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AQUAPORIN-4 MEDIATES COMMUNICATION BETWEEN ASTROCYTE AND MICROGLIA: IMPLICATIONS OF NEUROINFLAMMATION IN EXPERIMENTAL PARKINSON'S DISEASE

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Abstract—Aquaporin-4 (AQP4), a water-selective membrane transport protein, is up-regulated in astrocytes in various inflammatory lesions, including Parkinson disease (PD). However, the exact functional roles of AQP4 in neuroinflammation remain unknown. In the present study, we investigated how AQP4 participates in the neuroinflammation of PD using AQP4 knockout (KO) mice and astrocyte–microglial co-cultures. We found that AQP4 KO mice exhibited increased basal and inducible canonical NF-κB activity, and showed significantly enhanced gliosis (astrocytosis and microgliosis) in chronic MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)/probenecid PD models, companying with the increase in the production of IL-1β and TNF-α in the midbrain. Similarly, AQP4 deficiency augmented the activation of the NF-κB pathway and the production of IL-1β and TNF-α in midbrain astrocyte cultures treated with MPP^+ (1-methyl-4-phenylpyridinium). Furthermore, AQP4 deficiency promoted activation of microglial cells in the co-cultured system. Our data provide the first evidence that AQP4 modulates astrocyte-to-microglia communication in neuroinflammation, although its effect on astrocyte inflammatory activation remains to be explored.

Key words: aquaporin-4, Parkinson's disease, astrocyte, microglia, neuroinflammation.

INTRODUCTION

In recent years, numerous studies have confirmed that neuroinflammation plays a critical role in the pathogenesis of neurodegenerative diseases, including Parkinson disease (PD) (Gonzalez et al., 2014; Moehle and West, 2015; Rocha et al., 2015; Wang et al., 2015; Ward et al., 2015). In animal models of PD, the neuroinflammation occurs in the whole process of neurodegeneration (Cebrian et al., 2015). Activated microglia produces a variety of proinflammatory factors, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and reactive oxygen species (Gonzalez et al., 2014; Moehle and West, 2015). Over-activation of microglia and overproduction of proinflammatory factors may lead to neuronal death, while the role of astrocytes is controversial in neuroinflammation.

In the central nervous system (CNS), astrocytes constitute nearly half of the total cells, providing structural, metabolic and trophic support for neurons (Pekny and Pekna, 2014). Astrocytes can also function as immunocompetent cells in the CNS (Dong and Benveniste, 2001; Ben Haim et al., 2015). Both *in vitro* and *in vivo* studies have documented the ability of astrocytes to produce a wide variety of cytokines, chemokines and neurotrophic factors. Astrocytes are thought to play a key role in initiating and perpetuating the disruptive inflammatory process associated with multiple sclerosis and Alzheimer disease (Birch, 2014; Verkhratsky et al., 2014), but their roles in the inflammation of PD are not well understood.

Aquaporin-4 (AQP4) is a water-selective membrane transport protein expressed in glial cells in the brain (Fukuda and Badaut, 2012; Xiao and Hu, 2014). However, growing evidences showed that AQP4 expression in astrocytes differed markedly according to disease and was unnecessarily related to brain edema. In our previous studies (Fan et al., 2008; Chi et al., 2011), we established acute PD animal models by administration of

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Abbreviations: AQP4, aquaporin-4; CD11b-ir, CD11b-immunoreactive; CNS, central nervous system; DIV, day *in vitro*; DMSO, dimethyl sulfoxide; EAE, experimental autoimmune encephalomyelitis; ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acidic protein; GFAP-ir, GFAP-immunoreactive; IL-1β, interleukin-1β; KO, knockout; LDH, lactate dehydrogenase; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPTP/p, MPTP/probenecid; PD, Parkinson disease; qRT-PCR, quantitative real-time PCR; SNc, substantia nigra pars compacta; TBST, tris-buffered saline with tween; TH, tyrosine hydroxylase; TH-ir, TH-immunoreactive; TNF-α, tumor necrosis factor-α; WT, wildtype.

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1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and found that AQP4 knockout (KO) mice displayed marked astrogliosis and more robust microglial inflammatory responses in the midbrain compared with wildtype (WT) mice. In addition, the up-regulation of AQP4 in astrocytes was also found in various other inflammatory lesions (Fukuda and Badaut, 2012; Lan et al., 2015). However, the exact roles of AQP4 for neuroinflammation, especially in the astrocyte–microglia communication, remain unclear.

To explore how AQP4 participates in the neuroinflammation of PD, we compared the inflammatory profile of the midbrain from both genotype mice after a chronic MPTP/p (MPTP/probenecid) PD model. Secreted cytokines (IL-1β, TNF-α, and IL-6) were measured using quantitative real-time PCR (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA). Furthermore, astrocytes and microglias cultured from the midbrain of both genotype mice were stimulated by MPP^+ (1-methyl-4-phenylpyridinium), the putative toxic metabolite of MPTP, to explore the probable mechanisms.

EXPERIMENTAL PROCEDURES

Animals and treatments

AQP4 KO mice were generated as previously described (Fan et al., 2005). All experiments were performed on weight-matched littermates (24–32 g) produced by intercrossing of CD1 heterozygotes. Investigators were blinded to the genotype for all experiments. Mice were kept under environmentally controlled conditions (ambient temperature, 22 °C; humidity, 40%) on a 12-h light/dark cycle with food and water *ad libitum*. All experiments were approved by IACUC (Institutional Animal Care and Use Committee of Nanjing Medical University). All efforts were made to minimize animal suffering and to reduce the number of animals used for the experiments.

As previously reported (Liss et al., 2005; Lu et al., 2014), sixteen-week-old male mice were injected with probenecid (a clearance inhibitor, given to prolong MPTP neurotoxicity, 250 mg/kg in dimethyl sulfoxide [DMSO, D2650, Sigma, St. Louis, MO, USA], *i.p.*), 30 min prior to MPTP hydrochloride (25 mg/kg in saline, *s.c.*, Sigma, St. Louis, MO) administration, twice a week for a total of 10 doses over 5 weeks. Since neither probenecid nor DMSO used in our study significantly produced any effect on the striatal DA contents (Lau et al., 1990; Petroske et al., 2001; Barber-Singh et al., 2009), mice were treated similarly with probenecid alone as controls. The time schedule of intervention and analyses performed is shown in Fig. 1. Seven days after the last injection, mice were anesthetized with intraperitoneal pentobarbital sodium (60 mg/kg, Sigma–Aldrich, St. Louis, MO). For immunohistochemistry and stereology, mice (four or five per group) were perfused transcardially with 4% paraformaldehyde. Brains were dissected out and maintained in 4% paraformaldehyde overnight, cryopreserved in 30% sucrose in phosphate-buffered solution (PBS) and then stored at −80 °C until use. For qRT-PCR (four per group), western blotting (four per group) and ELISA (eight per group) analysis, mice were sacrificed by decapitation. Ventral midbrains were rapidly dissected on a cold plate, flash frozen in liquid nitrogen, and stored at −80 °C until use.

Immunohistochemistry and immunocytochemistry

For immunohistochemistry, coronal slices (30-μm thick) were cut to obtain serial sections of midbrain (anterior–posterior −2.54 to −3.64 mm) using bregma-based coordinates (Paxinos, 2001). Every third section (~12 sections per mouse) was processed for immunohistochemistry of tyrosine hydroxylase (TH, mouse anti-TH antibody, T1299, 1:4000, Sigma). The sections of substantia nigra pars compacta (SNc) adjacent to those used to test for TH immunoreactivity were processed for glial fibrillary acidic protein (GFAP, rabbit anti-GFAP antibody, MAB360, 1:800, Millipore, six sections per mouse) or CD11b (mouse anti-CD11b antibody, MCA711, 1:50, Serotec, six sections per mouse) for 24 h at 4 °C. Then, the sections were rinsed, incubated in an appropriate secondary antibodies (goat anti-mouse IgG, 074-1806, 1:800; goat anti-rabbit IgG, 074-1506, 1:800, KPL) for 1 h. For visualization, the Avidin–peroxidase protocol (ABC, Vector, UK) was applied, using 3,3′-diaminobenzidine as chromogen. Control staining was performed as described without the administration of primary antibodies.

For cultured cell immunofluorescence, cells were incubated with goat anti-AQP4 antibody (sc-9887, 1:800; Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse anti-GFAP antibody (ab10062, 1:500; Abcam, Cambridge, MA, USA), and mouse anti-CD11b antibody (MCA711, 1:200; Serotec, Raleigh, NC, USA).

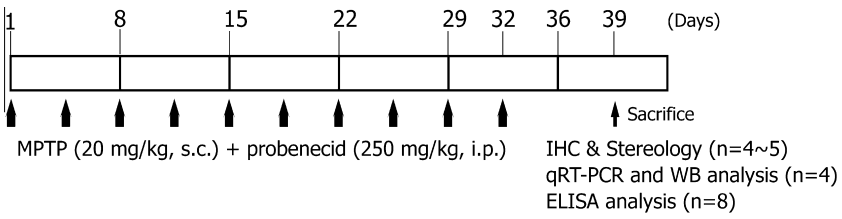

Fig. 1. Schematic diagram showed the time schedule of intervention and analyses. Mice were treated subcutaneously with 20 mg/kg MPTP and intraperitoneally with 250 mg/kg probenecid every 3.5 days over a period of 5 weeks. Control mice were treated subcutaneously with saline and intraperitoneally with probenecid. Seven days after the last injection, mice were sacrificed for indicated studies. Following were mice numbers used for this experiment, immunohistochemistry (n = 4–5), qRT-PCR studies (n = 4), Western blotting analysis (n = 4) and ELISA (enzyme-linked immunosorbent assay, n = 8) per each treatment group. MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; WB, Western blotting.