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BRAIN RESEARCH

Research Report

VTA neurons show a potentially protective transcriptional response to MPTP

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ABSTRACT

Parkinson's disease and its characteristic symptoms are thought to arise from the progressive degeneration of specific midbrain dopamine (DA) neurons. In humans, DA neurons of the substantia nigra (SN) and their projections to the striatum show selective vulnerability, while neighboring DA neurons of the ventral tegmental area (VTA) are relatively spared from degeneration. This pattern of cell loss is mimicked in humans, primates, and certain rodents by the neurotoxin MPTP. In this study, we aimed to test the hypothesis that there are factors in the VTA that are potentially neuroprotective against MPTP and that these factors change over time. We have found a dynamic transcriptional response within the cells of the VTA to sustained exposure to a low dose of MPTP. Specifically, the VTA has increased expression of 148 genes as an early response to MPTP and 113 genes as a late response to MPTP toxicity. This response encompasses many areas of cellular function, including protein regulation (*Phf6*) and ion/metal regulation (*PANK2* and *Car4*). Notably, these responses were largely absent from the cells of the SN. Our data show a clear dynamic response in maintaining the homeostasis and viability of the neurons in the VTA that is lacking in the SN after neurotoxin challenge.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease affecting more than 2% of the population over the age of 65 (Dauer and Przedborski, 2003). A progressive loss of specific midbrain dopamine (DA) neurons is thought to give rise to the characteristic symptomology of the disease. In humans, DA neurons of the substantia nigra (SN) are significantly affected, whereas DA neurons of the neighboring ventral tegmental area (VTA) are relatively spared from disease pathology. This selective pattern of cell loss is mimicked in humans, non-human primates, and certain rodents by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Elsworth et al., 1987; Hirsch et al., 1988; Jacobowitz et al., 1984; Schneider et al., 1987), raising the interesting hypothesis that there are factors intrinsic to the VTA that are neuroprotective against

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Abbreviations: LCM, Laser capture microdissection; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SN, substantia nigra; VTA, ventral tegmental area

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MPTP toxicity. Our lab has developed a transgenic mouse that expresses GFP driven by the human tyrosine-hydroxylase promoter (hTH-GFP) (Kessler et al., 2003), which specifically labels DA neurons of the ventral midbrain allowing for easy visualization of these structures, and uniquely positions us to test this hypothesis.

Using the hTH-GFP mouse and the neurotoxin MPTP, we have begun to analyze the molecular changes in these different DA cell populations in response to PD pathology and PD related injury. MPTP has been shown to cross the blood brain barrier where it is converted by glia to the active metabolite MPP+, which then can be taken up by the DA transporter (DAT) into DA neurons (Gainetdinov et al., 1997; Glover et al., 1986; Kopin, 1988). Once internalized, MPP+ inhibits complex I of mitochondria, severely inhibiting ATP production, triggering deleterious downstream effects(Ramsay et al., 1987). In addition, MPP+ toxicity causes the release of reactive oxygen species. Combined, the effects of MPTP lead to eventual cell death. Interestingly, the toxic effects of MPTP are mainly limited to the DA neurons of SN, sparing glial cells of the SN, and more importantly, DA neurons of the neighboring VTA (Schneider et al., 1987). Traditional dosing paradigms of MPTP cause acute cell loss in the DA neurons of the SN (Bezard et al., 1997). However, in order to model the progressive nature of SN cell death in PD, we have used a low dose of MPTP over time, resulting in a model in which to study molecular events linked to PD associated neurodegeneration.

Large scale oligonucleotide arrays, such as those used in this study, offer the opportunity to study transcriptional changes to the entire genome. This allows us the possibility to explore cellular adaptation to injury and cell death. In this study, we utilized laser capture microdissection (LCM) to isolate DA neurons from the VTA and SN of our progressive MPTP mouse model of PD followed by microarray analysis to study gene expression changes in these regions during MPTP toxicity.

2. Results

2.1. *Progressive DA neuron loss model*

Current MPTP mouse models of Parkinson's disease (PD) use acute doses of MPTP in the range of 80 mg/kg to induce DA cell death in the substantia nigra (SN) (Scheller et al., 2008; Zhang et al., 2009). However, this model results in a rapid loss of DA neurons in the SN. In an effort to recapitulate progressive neurodegeneration in the SN, in this study, a low dose of MPTP was used in a chronic fashion as first described by Bezard et al. (1997). Transgenic mice expressing hTH-GFP were given MPTP (4 mg/kg/day described in methods) for 2 or 10 days. Closely following pathological conditions, animals treated with MPTP showed little to no progression of DA neuron loss in the VTA. After 2 days of MPTP treatment, mice lost 9.8±2.7% of DA neurons in the VTA, and after 10 days of MPTP treatment, mice lost 9.5±2.7% of VTA neurons (Fig. 1). After 2 days of MPTP treatment, mice lost 27.0±3.9% of DA neurons in the SN, and after 10 days of MPTP treatment, mice lost 71.9±3.6% of SN neurons (Fig. 1). These time points were used to signify early and late stages of PD related neurodegeneration.

2.2. *Genomic-scale gene expression*

Utilizing microarray analysis to study PD is not a novel approach, as evidenced by the data derived from experiments done in untreated and acute MPTP treated animals (Greene et al., 2005; Miller et al., 2004). However, the relatively protected VTA has been largely viewed only in comparison to the SN, not as a responsive region that might be neuroprotective. The microarray data presented here aims to shed light on potentially neuroprotective genes in the VTA while addressing the progressive nature of DA neuron loss in the midbrain. In this study, we look at the gene expression of the VTA in response to low-dose chronic MPTP treatment. It is expected that genes in the VTA may show increased or decreased expression following toxin treatment. In this study, we have focused on our hypothesis that the VTA may up-regulate protective factors as a way to protect itself from the toxic insult of MPTP, and as such have concentrated on genes that are specifically up-regulated within the VTA in response to MPTP. Interestingly we have found a set of genes that are up-regulated within the VTA which does not show a similar expression pattern within the SN. These genes likely orchestrate the protective response of DA neurons in the VTA to MPTP.

There are several approaches to the analysis of microarray data. Due to the variability of inclusionary criteria in determining differentially expressed genes, we employed a fairly common and straight forward method. In this study, we selected for genes that have shown a minimum 2.0 fold change with a p-value of less than 0.05. Using Student's T-test and following the above mentioned criteria, we identified genes that were up-regulated in the VTA in response to MPTP. Following the generation of these gene lists, we used the Gene Ontology project's categories of molecular function and biological process to group our genes of interest.

2.2.1. *Differential gene expression in the VTA and SN*

Studies of gene expression in the VTA have mostly relied on a comparison to the SN. These studies have shown that neurons of the VTA and SN are similar in terms of overall gene expression (Chung et al., 2005; Greene et al., 2005). Despite their general transcriptional resemblance, however, some differences in specific genes have been repeatedly observed. Our microarray analysis has corroborated a number of these genes. Specifically, BDNF, Calbindin, neurotrophin 3, and α 1B adrenergic receptor show greater expression in the VTA whereas, Aldh1a7, GIRK2, IGF1, mGluR1, and parvalbumin all show greater expression in the SN (Table 1) (Alfahel-Kakunda and Silverman, 1997; Andersen et al., 1994; Garcia-Segura et al., 1991; Liang et al., 1996; Massi et al., 2000; McCaffery and Drager, 1994; Schein et al., 1998; Seroogy et al., 1994; Shigemoto et al., 1992). RT-PCR analysis confirmed previous reports (Greene, 2006) of greater expression of calbindin in the VTA (Liang et al., 1996) and greater expression of GIRK2 in the SN (Schein et al., 1998) (Fig. S1), confirming the positive acquisition of stated regions by LCM.

Previous studies have debated the role of dopamine transporter (DAT) in the differential cell death seen between the SN and VTA (Gonzalez-Hernandez et al., 2004; Hung et al., 1995). Of notable interest in our study, DAT expression