

Neuroprotective effect of vasoactive intestinal peptide (VIP) in a mouse model of Parkinson's disease by blocking microglial activation¹

MARIO DELGADO^{*,#,†,2} AND DOINA GANEA^{*}

^{*}Department of Biological Sciences, Rutgers University, Newark, New Jersey, USA;

[#]Departamento Biología Celular, Facultad de Biología, Universidad Complutense, Madrid 28040, Spain; and [†]Instituto Parasitología Biomedicina "Lopez Neyra", CISC, Granada 18001, Spain

SPECIFIC AIMS

Parkinson's disease (PD) is a common neurodegenerative disorder with no effective treatment, characterized by a massive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). In addition to other specific mechanisms, activated microglia cells, through the action of their derived cytotoxic factors, actively participate in the pathogenesis of PD. The present study investigates the potential neuroprotective effect in a murine model of PD of the vasoactive intestinal peptide (VIP), a neuropeptide with a potent anti-inflammatory effect, which has been found to protect from other inflammatory disorders. The involvement of activated microglia and their derived cytotoxic products in such effect has been also investigated.

PRINCIPAL FINDINGS

1. VIP protects from MPP⁺-induced dopaminergic neurodegeneration and microglia activation in vitro

To elucidate PD pathogenic factors, and thus to develop therapeutic strategies, a murine model was used. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) reproduces most of the clinical, biochemical, and neuropathological hallmarks of PD, including dramatic neurodegeneration of the nigrostriatal dopaminergic pathway. We first investigated the in vitro effect of VIP on MPP⁺-induced neurodegeneration in ventral midbrain cultures. Treatment of mesencephalic cultures with MPP⁺ results in a dramatic decrease in the number of dopaminergic TH⁺ (tyrosine hydroxylase) neurons and in [³H]dopamine uptake. The MPP⁺-induced dopaminergic cell loss was paralleled by an increase in the number of activated microglia and in TNF- α production. VIP prevented, in a dose-dependent manner, MPP⁺-induced TH⁺ neurodegeneration and microglia activation (not shown).

2. Neuroprotective effect of VIP on MPTP-induced nigrostriatal dopaminergic neuronal death in vivo

We next investigated the effect of VIP in the MPTP murine model of PD, where we determined dopaminergic neuronal cell loss in SNpc by counting TH⁺ neurons, 1 wk after administration of MPTP (Fig. 1A). The density of dopamine transporter (DAT, [³H]-mazindol) binding sites was used as an anatomical marker of nigrostriatal innervation (Fig. 1B). Levels of TH protein and catechols (dopamine and DOPAC) in striatum were determined as biochemical markers of dopaminergic nigrostriatal function (Fig. 1C–E). The content of striatal nitrotyrosine was evaluated as a measure for the nitric oxide (NO)-related oxidative damage of the MPTP neurotoxic process (Fig. 1F). MPTP administration results in a dramatic decrease in the number of SNpc dopaminergic neurons, a loss of dopaminergic terminals in the striatum (Fig. 1A, B), a decrease in striatal TH protein levels (Fig. 1C), a depletion in dopamine and its major CNS metabolite DOPAC (Fig. 1D, E), and an increase in the levels of striatal nitrotyrosine (a fingerprint of NO-derived modification of protein and one of the main markers of oxidative damage mediated by MPTP) (Fig. 1F). Stereotaxic administration of VIP into the left SN of MPTP-treated mice significantly prevented, in a dose-dependent manner, the ipsilateral SNpc dopaminergic neuronal cell death, the loss of striatal dopaminergic fibers, subsequent depletion of TH, dopamine and DOPAC, and the increase in nitrotyrosine levels (Fig. 1). The neuroprotective effect of VIP in the contralateral nigrostriatal system was much lower (not shown). Systemic administration of VIP (i.p. injection) was much less effective. Doses 15-fold higher were necessary for a significant effect, which was 50% less efficient than cerebral VIP administration (not shown).

¹ To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.02-0799fje>; to cite this article, use *FASEB J.* (March 5, 2003) 10.1096/fj.02-0799fje

² Correspondence: Department Cell Biology, School of Biology, Complutense University, Madrid 28040, Spain. E-mail: mariodm@bio.ucm.es

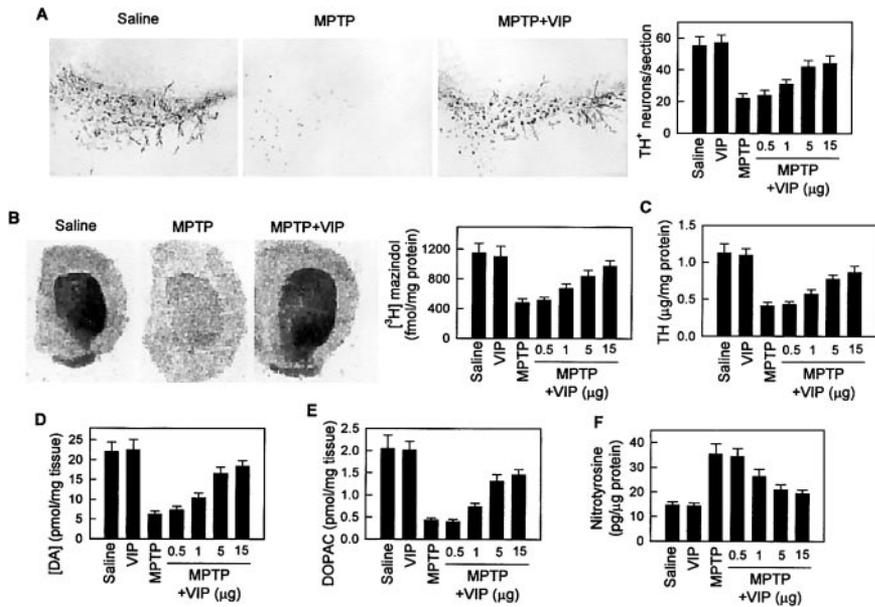


Figure 1. Neuroprotective effect of VIP on MPTP-induced nigrostriatal dopaminergic neuronal death in vivo.

3. VIP prevents MPTP-induced microglia activation in vivo

In addition to the dramatic loss of dopaminergic neurons, PD is characterized by microglial activation in SNpc and striatum, and several studies have associated cytotoxic factors produced by activated microglia with the ongoing dopaminergic neurodegeneration. Since

VIP inhibition of microglia activation under inflammatory conditions has been described, VIP-mediated microglia deactivation emerges as a possible mechanism mediating the protective effect of VIP on MPTP-induced neurodegeneration. As shown in **Fig. 2A**, MPTP administration results in a dramatic increase in the number of activated microglia in both striatum and SNpc, as indicated by high expression of Mac-1, a

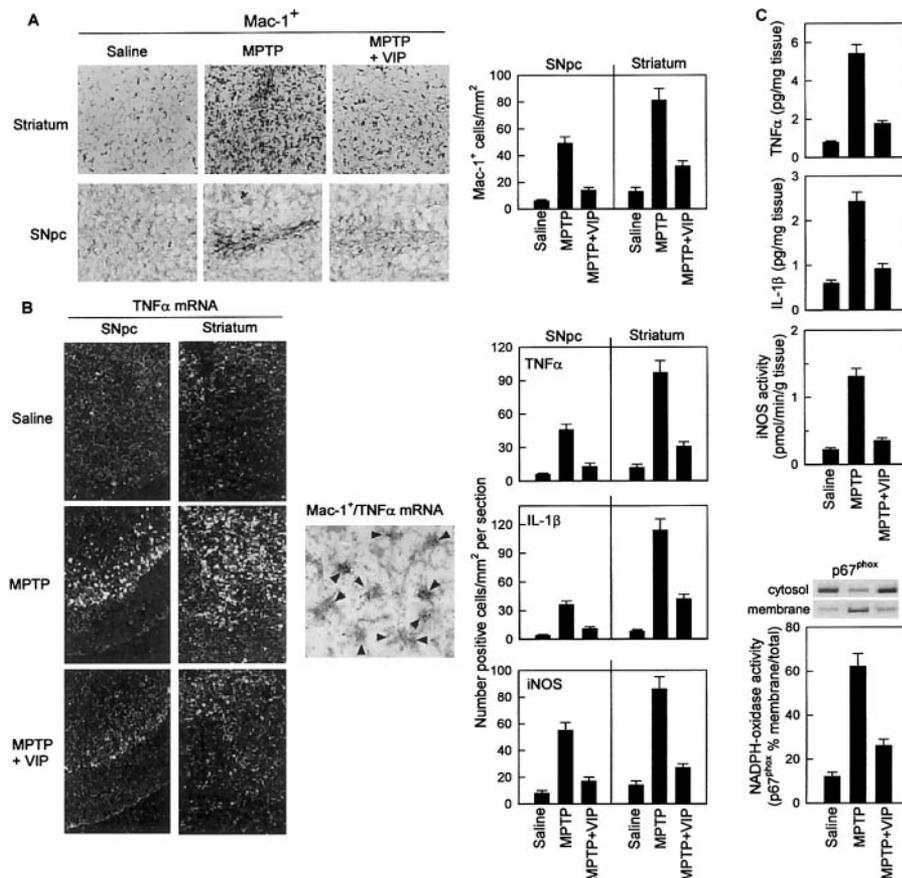


Figure 2. VIP prevents MPTP-induced microglia activation in vivo.

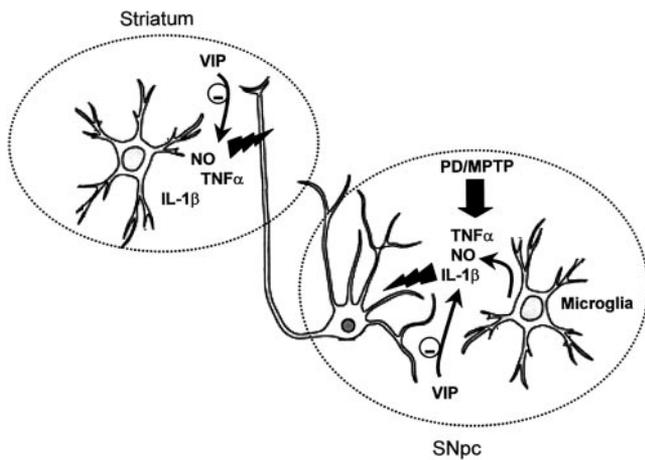


Figure 3. VIP prevents nigrostriatal dopaminergic neurodegeneration in a mouse model of Parkinson's disease by inhibiting microglia activation.

specific marker for activated microglia, and the change in morphology (large cell body with poorly ramified short and thick processes) compared with saline-injected mice, which showed minimal microglial activation (low Mac-1 expression and small microglial cell body with thin and ramified processes). MPTP-induced microglial activation is paralleled by increases in TNF- α , IL-1 β , and inducible NO synthase (iNOS) mRNA expression in striatum and SNpc (Fig. 2B), which correlates with an augmentation in TNF- α and IL-1 β protein levels and in iNOS activity in the ventral midbrain (Fig. 2C). Double in situ hybridization and immunocytochemical examination showed that the majority of cells expressing these inflammatory mediators are microglia (Fig. 2B). MPTP increased NADPH-oxidase activation, evidenced by translocation of the p67^{phox} subunit from the cytosol to the plasma membrane (Fig. 2C). iNOS and NADPH-oxidase are two prominent enzymes of activated microglia producing NO and reactive oxygen species (ROS), which together with TNF- α and IL-1 β are well-known microglial-derived noxious mediators in neurodegeneration. VIP administration to MPTP-treated mice abolished all these events. VIP significantly reduced MPTP-induced

microglia activation, expression of the neurotoxic factors TNF- α and IL-1 β , and enzymatic activity of iNOS and NADPH-oxidase (Fig. 2).

The involvement of astrocytes in the mediation of VIP, although minimal, should not be discounted, because VIP slightly reduced the MPTP-induced astrogliosis (not shown). Although it is unlikely that the reduction of MPTP-induced microglia activation by VIP is secondary to the attenuation of neuronal loss but rather the reverse, a direct action of VIP on neurons cannot be ruled out.

CONCLUSIONS AND SIGNIFICANCE

We propose that similar to after endotoxin-induced injury and brain trauma, in the MPTP murine model of PD and possibly PD itself, VIP, probably through the inhibition of proinflammatory noxious mediators (i.e., NO, ROS, TNF- α , and IL-1 β) produced by activated resident microglia, prevents the loss of dopaminergic cells and nerve fibers in the nigrostriatal pathway. Our study invites important future directions, including the possible therapeutic role of VIP in brain disorders such as multiple sclerosis, Alzheimer's disease, and AIDS dementia, where inflammatory responses play a major role. Since an inflammatory response is involved in PD, antioxidants or other newly developed, nonsteroidal anti-inflammatory drugs, such as iNOS inhibitors, cyclooxygenase inhibitors, or minocycline have been proposed for treatment in PD. However, although several drugs offered neuroprotection in animal models, there has been little or no success in the clinical treatment of PD. This may indicate that the animal models do not reflect the events in PD or that neuronal cell death involves a cascade of events that cannot be prevented by a single neuroprotective drug. Thus, consideration should be given to multidrug therapy, similar to the approach taken in AIDS and cancer therapy. It is also possible that agents such as VIP that affect a large spectrum of inflammatory mediators might be at an advantage compared with other anti-inflammatory agents. FJ