Psychosocial stress enhances susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in C57BL/6N mice

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(Received 6 September 2019; and accepted 28 September 2019)

ABSTRACT
Psychological stress is thought to be a risk factor for the onset or accelerate the progression of Parkinson’s disease. The main aim of this study is to explore the causative effect of confrontational housing (CH), a paradigm developed as an animal model of psychosocial stress, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice. When mice were housed confrontationally for 24 h, they displayed increased anxiety-like behavior in the light/dark box test. Administration of MPTP after CH for 24 h caused severe damage of striatal dopaminergic neurons as indicated by decreases in dopamine transporter and tyrosine hydroxylase proteins and an increase of glial fibrillary acidic protein levels compared to CH alone. The dose of MPTP used this study slightly affected these protein levels in the striatum of control mice, but they did not significantly change. Our results indicate that the striatal dopaminergic neurons are vulnerable to environmental risk factors that presumably have neurotoxin-like properties under psychological stress condition.

The pathogenesis of sporadic Parkinson’s disease (PD) is still unclear, but it is thought to result from the interaction between genetic and environmental causes (9). Neurodegeneration of nigrostriatal dopaminergic pathway in PD is believed to start long before the defining motor dysfunction have become apparent (16). Some non-motor symptoms, such as rapid eye movement sleep behavior disorder and olfactory disturbance, also seem to long antedate the onset of motor symptoms in PD patients (16). Physical and emotional stresses have been considered as risk factors in the etiology and pathophysiology of the disease (2, 4). For instance, a case report suggested that major stress may have triggered PD in a young woman who experienced extreme depression (22). A strong positive association between depression caused by stress and the subsequent incidence of PD has been reported (6, 18). However, the bases of the stresses or stress-related psychiatric disorders-induced impairments in this disease are still unclear.

In the mouse model of PD treated with a dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), several studies have indicated that emotional stress is involved in the vulnerability of striatal dopaminergic neurons to the neurotoxin. For instance, augmentation of the toxin-induced neurotoxicity by chronic mild stress has been reported (7, 8). Chronic restraint stress also exacerbates the motor deficit and neuroinflammation in the MPTP mouse model (10). We previously reported that Mg\[^{2+}\]-deficient mice, which display emotional behaviors such as depression- and anxiety-related behaviors, showed to increase the susceptibility to MPTP (14). A recent study has demonstrated that acute restraint stress enhances striatal dopaminergic neurodegeneration in the MPTP mouse model through the transport of toxin into the brain (12).
However, due to clinical relevance, it is necessary to approach the human psychological condition to evaluate the influence of the stresses on vulnerability of striatal dopaminergic neurons in animal models. We therefore investigated the effects of psychosocial stress, which was established using confrontational housing (CH) by Unno et al. (20, 21), on striatal dopaminergic neurons in mice treated with MPTP.

Eight-week-old male C57BL/6N mice were purchased from Charles River Japan (Atsugi, Japan) and housed in standard conditions. All use of the animal and protocol procedures were approved by the Animal Care Committee at Hokuriku University (No.19-02). Psychosocial stress was applied to mice by the CH method as reported by Unno et al. (20, 21). In brief, the residence of each mouse is first established in the cage while is divided into two areas using a partition. After 7 days, confrontation is initiated by removing the partition. Food and water were given ad libitum. Three mice were housed in a cage without a partition to provide control group.

In the behavioral experiment, anxiety-like effects were investigated after CH for 24 h using the light/dark box test (LDT) as previously described by our laboratory (13). The LDT apparatus (20.5 × 41 × 41 cm each; Sanplatec Corp., Osaka, Japan) consisted of a light compartment and a dark one that were connected by an opening (7 × 7 cm) located in the center of the partition at floor level. The light compartment was brightly illuminated with a 60 W light source, whereas the other was covered by a black top. The light intensity in the light compartment was 800 lux compared with 40 lux in the dark compartment. At the beginning of the test, each mouse was placed in the dark compartment facing away from the opening. The latency to the first entry into the lit compartment (latency), time spent in the lit compartment (time in light) and total number of transition between the two compartments were recorded for 10 min.

To investigate the effects of CH on MPTP neurotoxicity, mice received MPTP hydrochloride (20 mg/kg of free base; Sigma-Aldrich, St Louis, MO, USA) in saline intraperitoneally after CH for 24 h. The dose of MPTP used in this study was selected based on the previous our study, which showed that its dose causes only the weak toxicity (13). The striatal tissues of mice were collected 3 days after MPTP administration. For immunoblotting analysis, tissues were homogenized, and then protein concentration was determined using BCA Protein Assay Kit (Cat # 23227; Thermo Scientific, Rockford, IL, USA). The homogenates were solubilized with Laemmli’s sample buffer and were subjected to 10% SDS polyacrylamide gel electrophoresis at 10 μg protein per lane. Immunoblotting was performed using anti-tyrosine hydroxylase (TH) (AB152; Millipore, Temecula, CA, USA), anti-dopamine transporter (DAT) (MAB369, Millipore) and anti-glial fibrillary acidic protein (GFAP) (G3893, Sigma-Aldrich) antibodies. Actin and Na+/K+-ATPase α-1 subunit proteins were identified with antibodies (MAB1501 and 05-369, Millipore, respectively) to each protein and were used as housekeeping proteins for quantitative analysis of TH, DAT, and GFAP in the same membranes. The membranes were incubated with alkaline phosphatase-conjugated secondary antibody, and then developed in accordance with the manufacturer’s instructions. The densities of immunoreactive bands were analyzed by image analysis software (Image J v1.50, NIH). Densitometric analysis was performed to quantify relative protein level against the housekeeping proteins.

Data analysis was performed with the statistical analysis system StatMate III (ATMS Co., Ltd., Tokyo, Japan). The statistical significances of the differences with raw data were assessed by two-tailed t-test or one-way ANOVA followed by Tukey’s post-test. Differences of less than 5% were considered statistically significant.

Unno et al. reported that the immobility time was significantly longer in the tail suspension test (TST) in mice during CH, indicating that depression-like behavior was induced by the psychosocial stress (20). Here we first examined the effects of CH on anxiety-like behavior in the LDT. After CH for 24 h, the stressed mice exhibited a significant increase of latency to the first entry into the lit compartment and a tendency of shorter time spent in the lit compartment, but not statistically significant (Fig. 1). The total number of transition between the two compartments did not differ between the two experimental groups (Control: 11.3 ± 3.9; CH: 9.5 ± 4.9, means ± SD (n = 6)).

We investigated the effects of MPTP on striatal dopaminergic neurons with or without CH by evaluation of the striatal protein levels of DAT, TH and GFAP (Fig. 2A–E). The DAT and TH proteins are used as a marker of dopaminergic neurons, and GFAP is a well-known marker of reactive astrocytes in the response to neuronal damage (15). In fact, the dopaminergic neurodegeneration by neurotoxins, such as 6-hydroxydopamine and MPTP, causes rapid and profound changes in striatal astrocytes characterized by increased GFAP-immunoreactivity (19). These protein levels of DAT, TH and GFAP in the
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mechanisms are involved in this situation that dopaminergic neurons are easily damaged by MPTP administration with CH. First, abnormality of monoamine neurotransmission associated with the stress could be related to the vulnerability of dopaminergic neurons. There is now ample evidence that dopaminergic neurons respond to various acute stressors by heightened dopamine synthesis, release, and metabolism (1). The acceleration of dopamine turnover could increase auto-oxidation of dopamine and also leads to quinones formation which can induce oxidative stress (11). Second, it is known that severe psychological stress induces the proinflammatory networks of cytokines and chemokines, which in turn activate the hypothalamic-pituitary-adrenal axis (3). In PD patient, the concentrations of tumor necrosis factor, interleukins and β macroglobulin are increased in the substantia nigra (5). Therefore, the inflammatory process may also participate in the induction of vulnerability of dopaminergic neurons during CH.

Taken together, our study provides an evidence that emotional stresses or stress-related psychiatric disorders could be a risk factor for the onset and the development of PD. If this hypothesis is correct, alleviation of emotional stresses may be important to prevent or to delay the progression of the disease.

Acknowledgement

This work was supported by the Specific Research Foundation of Hokuriku University (No.250100).

Unno et al. previously showed that the weight of the adrenal gland increased significantly with CH, and the apparent hypertrophy continued for at least 1 week (20). Interestingly, the treatment of paroxetine, which is an anti-depressant used in clinical practice, significantly suppressed the adrenal hypertrophy of mice during CH. They also confirmed CH significantly increased the immobility time in the TST. These observations indicated that mice during CH displayed depressive-like behavior. We thought that the CH also could induce anxiety-like behavior because clinically depression and anxiety often coexist (17). Our results show that CH induces some anxiety-like behavior indicated by the increase of latency in the LDT. Therefore, the psychosocial stressed mice could be a useful animal model for studying psychiatric diseases such as depression and anxiety.

Our data here show that the alteration of DAT, TH and GFAP protein levels by MPTP administration was only seen in mice with CH, but not in control mice. Therefore, the psychosocial stress influences the susceptibility to neurotoxin. How the stress induces the vulnerability of dopaminergic neurons is still unknown. However, we believed that several mechanisms are involved in this situation that dopaminergic neurons are easily damaged by MPTP administration with CH. First, abnormality of monoamine neurotransmission associated with the stress could be related to the vulnerability of dopaminergic neurons. There is now ample evidence that dopaminergic neurons respond to various acute stressors by heightened dopamine synthesis, release, and metabolism (1). The acceleration of dopamine turnover could increase auto-oxidation of dopamine and also leads to quinones formation which can induce oxidative stress (11). Second, it is known that severe psychological stress induces the proinflammatory networks of cytokines and chemokines, which in turn activate the hypothalamic-pituitary-adrenal axis (3). In PD patient, the concentrations of tumor necrosis factor, interleukins and β macroglobulin are increased in the substantia nigra (5). Therefore, the inflammatory process may also participate in the induction of vulnerability of dopaminergic neurons during CH.

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Fig. 1 Effects of confrontational housing (CH) on light/dark test (LDT). For the CH, two mice were housed in a cage with a partition (single housing) for a week, and subsequently the mice were housed without a partition for 24 h. To provide control group, three mice were housed in a cage without a partition during the experiment. The evaluation was performed at 24 h after the start of CH. In the LDT, the latency to the first entry into the lit compartment (A) and time spent in the lit compartment (B) during the 10 min testing period are shown. Values are presented as the means ± SD (n = 6). *P < 0.05 vs. control group.
Fig. 2 Effects of MPTP on DAT, TH and GFAP protein levels in the striatum of C57BL/6N mice with or without confrontational housing (CH). Mice were received 20 mg/kg MPTP or saline at 24 h after the start of CH. They were killed at 3 days after the MPTP injection, and striatal tissue samples were prepared for immunoblot analyses. Representative immunoblots of DAT, TH and actin proteins in the striatum are shown in A. Representative immunoblots of GFAP and Na⁺/K⁺-ATPase α-1 subunit proteins in the striatum are shown in D. Immunoblots of DAT (B), TH (C) and GFAP (E) in the striatum were quantified in each group. Densitometric analysis was performed to quantify relative protein level against actin for DAT and TH or Na⁺/K⁺-ATPase α-1 subunit for GFAP. The data were expressed as % of control. Values are presented as the means ± SD (n = 3 or 4). *P < 0.05 and **P < 0.01 vs. CH alone.
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