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SCIENCE DIRECT

Molecular Brain Research 134 (2005) 170–179

MOLECULAR BRAIN RESEARCH

www.elsevier.com/locate/molbrainres

Research report

Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism

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Accepted 27 August 2004
Available online 25 September 2004

Abstract

Idiopathic Parkinson's disease (PD) affects 2% of adults over 50 years of age. PD patients demonstrate a progressive loss of dopamine neurons in the substantia nigra pars compacta (SNpc). One model that recapitulates the pathology of PD is the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Here we show that exposure to an enriched environment (EE) (a combination of exercise, social interactions and learning) or exercise alone during adulthood, totally protects against MPTP-induced Parkinsonism. Furthermore, changes in mRNA expression would suggest that increases in glia-derived neurotrophic factors, coupled with a decrease of dopamine-related transporters (e.g. dopamine transporter, DAT; vesicular monoamine transporter, VMAT2), contribute to the observed neuroprotection of dopamine neurons in the nigrostriatal system following MPTP exposure. This non-pharmacological approach presents significant implications for the prevention and/or treatment of PD.

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

Keywords: MPTP; Exercise; GDNF; Neurotrophic factors; Mouse

1. Introduction

Epidemiological studies have suggested that exposure to environmental toxins provides an increased risk to a number of neurological disorders [62,72]. Perhaps the most studied of these is PD, which affects 2% of adults over 50 years of age and is characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). Current treatments for PD are numerous, the most widely used being DA-replacement therapy. Amelioration of PD symptoms through this method is transient and for this reason, several non-pharmacological methods have been developed in an attempt to permanently reverse the symptoms of SNpc cell loss including transplantation of DA cells [25] and destruction of cerebral motor pathway nuclei [58]. None of the current therapies are aimed at preventing the disorder and some may exacerbate the condition [25]. Another problem in treating PD is that a majority of the SNpc neurons are lost at the onset of visible symptoms [6]. However, since PD generally presents in the 6th decade of life, identification of a mechanism or therapy that slows or ameliorates the cell loss for several years could, due to actuarial realities, eliminate symptomatic PD in many individuals.

One of the best models for generating experimental PD is the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes a specific loss of SNpc neurons identical to that seen in PD [42]. Exposure to MPTP has similar effects in many vertebrate species, including man and mouse. MPTP is metabolized by glial MAO-B to MPP^+. MPP^+ is transported into neurons through the dopamine transporter (DAT) where it interferes with Complex I respiration [44,55]. Once in cells, MPP^+ is sequestered into vesicles by the vesicular monoamine transporter (VMAT2), which may provide some cellular protection against this

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doi:10.1016/j.molbrainres.2004.08.008

toxin [28,73]. Thus, the relative expression of DAT and VMAT2 within a neuron may determine its potential for survival from an exogenous toxin [50].

In addition to the genetic control of MPTP-induced SNpc neuron loss [38], several studies have shown that environmental factors can alter the prevalence and outcome of neurodegenerative disease [75]. Some of these can be experimentally recapitulated through exposure to a modified or "enriched" environment (EE) that incorporates social interactions, learning and exercise [43,45].

The complexity of an animal's environment has been shown to affect brain structure and function. EE exposure leads to increases in neuron size, dendrite length, synaptic density and spine number [3,11,14,17,21,22,35,36,80]. It has also been demonstrated that animals raised in an EE have increased neurotrophin levels [40,53,59,66].

In this study, we examine the role of environment in modulating SNpc cell loss following administration of MPTP. We show that introducing animals to an enriched environment as adults totally protects against MPTP-induced Parkinsonism. Within the EE, exercise appears to be the critical component. The likely mechanism for this neuroprotection is a significant increase in specific growth factors coupled with a down-regulation of dopamine-related transporters in the nigrostriatal system.

2. Materials and methods

2.1. Animal procedures

All mice used in this study were C57Bl/6J (Jackson Laboratories, Bar Harbor, ME). Animals were maintained in a temperature-controlled environment with free access to food and water and kept on a 12-h light/dark cycle; lights on at 7.00 am. All animal procedures were in compliance with St. Jude Children's Hospital Institutional guidelines and were approved by the SJCRH Institutional Animal Care and Use Committee.

2.2. Environment preconditioning

The animals were preconditioned in environments termed either (1) "Enriched" (14 mice/cage) (2) "Exercise" (4 mice/cage) (3) and standard (4–6 mice/cage). The "Enriched Environment" cages (1×1 m) consisted of two running wheels, nesting material and a system of interchangeable tunnels re-arranged on a weekly basis [43]. The "Exercise cages" consisted of a standard mouse colony cage containing a running wheel. Running wheels in the "enriched" and "exercise" cages" were wire meshed with a diameter of 12.7 cm. These wheels were freely available, but the individual animals activity levels were not monitored. In a separate experiment, the daily activity of individual female C57Bl/6J mice was measured using exercise wheels from Lafayette Instruments (Lafayette, IN, Model 80820).

For the experimental animals, female mice were born and raised in "standard" cages. At 2–3 months, these mice were removed from their home cage and placed into either an EE or exercise cage. The reason we used only female mice is that we wanted to eliminate any bias arising from the use of a single litter. Thus, the production of mixed litter cages was only possible by using female mice since it is well known that they exhibit decreased stress and aggressive behavior in a mixed population [60]. In a previous study, we showed that there was no difference in MPTP-sensitivity in male and female C57Bl/6J mice [38].

2.3. MPTP treatment

All animals injected with MPTP were between 5 and 7 months. Animals received MPTP (4×20 mg/kg/2-h intervals) (Research Biochemical International, Natick, MA) in a 5-ml/kg volume or vehicle (sterile saline) alone. For the time course studies, animals were injected s.c., while animals in the EE experiments were injected i.p. Seven days (a time corresponding to maximal SNpc cell loss [41]) and 14 days after MPTP administration animals were anesthetized with tribromoethanol (250 mg/kg (i.p.)) and transcardially perfused with 0.1 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Brains were removed, post-fixed overnight, processed and sectioned at 5 μm and cut throughout the SNpc and mounted onto polyionic microscope slides (Superfrost-plus, Fisher). In addition, a number of animals were anesthetized, decapitated and the SN and striatum (STR) dissected out and rapidly froze on dry ice and stored at −80 °C until RNA processing.

2.4. Immunohistochemistry

Standard immunohistochemical techniques were used to identify cells positive for tyrosine hydroxylase (TH) in the SNpc. Paraffin sections were stained with a polyclonal antibody specific for TH (1:250; Pel Freez, Rogers, AR). Immunopositive cells were subsequently visualized using a peroxidase-anti-peroxidase system (Vector Laboratories, CA) and DAB (KPL, Gaithersburg, MD). Slides were then counterstained with the Nissl stain Neutral Red, dehydrated through a graded series of alcohol, mounted in Permount and coverslipped.

2.5. Cell quantitation

The major neuron-type in the SNpc is dopaminergic and can be visualized by immunolabeling with tyrosine hydroxylase (Fig. 2A). In order to make sure that we counted all neurons in the SNpc and not just those exhibiting the phenotypic marker (TH), we counterstained every immunostained section with the Nissl marker Neutral Red. All cells in the SNpc having a the appearance of dopaminergic neurons (TH+Nissl) were counted as previously described using both stereological and 2D-measurement techniques [12,38]. As