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Research Report

Impaired *in vivo* dopamine release in *parkin* knockout mice

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ABSTRACT

parkin is the most frequent causative gene among familial Parkinson's disease (PD). Although *parkin* deficiency induces autosomal recessive juvenile parkinsonism (AR-JP, PARK2) in humans, *parkin* knockout (PKO) mice consistently show few signs of dopaminergic degeneration. We aimed to directly measure evoked extracellular dopamine (DA) overflow in the striatum with *in vivo* voltammetry. The amplitude of evoked DA overflow was low in PKO mice. The half-life time of evoked DA overflow was long in PKO mice suggesting lower release and uptake of dopamine. Facilitation of DA overflow by repetitive stimulation enhanced in the older PKO mice. Decreased dopamine release and uptake in young PKO mice suggest early pre-symptomatic changes in dopamine neurotransmission, while the enhanced facilitation in the older PKO mice may reflect a compensatory adaptation in dopamine function during the late pre-symptomatic phase of Parkinson's disease. Our results showed *parkin* deficiency may affect DA release in PKO mice, although it does not cause massive nigral degeneration or parkinsonian symptoms as in humans.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. The main pathology in PD is the loss of dopaminergic neurons in the substantia nigra pars compacta. Although most PD cases are sporadic, to date, 16 subtypes of familial forms of parkinsonism and 8 causative genes have been identified (Mizuno et al., 2006; Satake et al., 2009; Thomas and Beal, 2007; Vila and Przedborski, 2004). Recent evidence suggests that the interaction between genetic and environmental factors could contribute to the pathogenesis of PD (Hattori and Mizuno, 2004). Thus, the pathogenesis of familial forms of PD foreshadows a common pathway of nigral degeneration in not only various types of familial forms of PD but also the sporadic form. *parkin* is the gene responsible for PARK2, an autosomal recessive type of a familial form of parkinsonism (Kitada et al., 1998). This form is the most frequent among young-onset PD, and is

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Abbreviations: AR-JP, autosomal recessive early-onset parkinsonism; DA, dopamine; MFB, medial forebrain bundle; PKO, *parkin* knockout; WT, wild type

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characterized by L-DOPA responsive dystonia and parkinsonism (Hattori and Mizuno, 2004).

parkin deficiency is expected to be associated with loss-of-function effect based on the recessive mode of inheritance. However, the *parkin* knockout (PKO) mouse lacks extensive nigral degeneration or marked parkinsonism-like motor deficits (Goldberg et al., 2003; Itier et al., 2003; Perez and Palmiter, 2005; Sato et al., 2006; Von Coelln et al., 2004). On the other hand, these mice show mild changes in extracellular dopamine (DA) (Goldberg et al., 2003; Itier et al., 2003) and upregulation of DA receptor binding in the striatum (Sato et al., 2006). Other studies also reported non-motor manifestations of PD (Zhu et al., 2007) and certain cognitive changes in PKO mice (Goldberg et al., 2003; Itier et al., 2003; Zhu et al., 2007).

In spite of previous failures detecting PD-like changes in PKO mice in vivo, physiological dysfunction of dopamine neurotransmission remains to be evaluated. In mouse species, lack of motor deficit does not suggest intact dopaminergic functions (Jackson-Lewis and Przedborski, 2007). In this study, we aimed to measure evoked extracellular DA overflow in the striatum in vivo by a high-speed electrochemical technique using carbon-fiber microelectrodes (Nakazato and Akiyama, 1999; Natori et al., 2009; Suzuki et al., 2007; Yavich et al., 2004).

2. Results

2.1. *General growth and behavioral tests of PKO mice*

In agreement with previous studies (Goldberg et al., 2003; Itier et al., 2003; Perez and Palmiter, 2005; Sato et al., 2006; Von Coelln et al., 2004), PKO mice developed normally except for their smaller body weight (WT; 34.3±1.0 g, PKO; 30.0±0.7 g), the main effects of gene and age were significant ($F_{(1,62)}=16.3$, $P<0.001$; $F_{(3,62)}=23.3$, $P<0.001$), but the general behavior seemed to be normal.

The pole and accelerating rotarod tests were used to evaluate motor function prior to voltammetry tests. In the pole test, neither age nor genotype had significant effects on the time for turning on the pole (T_{Turn}) ($F_{(3,55)}=0.32$, $P=0.821$; $F_{(1,62)}=2.38$, $P=0.149$, Fig. 1A). With regard to the time required for landing (T_{Land}), the main effect of age was significant ($F_{(3,55)}=12.74$, $P<0.001$) but the effect of genotype was not ($F_{(1,62)}=2.174$, $P=0.146$, Fig. 1B). The effect of age on the time for landing could be due to the difference in body weight, because heavier mice went down the pole faster (correlation between T_{Land} and bodyweight, $r=-0.49$, $P<0.01$). In the accelerating rotarod test, the main effect of age on the falling latency was significant ($F_{(3,55)}=9.54$, $P<0.001$, Fig. 1C), but the main effect of genotype was not ($F_{(1,62}=0.36$, $P=0.55$). The above results are consistent with previous studies (Goldberg et al., 2003; Itier et al., 2003; Perez and Palmiter, 2005; Sato et al., 2006; Von Coelln et al., 2004) and indicate that the genotype does not significantly affect general motor function in PKO mice.

2.2. *Evoked DA overflow*

During the 2-s stimulation of the nigrostriatal fibers in the medial forebrain bundle (MFB), a fast and short-lasting increase in electrochemical current was observed (Fig. 2A). The maximum amplitude of the evoked dopamine overflow ($\Delta[DA]_{max}$) was lower in PKO than in WT (Fig. 2C). The main genotype effect was significant ($F_{(1,62)}=11.4$, $P=0.0016$), but that of age was not ($F_{(3,55)}=0.843$, $P=0.48$). Administration of nomifensine, a competitive DA uptake inhibitor, increased the maximum amplitude (Fig. 2B,D). The genotype effect was again significant after nomifensine ($F_{(1,59)}=18.23$, $P<0.001$), but that of age was not ($F_{(3,52)}=0.52$, $P=0.67$). There were significant differences ($P<0.05$) in $\Delta[DA]_{max}$ before nomifensine between WT and PKO at 3 and 6 months of age (Fig. 2C), and in $\Delta[DA]_{max}$ after nomifensine at 3, 6 and 9 months of age (Fig. 2D). However, there were no differences either before or after nomifensine at 12 months of age. In the half-life time ($T_{1/2}$) of the evoked DA overflow, the main genotype effect was significant before nomifensine ($F_{(1,62)}=6.89$, $P=0.01$) but that of age was not ($F_{(3,55)}=0.634$, $P=0.597$) (Fig. 2E). The administration of nomifensine significantly increased $T_{1/2}$ both in WT

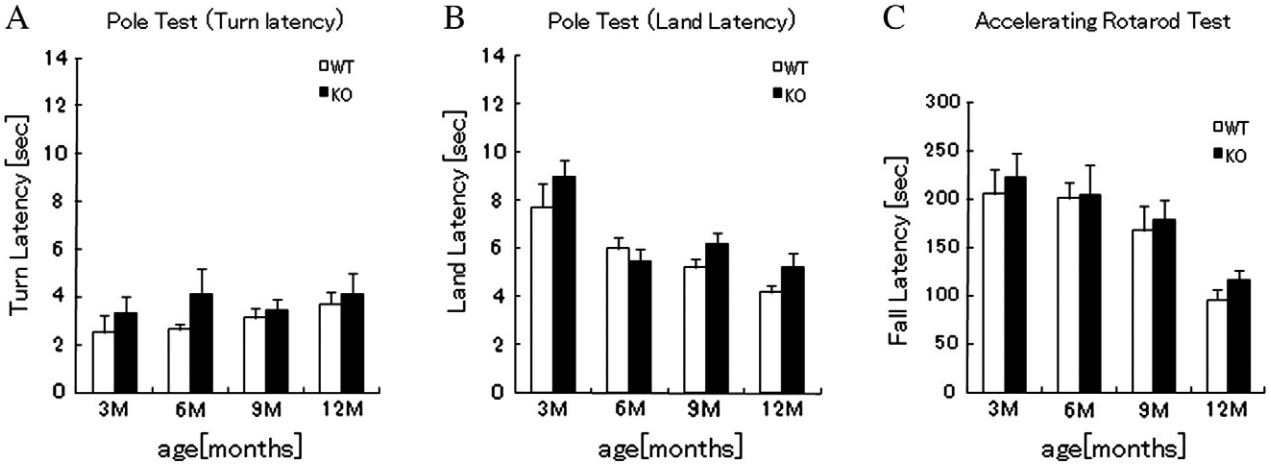

Fig. 1 – Behavioral tests performed before voltammetric measurement. (A) The latency before the mouse turned toward the ground (T_{Turn}) and (B) the time from turn to landing (T_{Land}) in the pole test. (C) Accelerating rotarod test (3-cm diameter), in which the rod accelerated from 3 to 35 rpm over 5 min. Data are mean±SEM of 8 to 10 mice.