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GPER1-mediated immunomodulation and neuroprotection in the myenteric plexus of a mouse model of Parkinson's disease

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ABSTRACT

Lewy pathology affects the gastrointestinal tract in Parkinson's disease (PD) and recent reports suggest a link between the disorder and gut inflammation. In this study, we investigated enteric neuroprotection and macrophage immunomodulation by 17β-estradiol (E2) and the G protein-coupled estrogen receptor 1 (GPER1) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse PD model. We found that both E2 and the GPER1 agonist G1 are protective against the loss of dopamine myenteric neurons and inhibited enteric macrophage infiltration in MPTP-treated mice. Coadministration of GPER1 antagonist G15, while completely blocking the neuroprotective and anti-inflammatory effects of G1 also partially prevented those of E2. Interestingly, we found that E2 and G1 treatments could directly alter MPTP-mediated immune responses independently from neurodegenerative processes. Analyses of monocyte/macrophage NF-κB and iNOS activation and FACs immunophenotype indicated that 1-methyl-4-phenylpyridinium (MPP^+) treatment induces a strong immune response in monocytes, comparable to that of canonical challenge by lipopolysaccharide. In these cells, G1 and E2 treatment are equally potent in promoting a shift toward an anti-inflammatory "M2" immunophenotype reducing MPP^+-induced NF-κB and iNOS activation. Moreover, G15 also antagonized the immunomodulatory effects of G1 in MPP^+-treated macrophages. Together these data provide the first evidence for the role of GPER1 in enteric immunomodulation and neuroprotection. Considering increasing recognition for myenteric pathology as an early biomarker for PD, these findings provide a valuable contribution for better understanding and targeting of future therapeutic strategies.

Introduction

Parkinson's disease (PD) is a progressive neurological disorder characterized by motor behavior dysfunctions such as tremor, muscle rigidity, postural instability and bradykinesia. Although people affected by PD are subjected to a significant preclinical period of 5–6 years (Schapira and Obeso, 2006; Hawkes et al., 2009; Savica et al., 2010), there are numerous non-motor manifestations including autonomic dysfunction, sensory problems, sleep disorders and neuropsychiatric symptoms which that precede the onset of motor disabilities by 20 years or more (Poewe, 2006; Reichmann, 2010). In particular, delayed gastric emptying, bloating from small bowel coordination, constipation and defecatory dysfunction are common occurrences in PD and are predicted to predate motor symptoms by at least a decade (Abbott et al., 2001; Savica et al., 2009). Increasing evidence suggests that delayed colonic transit in Parkinson's disease is caused by disordered autonomic regulation due to central and peripheral degeneration of parasympathetic nuclei. They are found in the vagal and intermediolateral nuclei as well as in the myenteric and submucous plexii of the gastrointestinal tract. Within the latter structures, the presence of Lewy bodies and a depletion of dopamine-producing neurons may be rated as characteristic morphologic lesions (Wakabayashi et al., 1990; Singaram et al., 1995).

Recent studies suggest that the enteric nervous system (ENS) is severely affected by PD, as demonstrated by the presence of α-synuclein in colonic biopsies from PD patients (Lebouvier et al., 2008, 2009, 2010a,b, Shannon et al., 2012). The latter exhibits significant increases in intestinal permeability (gut leakiness) accompanied by bacterial translocation and indicators of oxidative stress (Forsyth et al., 2011). Interestingly, this hyper-permeability correlates with increased intestinal

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mucosa presence of *Escherichia coli* bacteria, nitrotyrosine, and α-synuclein as well as serum lipopolysaccharide (LPS) binding protein levels in PD patients, suggesting the presence of ongoing inflammation. Considering the high exposure of enteric DAergic neurons to toxins or pathogens like *E. coli*, it is critical to understand the mechanisms of gut neurodegenerative processes in PD. An in-depth analysis of the peripheral aspects of DAergic alterations might provide the key to understanding both PD etiology and progression.

Progressive and chronic inflammatory processes have long been observed in PD brains and suggested to contribute to neuronal damage. For instance, activated microglia, proinflammatory cytokines and T lymphocytes can be detected in the cerebrospinal fluid, substantia nigra or caudate putamen of PD patients (Mogi et al., 1996; Nagatsu et al., 2000). Parallel to central inflammation, recent studies indicate similar processes at peripheral sites. For example, elevations in proinflammatory cytokines levels in colonic biopsies provide strong evidence for gastrointestinal inflammation in PD patients (Devos et al., 2013). The contribution of inflammation to neuronal pathology in the ENS, however, still remains largely uninvestigated. Notably, it is not clear whether the innate immune response associated with neurodegeneration is a primary or secondary event contributing to disease progression.

We recently addressed this issue by exploring inflammation and myenteric DAergic neuronal degeneration caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) challenge in mice with intact immune system, or those deficient in MyD88, a major protein implicated in the innate immunity cascade (Cote et al., 2011). Our results showed that MPTP-treated MyD88 knock out (MyD88($^{-/-}$)) mice are protected against the toxin-induced TH-immunoreactive neuronal damage in the myenteric plexus of the distal ileum (Cote et al., 2011). Importantly, in MPTP-treated MyD88$^{-/-}$ mice, resident macrophages predominantly exhibit an anti-inflammatory "M2" phenotype, which may contribute to myenteric DAergic neuron protection (Cote et al., 2011). Further investigations into the role of the innate immune system in peripheral neuronal damage revealed that partial depletion of proinflammatory "M1" monocytes also inhibits the MPTP-induced myenteric alterations (Cote et al., 2015). Taken together, our recent results suggest a critical role for inflammation in the gastrointestinal DAergic degeneration induced by MPTP.

Given this striking evidence, we moved forward to seek therapeutic molecules that, in this model, could both provide myenteric neuroprotection and induce a beneficial shift toward an anti-inflammatory "M2" macrophage immunophenotype. Several interesting studies support a beneficial role of estrogen exposure in PD and suggest a potential application for estrogen as a neuroprotective agent (Liu and Dluzen, 2007). Estrogens play a complex role in inflammation as they possess both anti-inflammatory and proinflammatory roles depending on various factors, in addition to their ability to specifically alter the response of different immune cell types (Straub, 2007). Moreover, studies in the MPTP mouse model of PD indicated that estrogen is centrally neuroprotective (Bourque et al., 2009). While the mechanism of 17β-estradiol (E2)-related neuroprotection against MPTP implicates estrogenic receptor (ER) α, the discovery of a new estrogenic receptor, the G protein-coupled ER 1 (GPER1), offers the possibility of an additional mechanism of E2 action. Since the activation of GPER1 does not stimulate uterine and mammary gland epithelial cell proliferation, which may lead to cancers (Otto et al., 2008), the benefits stemming from specifically targeting GPER1 represent potential therapeutic avenues. Indeed, in a recent article we demonstrated that G1 is as potent as E2 in mediating powerful neuroprotective effects in the central nervous system of MPTP-treated mice (Bourque et al., 2013). The present study supplements these findings by evaluating the estrogenic immunomodulatory and neuroprotective mechanisms in the ENS and the contributions of GPER1 using the GPER1-specific agonist G1 and antagonist G15 (Bologa et al., 2006; Dennis et al., 2009).

Materials and methods

Animals

C57BL/6 male mice (10 weeks) were purchased from Charles River Canada (Montreal, QC, Canada). Mice were housed in cages under standard laboratory conditions, allowed free access to food and water, and acclimated to a controlled-temperature environment maintained under a 12 h light/dark cycle. The Laval University Animal Care Committee approved all of the animal studies. All efforts were made to minimize animal suffering and to reduce the number of mice used.

Treatments

Mice were divided in six groups (n = 14): Control, MPTP treatment, MPTP with E2 treatment, MPTP with GPER1 agonist (G1), MPTP with 17β-estradiol and GPER1 antagonist (G15) and MPTP with G1 and G15. Each group received a 5-day pre-treatment with E2, G1, G15 or vehicle prior to MPTP injections. For the pre-treatment, two subcutaneous injections per day of E2 (1 μg; Sigma Chemical, St. Louis, MO, USA), G1 (5 μg; Tocris, Ellisville, MO, USA), G15 (10 μg; Tocris) or vehicle (0.9% saline with 0.3% gelatin) were performed. On day 5, mice received four intraperitoneal injections of MPTP (4.75 mg/kg; Sigma Chemical) or a saline solution separated by a 2-hour interval. The treatments (17β-estradiol, G1 and G15) were continued until day 10. Each concentration used was determined following dose–response analysis as described in Bourque et al., (2013).

Tissue preparation

On day 11, four mice in each experimental group were deeply anesthetized with isoflurane. Peritoneal cells were collected by lavage using 5 ml of ice-cold complemented Dulbecco's Modified Eagle Medium (DMEM with 10% fetal bovine serum), washed and resuspended in 1 ml for cytometry analysis. The remaining mice were anesthetized and then decapitated. The ileum, part of the small intestine immediately adjacent to the cecum, was excised and microdissected to isolate the myenteric plexus as described in our previous publication (Cote et al., 2011). A second group of mice (n = 5) received saline, G1, MPTP or MPTP with G1 treatment to validate the effect of GPER1 activation on the phenotype of circulating monocytes. Animals were deeply anesthetized with isoflurane before cardiac puncture and blood cells were immediately processed for flow cytometry analysis.

Immunohistochemistry

Free-floating sections of myenteric plexus were subjected to immunohistochemistry as reported (Cote et al., 2011). Myenteric neurons were stained by the Cuprolinic blue coloration (Holst and Powley, 1995). Macrophages were identified by the pan-macrophage/microglia marker ionized calcium binding adaptor molecule 1 (Iba-1; 1:1000; Cedarlane, ON, Canada) and DAergic neurons were localized by a polyclonal Tyrosine Hydroxylase antibody (TH; 1:1000; Cedarlane). A biotinylated goat anti-rabbit IgG (Cedarlane) was used as secondary antibody. The signal was revealed with the streptavidin–biotin peroxidase reaction method using an ABC Vectastain elite kit (Vector Laboratories Inc, ON, Canada) and 3–3′diaminobenzidine (DAB, Vector Laboratories Inc) as chromagen.

A Nikon C80i microscope equipped with a MicroFire digital camera (MBF bioscience, Williston, VT) was used to acquire pictures. Some viewing adjustments have been made with Photoshop CS3 (Adobe system) and figures were assembled using Adobe Illustrator CS3. Quantification of the total number of labeled cells and the areas of TH-immunoreactive fibers were analyzed by stereology as previously described (Cote et al., 2011).

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