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STRIATAL TYROSINE HYDROXYLASE-POSITIVE NEURONS ARE ASSOCIATED WITH L-DOPA-INDUCED DYSKINESIA IN HEMIPARKINSONIAN MICE

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Abstract—L-3,4-Dihydroxyphenylalanine (L-DOPA) is the therapeutic gold standard in Parkinson's disease. However, long-term treatment is complicated by the induction of debilitating abnormal involuntary movements termed L-DOPA-induced dyskinesias (LIDs). Until today the underlying mechanisms of LID pathogenesis are not fully understood. The aim of this study was to reveal new factors, which may be involved in the induction of LID. We have focused on the expression of striatal tyrosine hydroxylase-positive (TH+) neurons, which are capable of producing either L-DOPA or dopamine (DA) in target areas of ventral midbrain DAergic neurons. To address this issue, a daily L-DOPA dose was administered over the course of 15 days to mice with unilateral 6-hydroxydopamine-induced lesions of the medial forebrain bundle and LIDs were evaluated. Remarkably, the number of striatal TH+ neurons strongly correlated with both induction and severity of LID as well as ΔFosB expression as an established molecular marker for LID. Furthermore, dyskinetic mice showed a marked augmentation of serotonergic fiber innervation in the striatum, enabling the decarboxylation of L-DOPA to DA. Axial, limb and orolingual dyskinesias were predominantly associated with TH+ neurons in the lateral striatum, whereas medially located TH+ neurons triggered locomotive rotations. In contrast, identified accumbal and cortical TH+ cells did not contribute to the generation of LID. Thus, striatal TH+ cells and serotonergic terminals may cooperatively synthesize DA and subsequently contribute to supraphysiological synaptic DA concentrations, an accepted cause in LID pathogenesis.

Key words: Parkinson's disease, dopamine, abnormal involuntary movement, striatum, accumbens, TH.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder and clinically characterized by akinesia, rigidity and resting tremor. These motor symptoms are related to reduced dopamine (DA) levels in the striatum (Bernheimer et al., 1973), resulting from a progressive loss of terminals of degenerating neuromelanin-containing DAergic neurons in the substantia nigra pars compacta (SNpc) (Hirsch et al., 1988). Since its discovery in the 1960s, DA replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA) has remained the gold standard symptomatic treatment for PD (Birkmayer and Hornykiewics, 1961; Smith et al., 2012). Unfortunately, its chronic administration leads to debilitating abnormal involuntary movements (AIMs) termed L-DOPA-induced dyskinesia (LID) that occur in nearly 90% of PD patients within a decade (Ahlskog and Muenter, 2001).

Several pre- and postsynaptic mechanisms are supposed to contribute to LID development and expression. As a pivotal factor in pathogenesis, fluctuations in central DA levels cause aberrant plasticity in DAergically innervated brain structures, with the striatum playing a crucial role among them (de la Fuente-Fernández et al., 2004; Lindgren et al., 2010; Feyder et al., 2011; Huang et al., 2011). Interestingly, recent evidence has implicated raphe-striatal serotonergic neurons as an additional source of striatal DA following peripheral administration of L-DOPA, as they contain the requisite transport and enzymatic machinery to take up, convert and release L-DOPA-derived DA (Arai et al., 1995; Tanaka et al., 1999; Maeda et al., 2005). Unfortunately, these neurons lack DA transporters and D2 autoreceptors and are thus incapable of regulating

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Abbreviations: 6-OHDA, 6-hydroxydopamine; AADC, aromatic acid decarboxylase; AIMs, abnormal involuntary movements; DA, dopamine; L-DOPA, L-3,4-dihydroxyphenylalanine; LID, L-DOPA-induced dyskinesia; MFB, medial forebrain bundle; NHS, normal horse serum; OD, optical density; PD, Parkinson's disease; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH+, TH-positive; ΔFosB, delta isoform of oncogene FosB.

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the exaggerated DA efflux that triggers LID (Carta et al., 2007; Eskow et al., 2009; Navailles et al., 2010).

In addition to the "classical" catecholaminergic brainstem nuclei synthesizing DA, norepinephrine or epinephrine as neurotransmitters, neurons partially expressing individual enzymes of DA biosynthesis, namely tyrosine hydroxylase (TH) and/or aromatic acid decarboxylase (AADC), are found in different areas of the central nervous system (Weihe et al., 2006; Ugrumov, 2013). TH-positive (TH+) neurons exist widely spread throughout the brain and are reactive to DAergic perturbations. Following DAergic denervation in PD patients (Porritt et al., 2000) and in corresponding animal models (Tashiro et al., 1989; Betarbet et al., 1997; Meredith et al., 1999; Lopez-Real et al., 2003) there was a compensatory increase in TH+ neurons in the striatum. This increment does not result from neurogenesis of TH+ cells but from a phenotypic shift of pre-existing GABAergic intrastriatal neurons (Tandé et al., 2006; Darmopil et al., 2008). Additionally, stereotactic injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) of mice lead to an increment of TH+ cell numbers and fiber sprouting in target areas (Depboylu, 2014; Depboylu et al., 2014). Thus, they might provide an additional source of L-DOPA and possible DA in these areas which may aggravate dyskinesia. Here, we aim to elucidate a possible functional involvement of these striatal, accumbal and cortical TH+ neurons in LID pathogenesis in mice.

EXPERIMENTAL PROCEDURES

Animals

50 male wildtype C57Bl/6 mice (10–12 weeks old, weighing 25–30 g) were obtained from Charles River (Sulzfeld, Germany) and housed at the animal facility of the Biomedical Research Centre at Philipps University Marburg under standardized conditions with 12:12-h light/dark cycle, room temperature 23 ± 1 °C and *ad libitum* access to food and water. All animal experiments were performed according to the EU Council Directive 2010/63/EU and approved by the local animal care committee (Regierungspräsidium Giessen, Germany).

Experimental design

The experimental design is outlined in Fig. 1. Initially, all animals received a unilateral 6-OHDA lesion of the MFB in order to achieve a complete depletion of the nigrostriatal DAergic pathway. After three weeks the mice were screened using cylinder test and amphetamine-induced rotation test and the animals with the severest lesion were selected for the experiment. Inclusion criteria were a forelimb use asymmetry showing < 20% left forelimb touches of total, as well as amphetamine-induced exhibition of > 3.5 net full body turns per minute ipsilateral to the lesioned side. Six weeks post-lesion animals were treated daily with L-DOPA in combination with the DOPA decarboxylase inhibitor benserazide hydrochloride for 15 days to induce stable AIMs. Mice receiving daily injections of saline served as a control group. AIMs were evaluated at days 1, 7 and 15 of the treatment period. 48 h after the last L-DOPA dose animals were sacrificed and the brains were processed for immunohistochemistry.

6-OHDA lesion

Stereotactic injections were conducted under general anesthesia induced by intraperitoneal (i.p.) injection of 8 ml/kg ketamine (2%; Pfizer, Karlsruhe, Germany) with 2 ml/kg xylazine (Bayer Healthcare, Leverkusen, Germany). At 30 min before cerebral injections of 6-OHDA, the mice received an i.p. injection of 25 mg/kg desipramine (Sigma-Aldrich, Munich, Germany) to protect norepinephrinergic neurons and fibers. To induce a severe DAergic lesion, 2-µl 6-OHDA (Sigma-Aldrich; 2 µg/µl in 0.9% NaCl with 0.2% ascorbic acid) was injected into the MFB at following coordinates: anterior–posterior −0.82 mm and medio-lateral −1 mm from bregma, −4.8 mm ventral to the dura. All injections were made at a rate of 200 nl/min, and the needle (33 gauge; Hamilton, Bonaduz, Switzerland) was kept in place for additional 5 min before retracted. Stereological coordinates were determined according to the mouse atlas of Paxinos and Franklin (2001). To minimize the post-operative mortality of 80% reported in mice (Lundblad et al., 2002), an intensive care was performed for two weeks following lesion by daily subcutaneous (s.c.) injections of 1–2 ml 0.9% NaCl against dehydration and by soaking the food pellets in water to allow eating for weak mice. This preventive protocol led to a survival rate of 76%.

Behavioral analysis

Cylinder test. To evaluate the extension of the lesion, at three weeks after the 6-OHDA lesion spontaneous forelimb use was evaluated in the cylinder test according to the previously described test paradigm for rats (Schallert and Tillerson, 1999; Carlsson et al., 2007). The mice were placed individually in a glass cylinder (10.5-cm diameter, 14-cm height) and video recorded while performing 20 weight-shifting movements of the forepaws in contact with the cylinder wall. The numbers of the left or right forepaw contacts were scored by an observer blinded to the animals' identity and presented as left (impaired) touches in percentage of total touches. Normal mice will score 50% in this test.

Amphetamine-induced rotation test. At 3 weeks after the 6-OHDA lesion rotational behavior induced by amphetamine (5 mg/kg, i.p.; Sigma-Aldrich) was observed in an open-field arena with an area of 52 cm × 52 cm. The testing sessions were performed and video recorded over 30 min and the animals' right and left full body turns were checked by an experimentally blinded investigator. The data were expressed as net full body turns per minute, with negative values indicating rotation contralateral to the lesion side.