Neuroscience 359 (2017) 258–266

SUBCELLULAR EXPRESSION OF AQUAPORIN-4 IN SUBSTANTIA NIGRA OF NORMAL AND MPTP-TREATED MICE

AGNETE PRYDZ,[a] KATJA STAHL,[a] MAJA PUCHADES,[a] NINA DAVARPANEH,[a] MARIA NADEEM,[a] OLE PETTER OTTERSEN,[a] VIDAR GUNDERSEN[a,b] AND MAHMOOD AMIRY-MOGHADDAM[a]*

[a] *Division of Anatomy and Healthy Brain Ageing Center Regional Research Network, Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, PO Box 1105, Blindern, 0317 Oslo, Norway*

[b] *Department of Neurology, Oslo University Hospital, Rikshospitalet, PO Box 4950 Nydalen, 0424 Oslo, Norway*

Abstract—Aquaporin-4 (AQP4) is the predominant water channel in mammalian CNS where it is localized at the perivascular astrocytic foot processes abutting brain microvessels. Several lines of evidence suggest that AQP4 is involved in important homeostatic functions and that mislocalization of the perivascular pool of AQP4 is implicated in several different brain disorders. A recent study suggests that the differential susceptibility of midbrain dopaminergic neurons to the parkinsonogenic toxin 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP) depends on the expression of AQP4. Further, MRI studies of patients with Parkinson's disease (PD) point to an excessive water accumulation in the substantia nigra (SN). This prompted us to investigate the cellular and subcellular distribution of AQP4 in mouse SN using immunofluorescence and quantitative immunogold cytochemistry. Compared with neocortex, SN exhibits a higher concentration of AQP4. Specifically, judged by electron microscopic immunogold analysis, the perivascular density of AQP4 in SN exceeds by 70% the perivascular density of AQP4 in the neocortex. An even larger difference in AQP4 labeling was found for astrocytic processes in the neuropil. Treatment with MPTP further increased (by >30%) the perivascular AQP4 density in SN, but also increased AQP4 labeling in the neocortex. Our data indicate that the perivascular AQP4 pool in SN is high in normal animals and even higher after treatment with MPTP. This would leave the SN more prone to water accumulation and supports the idea that AQP4 could be involved in the pathogenesis of PD.

Key words: aquaporin-4, astrocyte, MPTP, Parkinson's disease, immunogold histochemistry.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the Western hemisphere, characterized by progressive neuronal cell death predominantly of the dopaminergic neurons (DA) in substantia nigra pars compacta (SNpc) (Hirsch et al., 2013). More than 90% of all cases with PD have unknown etiology and the mechanisms underlying the selective loss of nigral DA neurons are not understood. Evidence is accumulating to suggest that astrocytes might play a role in the pathophysiology of PD (Mena and García De Yébenes, 2008) and recent studies in animal models of PD have indicated an involvement of the astrocytic water channel aquaporin-4 (AQP4) (Fan et al., 2008; Yang et al., 2011; Sun et al., 2016; Zhang et al., 2016). Moreover, MRI studies of patients with PD have revealed an abnormal water accumulation in the SN (Ofori et al., 2015a). However, the cellular and subcellular expression of AQP4 in the SN is unknown, as is the expression level of AQP4 in the parkinsonian brain.

Studies of other brain regions have revealed that AQP4 is concentrated in astrocytic endfeet that encompass the blood–brain and brain–liquid interfaces (Nielsen et al., 1997; Rash et al., 1998; Amiry-Moghaddam and Ottersen, 2003). The polarized expression of AQP4 in astrocytes is crucial for homeostatic processes maintaining normal function, including water and potassium homeostasis (Nagelhus et al., 1999). Further, polarized expression of AQP4 has been shown to be important for cell migration (Saadoun et al., 2005) and possibly astrocytic Ca^{2+} signaling (Thrane et al., 2011). Loss of the polarized expression of AQP4 has been shown in several pathological conditions, such as stroke (De Castro Ribeiro et al., 2006; Frydenlund et al., 2006), temporal lobe epilepsy (Alvestad et al., 2013) and Alzheimer's disease (Yang et al., 2011). Here we used immune-microscopy in healthy mice and mice treated with the parkinsonogenic toxin 1-methyl-4-phenyl-1,2, 3,6-tetrahydropyridine (MPTP) to ask whether the SN differs from other brain regions with respect to the expression of AQP4 and whether the expression of AQP4 in SN is sensitive to MPTP.

Our data indicate that astrocytes in the SN differ from those in neocortex by showing a higher level of AQP4, particularly in those endfoot membrane domains that mediate water exchange between brain and blood. Nine

*Corresponding author.
E-mail address: mahmo@medisin.uio.no (M. Amiry-Moghaddam).
Abbreviations: AQP4, aquaporin-4; DA, dopaminergic; GFAP, glial fibrillary acidic protein; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; SN, substantia nigra; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase.

http://dx.doi.org/10.1016/j.neuroscience.2017.07.029
0306-4522/

days after treatment with MPTP the AQP4 pool in these membrane domains has increased further, and more so than the AQP4 pools in other domains of the astrocyte membrane. AQP4 over expression in specific astrocyte membrane domains may contribute to the water dyshomeostasis observed in patients with PD.

EXPERIMENTAL PROCEDURES

Animals

Male wild-type C57BL/6 mice 8–12 weeks were used in this study. Experimental protocols were approved by the Institutional Animal Care and Use Committee and conform to National Institutes of Health guidelines for the care and use of animals.

Tissue preparation

Untreated animals. Animals were anesthetized deeply with hypnorm dormicum (0.105 mg/kg) followed by intracardial perfusion. For light microscopy of untreated SN and neocortex, the animals (n = 5) were perfusion-fixed with 4% formaldehyde (FA) in 0.1 M phosphate buffer (PB) (Sigma–Aldrich, St. Louis, MO, USA) using pH-shift (Eilert-Olsen et al., 2012) starting with pH 6.0 for 5 min, then shifting to pH 10.5 for 10 min. The brains were dissected out and post-fixed overnight in 4% FA with pH 10.5. Animals used for electron microscopy (n = 3) were perfusion-fixed with 4% FA and 0.1% glutaraldehyde (GA) in 0.1 PB (Sigma–Aldrich), and then post-fixed in the same solution overnight. All brains were stored in a 1:10 dilution of their respective fixative at 4°C until further preparations.

MPTP- and saline-treated animals. Mice were treated with either MPTP or saline, following an acute protocol as previously described (Jackson-Lewis et al., 1995; Przedborski and Jackson-Lewis, 2007; Puchades et al., 2013). One series of MPTP-treated mice and controls was used for EM studies and another series was used for light microscopic immunohistochemistry. For EM experiments, male C57 BL/6 mice (n = 5; 8 weeks of age; Taconic, Denmark) received MPTP–HCl free base (Sigma–Aldrich) in an acute paradigm (four injections subcutaneously at 2-h intervals, 18 mg/kg for each injection) (Przedborski and Jackson-Lewis, 2007). The control mice (n = 5) received injections of saline (4 mL/kg). For light microscopic immunohistochemistry, male C57 BL/6 mice (n = 4; 12 weeks of age; Taconic, Denmark) received MPTP–HCl free base (Sigma–Aldrich) in an acute paradigm (three subcutaneous injections of 15 mg/kg at 2-h intervals). The control mice (n = 3) received injections of saline (4 mL/kg). The MPTP dosage was reduced in the second series in order to reduce the acute mortality associated with the higher MPTP concentration. Based on Jackson-Lewis et al. (Jackson-Lewis et al., 1995) who showed that the degeneration of dopamine neurons started within the first day after MPTP treatment, reached a plateau at day 4, and was thereafter stable until day 28, we chose a survival time of 9 days after the last MPTP injection. The choice of this time point is also in line with the recommendations of Przedborski and Jackson-Lewis (2007). From day 7 until day 28 TH-immunoreactivity can be used to determine accurately the number of living neurons in SNpc (Jackson-Lewis et al., 1995). Nine days after the last injection, both groups of animals were deeply anesthetized with pentobarbital (0.1 mL/50 g body weight) followed by transcardial perfusion of either 4% FA and 0.1% GA or 4% FA for 10 min and post-fixed *in situ* overnight (Puchades et al., 2013). Tissue samples from the 4% FA-fixed brains were used for immunofluorescence analysis and samples from both fixatives were used for the immunogold analysis. The difference in the fixatives did not have any impact on the density of the immunogold labeling (data not shown). The brains were dissected out and either processed for electron microscopy or cut into 30 μm coronal sections using a Vibratome (Microm, Jacksonville, FL, USA) for immunohistochemistry and stored at 4°C in 1/10 fixative until further preparations.

Immunofluorescence and confocal microscopy. The immunofluorescence labeling was performed as described previously (Yang et al., 2011). In brief, the sections were treated with 10% normal goat serum (NGS) and 1% bovine serum albumin (BSA) upon incubation with primary antibodies overnight in 3% NGS, 1% BSA, 0.5% Triton-X-100 and 0.05% sodium azide in 0.01 M phosphate-buffered saline (all chemicals from Sigma–Aldrich). Following washing, the sections were incubated with secondary antibodies, washed in PBS, and mounted with Prolong Gold Antifade Reagent with DAPI (Invitrogen, Norway). A complete list of the primary and secondary antibodies used in this study is presented in Table 1. Images were collected with a LSM 510 META Confocal Microscope (Zeiss, Jena, Germany) using a

Table 1. Antibodies

Method	Primary antibody	Secondary antibody
Immunogold	AQP4, host: rabbit, Sigma-Aldrich, diluted 1:400	Goat anti-rabbit 15 nm gold, Abcam, diluted 1:20
Immunofluorescence	AQP4, host: rabbit, Sigma-Aldrich, diluted 1:400	Cy3 donkey anti-rabbit, Jackson, diluted 1:500
	TUJ1, host: mouse, Covance, diluted 1:1000	Alexa 488 donkey anti-mouse, Invitrogen, diluted 1:500
	TH, host mouse, Chemicon, diluted 1:1000	Alexa 488 donkey anti-mouse, Invitrogen, diluted 1:500
	GFAP, host chicken, Nordic BioSite, diluted 1:500	Cy2 donkey anti-chicken, Jackson, diluted 1:500