported that acute administration of rotenone increases DA turnover [11], whereas subchronic intermittent treatment (by ip injection) only moderately lowers DA content in the striatum [12,13]. However, these treatments might not reflect the changes with continuous exposure to a moderate dose of rotenone, which probably more adequately mirrors the environmental toxicity. Moreover, it is not known how the presynaptic function of dopaminergic neurons, i.e., the release of DA, is changed, and how vulnerable dopaminergic neurons are to oxidative stress in terms of their ability to release. This latter aspect seems to have particular interest because H_2O_2 releases a higher amount of DA from striatum than noradrenaline (NA) from the cortex [14]. Furthermore, DA is more harmful than other physiologically relevant monoamines [3,15], because its metabolism by MAO produces more H_2O_2 and could also give rise to the formation of toxic quinones and semiquinones [16].

Therefore, this study was designed to explore how striatal neurotransmission is altered in response to conventional neuronal activity and oxidative stress in the rotenone model of PD.

Materials and methods

Implantation of osmotic pumps and animal care

All animal experiments were performed in accordance with the *National Institute of Health Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of the Hungarian Academy of Sciences.

We used male Sprague-Dawley rats (280–320 g). Alzet osmotic minipumps (Alzet Corporation, Palo Alto, CA) were filled with rotenone dissolved in equal volumes of dimethyl sulfoxide (DMSO) and polyethylene glycol (PEG-300) or with the solvent alone. Pumps were kept in sterile 0.9% saline at 37°C overnight before the operation. Ketamine (75 mg/kg), rompum (10 mg/kg), and xylazine (10 mg/kg) were injected intramuscularly as anesthetics. Pumps were implanted under the skin on the back of each animal and were attached to the right jugular vein by a catheter. Control rats received DMSO:PEG (1:1) only. The treated rats received 2–3 mg of rotenone/kg per day (calculation based on weight at the time of surgery). Following surgery, rats were monitored for behavior, weight, and overall health. When rotenone-treated rats showed weight loss, their diet was supplemented with oral administration of Nutrical (Evsco Pharmaceuticals, Buena, NJ). Subcutaneous lactated Ringer's solution (Rindex 5; Reanal, Hungary) injection was given when rats showed signs of dehydration.

Immunohistochemistry

Control, sham-operated, and rotenone-treated animals were decapitated after a short ether stress and the brain was quickly removed. The upper part of the striatum with adjacent cortex was immersed in 4% paraformaldehyde, 0.5% glutaraldehyde, and 15% saturated picric acid in 0.1 M phosphate buffer (PB), pH 7.4, for 6 h. The fixative was changed every half hour. The tissue block was rinsed and washed in 0.1 M PB overnight at 4°C. Forty-micrometer sections were cut by a vibratome in the same buffer. Sections were immersed in 0.05 % Triton X-100 in 0.1 M PB for 1 h at room temperature and, after several washing steps in PB, were blocked for endogenous peroxidase activity with 3 % H_2O_2/PB for 15 min. This was followed by incubation in blocking buffer (5% normal horse serum in PB) with gentle agitation at room temperature for 1 h. Floating sections were then incubated in 1:1000 dilution of the TH antibody (Sigma) overnight at 4°C. One set of sections was incubated in the absence of the antibody as a control. After removal of the solution of TH antibody and several washing steps, sections were incubated at room temperature for 1 h with biotinylated anti-mouse IgG (Vector Laboratories) at a 1:200 dilution. ABC reaction and detection of antigen–antibody complexes were performed according to the Vector Laboratories (Burlingame,