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Research Report

MPTP induces intranuclear rodlet formation in midbrain dopaminergic neurons

Wiplove Lamba [a], Wendy Prichett [b], David Munoz [c], David S. Park [a,b], John M. Woulfe [a,b,d,*]

[a] The University of Ottawa, Ottawa, Ontario, Canada
[b] The Ottawa Health Research Institute, Ottawa, Ontario, Canada
[c] St. Michael's Hospital, Toronto, Ontario, Canada
[d] The Ottawa Hospital, Ottawa, Ontario, Canada

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Abstract

Neuronal intranuclear rodlets (INRs; rodlets of Roncoroni) have been known to neuroanatomists since the turn of the century. However, the functional and/or pathological significance of these structures has remained enigmatic. We recently demonstrated that these structures are immunoreactive for class III β tubulin and for glucocorticoid receptor. Moreover, they are markedly reduced in the temporal cortex of patients with Alzheimer's disease relative to age-matched controls and those with dementia with Lewy bodies, thereby implicating these structures in neurodegenerative disease pathogenesis. The present report represents an experimental pilot study to investigate the possible involvement of INRs in Parkinson's disease (PD). Specifically, we demonstrate significantly increased INRs in dopaminergic neurons in the substantia nigra pars compacta and ventral tegmental area in mice treated with the selective catecholaminergic neurotoxin MPTP, relative to saline-treated controls. We have hypothesized that INRs represent an intranuclear sequestrum of monomeric β-tubulin and that their alteration in neurodegeneration may reflect disrupted or abnormal microtubule dynamics. We propose that the increased formation of INRs is related to the demonstrated ability of MPTP to cause microtubule disruption. Because tubulin has also been implicated in the pathogenesis of human PD, it is possible that the results of this study will have important implications for this most common neurodegenerative movement disorder.

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

Keywords: Intranuclear inclusion; Intranuclear rodlet; MPTP; Parkinson's disease; Class III beta tubulin

1. Introduction

The formation of proteinaceous intraneuronal inclusions is an emerging theme in the pathogenesis of neurodegenerative disorders [26]. These inclusion bodies consist, at least in part, of aggregated pathogenic protein. In several neurodegenerative disorders, most notably, but not exclusively, the polyglutamine repeat disorders, the inclusions are localized to the neuronal nucleus. There are several lines of evidence that these neuronal intranuclear inclusion bodies (NII's) are not pathogenic in and of themselves but, instead, may subserve a protective role by sequestering mutant oligomeric or protofibrillar protein species [2,11,22].

Abbreviations: AD, Alzheimer's disease; β3-tubulin, class III beta tubulin; DAPI, 4′-6-Diamidino-2-phenylindole; GR, glucocorticoid receptor; INR, intranuclear rodlet; MPP+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NII, neuronal intranuclear inclusion; PD, Parkinson's disease; SN, substantia nigra; TH, tyrosine hydroxylase

* Corresponding author. Department of Pathology, The Ottawa Hospital, Civic Campus, 1053 Carling Ave., Ottawa, Ontario, Canada K1Y 4E9. Fax: +1 613 761 4846.
E-mail address: jwoulfe@ottawahospital.on.ca (J.M. Woulfe).

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Intranuclear inclusion bodies are also present in normal brain where their status is uncertain [13,20]. We recently described the existence of rod-shaped neuronal intranuclear inclusions (INRs) in the non-diseased human brain [33]. These inclusion bodies are equivalent to the intranuclear rodlets (rodlets of Roncoroni) described by the classical neuroanatomists as early as 1894 [5,19]. They are intensely immunoreactive for the neuron-specific cytoskeletal protein β3-tubulin as well as for the α-isoform of the human glucocorticoid receptor (GR) [33,34], thereby providing modern tools for identifying them at the light microscopic level. Ultrastructurally, INRs consist of parallel arrays of 7 nM longitudinal filaments [29,34]. We have demonstrated that INRs are widespread in the human brain wherein they display a stereotyped, heterogeneous topographical pattern of distribution. INRs are particularly prevalent in pigmented neurons of the substantia nigra. The functional and/or pathological significance of these structures is unknown. We have hypothesized that they represent a non-pathological counterpart to the NIIs found in neurodegenerative disease. In this context, they may reflect a dynamic intranuclear sequestration of β-tubulin in neurons of the normal human brain.

We are interested in investigating the functional and/or pathological significance of these structures. INRs are markedly reduced in the temporal cortex of patients with Alzheimer's disease (AD) relative to age-matched controls and those with dementia with Lewy bodies, indicating their involvement in the pathogenesis of this neurodegenerative disease [34]. In light of their possible involvement in neurodegenerative disease as well as their prominent localization in the substantia nigra (SN) [34,35], the constituent dopaminergic neurons of which undergo selective degeneration in Parkinson's disease (PD), we were interested in investigating whether changes in INRs might be related to the pathogenesis of PD. Because human PD is associated with massive neuronal loss in the SN, confounding interpretation of changes in INR density, we chose to examine these changes in the MPTP mouse model. Since the discovery that MPTP and its toxic metabolite MPP^+ induce selective loss of nigral dopamine neurons and produce parkinsonism in man [18], MPTP has been used as a model substance in studies on PD. In the present study, we employ immunofluorescence double-labeling for the simultaneous localization tyrosine hydroxylase (TH) immunoreactive dopamine neurons and INRs, as a tool to examine whether MPTP causes changes in the density of nigral INRs.

2. Results

Microscopic inspection revealed abundant TH-positive neurons in the A8, A9, and A10 dopaminergic cell groups as described previously. Although fewer TH-positive neurons were counted in MPTP-treated animals than controls (1531 ± 160 vs. 1838 ± 175), neither this difference (P = 0.21) nor the difference between A9 and A10 (P = 0.17) or the interaction of the factors (P = 0.12) was significant in two-way ANOVA analysis. Analyzed separately, fewer TH-positive neurons were counted in the A9 cell group of MPTP-treated animals than controls (1169 ± 251 vs. 1866 ± 340, respectively, P = 0.004). In contrast, there was no significant difference in total TH-positive neuronal numbers in the A10 cell group among MPTP-treated and control mice (1894 ± 914 vs. 1780 ± 384, respectively, P = 0.85). TH-positive neurons in MPTP-treated animals displayed no significant morphological abnormalities.

In both control and MPTP-treated animals, a proportion of TH-positive neurons in both the A9 and A10 dopaminergic cell groups contained intensely GR- and TH-immunoreactive intranuclear structures (Fig. 1). Double immunostaining for GR and β3-tubulin confirmed that all INRs were double-immunostained for these two antigens in both control and MPTP-treated animals (Fig. 2). As described in the human substantia nigra, these were predominantly rod-shaped and were frequently identified in apposition to the nucleolus. Dot-like, sheet-like, and crystalloid morphologies were also seen.

The frequency of GR-immunoreactive INRs in TH-positive neurons in the A9 and A10 dopaminergic cell groups is compared among MPTP-treated and saline-treated mice in Fig. 2. There was substantial inter-individual variation, both within control and treated groups in the proportion of INR-bearing dopaminergic neurons (INR/TH). Two-way ANOVA analysis revealed a significant

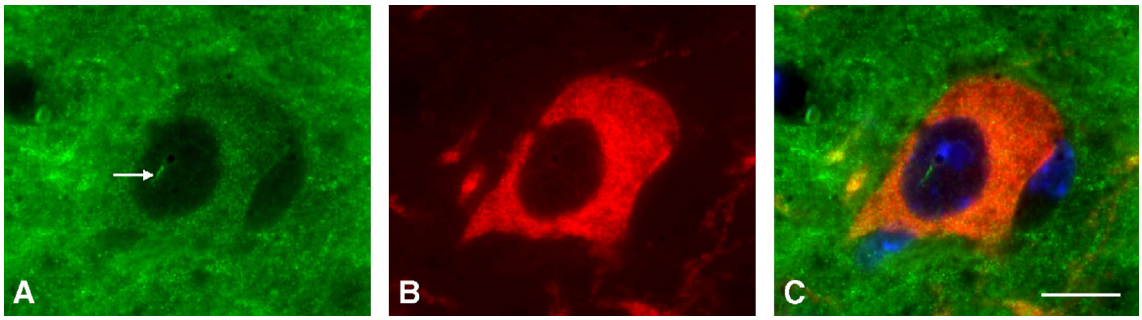

Fig. 1. TH-immunopositive neuron (A; red) in the A9 dopaminergic cell group of an MPTP-treated mouse contains a rod-shaped β3-tubulin-immunoreactive intranuclear rodlet (arrow in panel B; green). Merged image with DAPI counterstain in panel C. Scale bar = 10 μm.