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Different susceptibility to 1-methyl-4-phenylpyridium (MPP+)-induced nigro-striatal dopaminergic cell loss between C57BL/6 and BALB/c mice is not related to the difference of monoamine oxidase-B (MAO-B)

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ABSTRACT

Subcutaneous and intraperitoneal administrations of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induce selective dopaminergic (DA-ergic) neuronal death in many animal species. After passing through the blood–brain barrier (BBB), MPTP is converted to 1-methy-4-phenylpiridinium (MPP^+) by astrocytic monoamine oxidase-B (MAO-B). MPP^+ then induces the dopaminergic neuronal death. In mice, marked strain differences in the susceptibility to MPTP-injection have been reported. To clarify which factor(s) cause the strain differences, MPTP or MPP^+ was intracerebroventricularly (icv) injected into adult C57BL/6 (highly susceptible to MPTP) and BALB/c (resistant to MPTP) mice. The brain tissues including the striatum and substantia nigra pars compacta (SNpc) were examined immunohistochemically using an antibody to tyrosine hydrocyrase (TH). MPP^+-injected C57BL/6 mice showed a significant decrease in TH-immunopositive areas in the striatum at Day 3 post injection ($p < 0.01$), and TH-positive cells in the SNpc at Days 1 and 3 ($p < 0.01$), respectively, compared to saline-injected control mice. In addition, MPP^+-injected BALB/c mice showed a significant decrease in TH-positive areas in the striatum at Days 1 and 3, and SNpc TH-positive cells in the SNpc at Day 3, respectively ($p < 0.05$). However, the decrease rates in the BALB/c mice were lower than that in C57BL/6 mice. MPTP-injected C57BL/6 mice, however, showed no lesions in the striatum and SNpc at Days 1 and 7 after icv injection. All the present findings indicate that factors other than MAO-B can influence the strain susceptibility between C57BL/6 and BALB/c mice after the conversion from MPTP to MPP^+.

1. Introduction

Several animal models have been developed to study the underlying mechanisms that lead to idiopathic Parkinson's disease (PD), including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-injected mice. MPTP induces most of the cardinal histopathological features of PD, except intraneuronal inclusions like Lewy bodies, by subcutaneous (sc) and intraperitoneal (ip) administration. MPTP is metabolized by an enzyme, monoamine oxidase B (MAO-B) to 1-methy-4-phenylpiridinium (MPP^+) in liver cells and endothelial cells of microvasculature responsible for the blood-brain barrier (BBB) (Riachi et al., 1988; Yoshihara and Ohta, 1998). Since MPP^+ cannot pass through the BBB, MPTP that had escaped from the metabolism can enter into the brain parenchyma (Riachi et al., 1990). There MPTP is converted into MPP^+ by astrocytic MAO-B, and MPP^+ enters into DA-ergic neurons with a dopamine transporter (DAT) on the plasma membrane. Inside the DA-ergic neurons, MPP^+ injures the mitochondrial respiratory complex I, causes energy depletion, and finally leads to neuronal death (Smeyne and Jackson-Lewis, 2005).

There are species differences in the susceptibility to MPTP injection; for example, human and monkeys are susceptible, whereas rats and hamsters are relatively resistant (Burns et al., 1983; Chiueh et al., 1984; Langston et al., 1984; Mitra et al., 1994). Mice are middle susceptible, but show marked strain differences (Sundstrom et al., 1987; Riachi and Harik, 1988; Hamre et al., 1999); C57BL/6 mice are susceptible, while BALB/c mice are resistant, to MPTP toxicity (Sedelis et al., 2000; Yasuda et al., 2008; Filipov et al., 2009). However, the reason for such strain differences remains unclear. To clarify factor(s) influencing the mouse strain differences, MPTP and MPP^+ were intracerebroventricularly (icv) injected into susceptible C57BL/6 and resistant BALB/c mice, and the lesions were histologically examined.

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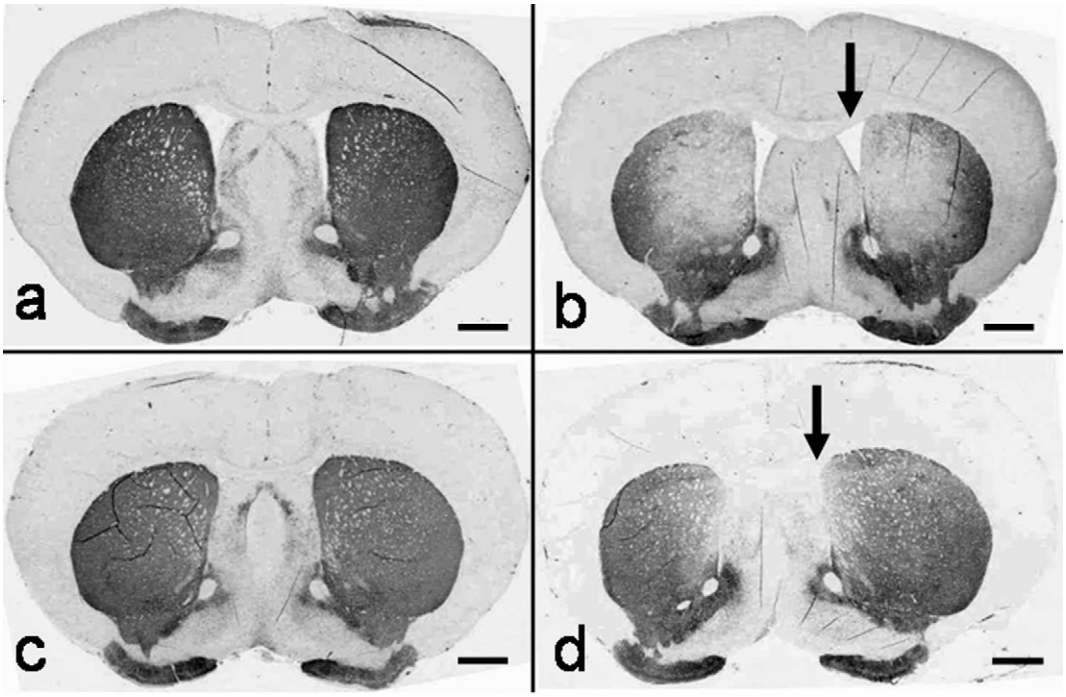

Fig. 1. TH-immunoreactivity in the striatum at Day 7 after injection. In MPP^+-treated mice, a reduction in TH-positive areas was observed at the injection (arrows) and contralateral sides of the striatum, especially adjacent to the lateral ventricles in C57BL/6 (b) and BALB/c (d) mice. However, no lesions were found in control C57BL/6 (a) and BALB/c (c) mice. EnVison method. Counterstained with methyl green. Bar = 1 mm.

2. Materials and methods

2.1. Animals

Eight-week-old male C57BL/6J and BALB/cByJ mice, weighting 21–24 g and 26–29 g, respectively, were purchased from Japan CLEA (Tokyo, Japan). The mice were housed in a room maintained under constant temperature ($23 \pm 2\,^{\circ}C$) and humidity ($55 \pm 5\%$) conditions with a 12-h light/dark cycle using an isolator caging system (Niki Shoji, Tokyo). Water and food were accessible ad libitum. All the experimental procedures used in the present study were approved by the Committee of Animal Experiments of the Graduate School of Agricultural and Life Sciences, the University of Tokyo.

2.2. Drug injection

MPP^+ iodide (Sigma, St. Luis, MO) was dissolved in saline. After being anesthetized, C57BL/6 and BALB/c mice ($n = 3$–5) were injected with 10 μl of solution containing 18 μg or 22 μg of MPP^+ iodide (0.8 mg/kg), respectively, into the unilateral ventricle. Control mice were injected with 10 μl of saline. The stereotaxic coordinates of injection site were as follows: bregma −0.5 mm, lateral 1.0 mm, depth 2.0 mm (C57BL/6) and bregma −1.0 mm, lateral 1.0 mm, depth 2.0 mm (BALB/c), respectively. The mice were sacrificed by cervical dislocation under ethyl ether anesthesia at 1, 3, and 7 days after injection (Days 1, 3 and 7). In addition, 10 μl of a solution containing 36 μg or 162 μg of MPTP-HCl (Sigma, St. Luis, MO, 1.54 mg/kg and 7.14 mg/kg respectively) or 10 μl of saline (control) were icv injected into C57BL/6 mice ($n = 3$). The mice were killed by cervical dislocation at Days 1 and 7 after injection.

2.3. Tyrosine hydroxylase (TH) immunohistochemistry

The brain samples were fixed in a 10% neutral buffered formalin solution for 4 days, processed routinely, and embedded in paraffin. Four μm-thick transverse sections encompassing the striatum and the SNpc were used for immunohistochemical examination. The primary antibody used was rabbit anti-TH (1:500, Millipore, Temecula, CA). Following the treatment with 8% skim milk at 37 °C for 40 min, sections were incubated with the primary antibody at 4 °C overnight, followed by secondary antibody reactions at 37 °C for 40 min using an EnVision Anti-rabbit conjugation system (Dako, Carpenteria, CA). Finally, the positive reaction was visualized with 0.05% 3,3′-diaminobenzidine (DAB) and 0.03% hydrogen peroxide in a Tris–HCl buffer, and the sections were counterstained with methyl green. TH-positive areas in the striatum were measured with an image analyzing program, NIH Image-J. In addition, TH-positive neurons in the SNpc were counted as previously described (Muthane et al., 1994). The boundary between the SNpc and ventral tegmental area was defined with the aid of the mouse brain atlas (Franklin and Paxinos, 2007). The number of TH-positive neurons on each representative mesencephalic section was counted under a magnification of 200×. The mean number of total TH-positive neurons was calculated for each mouse. Statistical significance was evaluated using the Student's *t*-test.

3. Results

3.1. Different susceptibility to MPP^+ in C57BL/6 and BALB/c mice

After MP^+ icv injection, the lesions were found mainly adjacent to the lateral ventricles in the striatum and in the ventral cells of the SNpc in both C57BL/6 and BALB/c mice (Figs. 1 and 2). In C57BL/6 mice, TH-positive areas in the striatum were significantly decreased at Days 3 and 7 after MPP^+ injection, whereas TH-positive cells in the SNpc were significantly decreased at Days 1, 3 and 7 (Fig. 3). The results suggest that the MPP^+ first affects specifically DA-ergic cells in the SNpc and that the lesion then spread to the striatum. In BALB/c mice, both TH-positive areas in the striatum and the number of TH-positive cells in the SNpc also decreased significantly at Days 1 and 3, and at Day 3, respectively (Fig. 3). Using one-way analysis of variance (ANOVA) between MPP^+-injected C57BL/6 and