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Research Report

Lesion of the locus coeruleus aggravates dopaminergic neuron degeneration by modulating microglial function in mouse models of Parkinson's disease

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ABSTRACT

The degeneration of noradrenergic neurons in the locus coeruleus (LC) commonly occurs in patients with Parkinson's disease (PD), which is characterized by a selective injury of dopaminergic neurons in the substantia nigra (SN). The pathological impact of the LC on the SN in the disease is unknown. In the present study, we used a noradrenergic toxin, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4), to deplete noradrenaline (NA) derived from the LC to explore its influence on degeneration or injury of dopaminergic neurons in the SN in mouse model produced by intraperitoneal injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or lipopolysaccharide (LPS). Our results demonstrated that lesion of the LC could change microglial function in the brain, which led to enhanced or prolonged expression of pro-inflammatory cytokines, diminished neurotrophic factors, and weakened ability of anti-oxidation in the SN. The in vitro experiments further confirmed that NA could reduce the inflammatory reaction of microglia. The selective

Abbreviations: AD, Alzheimer disease; BDNF, brain-derived neurotrophic factor; CBA, cytometric bead array; CCR2, C-C chemokine receptor type 2; CD11b/ITGAM, integrin alpha M; CD74, HLA class II histocompatibility antigen gamma chain; COX2, cyclooxygenase-2; Ctx, cortex; DAB, 3,3'-diaminobenzidine; DSP4, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GDNF, glial cell-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; Hip, hippocampus; GSH, glutathione; HRP, horseradish peroxidase; i.p., intraperitoneally; Iba1, ionized calcium-binding adaptor molecule 1; IL-13, Interleukin-13; IL-1β, interleukin-1 beta; IL-4, interleukin-4; IL-6, interleukin-6; LC, locus coeruleus; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NA, noradrenaline; NGF-β, nerve growth factor-β; NOS2, nitric oxide synthetase-2; PD, Parkinson's disease; REM, rapid eye movement; ROS, reactive oxygen species; SN, substantia nigra; SOD, superoxide dismutase; Str, striatum; TH, tyrosine hydroxylase; TNF, tumor necrosis factor

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injury of dopaminergic neurons by inflammation, however, was due to the inflammation in different brain regions rather than the depletion of NA. Our results indicate that the lesion in the LC is an important factor in promoting dopaminergic neuron degeneration by impacting the function of microglia in the midbrain.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disease characterized by specific loss of dopaminergic neurons in the substantia nigra (SN) with the appearance of serious motor symptoms (Braak et al., 2003). It has been found, however, that a proportion of noradrenergic neurons in the locus coeruleus (LC) also degenerate significantly in PD patients (Marien et al., 2004). The degree of loss of LC neurons is even greater and earlier than that of nigral dopaminergic neurons (Zarow et al., 2003). Lewy bodies, a pathological feature of PD, are formed in the LC even years prior to their formation in the SN (Braak et al., 2003). In addition, certain non-motor preclinical symptoms, including REM sleep behavior disorder (Boeve et al., 2003), depression (Leentjens et al., 2003), and autonomic nervous dysfunction (Boeve et al., 2003), develop before the emergence of motor symptoms in PD and have been shown to be related to LC degeneration.

The noradrenergic neurons in the LC modulate distinctive neurological functions by spread of their terminal varicosities to extensive brain areas, including the SN, striatum (Str) and cortex (Ctx) (Lategan et al., 1992, Fornai et al., 1998). Noradrenaline (NA) released from LC neuron varicosities diffuses into the microenvironment and affects both neurons and glial cells (Dzirasa et al., 2010; Lee, 2013). In addition, β2-adrenergic receptors on microglia can inhibit brain inflammation (Qian et al., 2011).

Inflammation of the brain caused by dysfunction of microglia is widely accepted as an important hypothesis of pathogenesis of PD (Qian et al., 2011). It is confirmed in both PD patients and PD animal models that abnormally activated microglia release a number of neurotoxic factors, such as pro-inflammatory cytokines and reactive oxygen species (ROS), which mediate the neurodegeneration in the SN and Str (Mosley et al., 2012). Similarly in Alzheimer disease (AD), microglial inflammation also plays a critical role in neurodegeneration in the cortex and hippocampus (Hip). In this case, the reduction of LC noradrenergic neurons occurs prior to the degeneration in the Ctx and Hip, and this has been related to the abnormal activation of microglia (Heneka et al., 2010). However, whether the depletion of NA in the LC could impact the pathogenesis of PD via modulating microglial inflammation in the SN has not been investigated.

In this study, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP4) was used to deplete the LC noradrenergic terminals, and followed by injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or lipopolysaccharide (LPS) to produce different DA neuron injured models. After that, microglial inflammation and alteration of dopaminergic neuron degeneration in the SN were evaluated. Our data suggested that the lesion in the LC might aggravate the microglial inflammation in the SN/Str area, which could subsequently enhance degeneration of dopaminergic neurons in the SN.

2. Results

2.1. *Intraperitoneal injection of DSP4 reduced level of NA in the SN/Str*

The HPLC analysis showed that the 2 injections of DSP4 could effectively reduce the level of NA in the Str and SN at the 7th day after the final injection, i.e., the NA in the Str and SN was reduced to about 80% and 40%, respectively (data not shown). The LC neuronal number showed no significant difference between DSP4-treated and saline-treated mice (data not shown).

2.2. *Noradrenergic depletion enhanced degeneration of DA neurons in the SN and injury of the DA terminals in the Str in PD models*

In the mice treated with DSP4 compared with saline-treated mice before MPTP injection, no significant differences were found in the number of dopaminergic neurons in the SN or the TH-positive (TH+) fiber density in the Str. ("Before" histogram bars in our figures represent those saline or DSP4 treated animals sacrificed on the same day when the first MPTP injection should be given but not.) After subacute MPTP treatments (7 d and 14 d), the TH+ staining for dopaminergic cell bodies in the SN and fibers in the Str was reduced compared to that before MPTP injection in both saline and DSP4 groups (Fig. 1A and C). However, the animals pretreated with DSP4 lost more dopaminergic neurons in the SN than saline-treated mice (Two-way ANOVA, $F=48.68$, $p<0.01$) at 7 d (saline vs. DSP4, $p<0.01$) and 14 d (saline vs. DSP4, $p<0.05$), i.e., lost about 1000 more at 7 d and 700 more at 14 d (Fig. 1B). The density of TH+ fibers in the Str was reduced significantly (Two-way ANOVA, $F=7.79$, $p<0.05$) at 14 d in DSP4 group (saline vs. DSP4, $p<0.05$) (Fig. 1D). The change in TH-protein levels measured by western blot in the Str in MPTP-treated mice also demonstrated that noradrenergic depletion aggravated the degeneration of dopaminergic neurons (Fig. 7A and B). Injection of DSP4 significantly decreased (Two-way ANOVA, $F=16.25$, $p<0.01$) the TH level at 7 d (saline vs. DSP4, $p<0.01$) and 14 d (saline vs. DSP4, $p<0.01$) after MPTP treatment (Fig. 7B), which mirrored the immunohistochemical staining results.

In the Str for the LPS-treated mice, at 1 d after LPS injection, quite a few deeply stained plaques, indicating the injury of dopaminergic fibers, appeared in the Str in both saline and DSP4 pretreated mice, but the size and color intensity of these plaques were larger and darker in the DSP4-treated group (Fig. 2A). The total area of these deeply stained plaques in the Str in the DSP4-treated group increased to a peak at 7 d (Two-way ANOVA, $F=9.71$, $p<0.01$; saline vs. DSP4, $p<0.01$) and reduced from 14 d (Fig. 2B), as shown by analysis with Image-Pro Plus software. At 28 d after LPS treatment, DSP4-treated mice still showed a few deeply stained plaques in the Str, while the saline