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Research report

Cardiovascular dysfunction associated with neurodegeneration in an experimental model of Parkinson's disease

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ABSTRACT

Patients with Parkinson's disease (PD) exhibit both motor and non-motor symptoms. Among the non-motor symptoms, cardiovascular autonomic dysfunction is frequently observed. Here, we evaluated baroreflex function, vascular reactivity and neuroanatomical changes in brainstem regions involved in the neural control of circulation in the 6-hydroxydopamine (6-OHDA) model of PD. Male Wistar rats received a bilateral injection of 6-OHDA or vehicle into the striatum. After 61 days, baroreflex function and vascular reactivity were assessed. The 6-OHDA and vehicle groups showed similar increases in mean arterial pressure (MAP) in response to phenylephrine (PE). However, the bradycardia observed in the vehicle group was blunted in the 6-OHDA-treated rats. Injection of sodium nitroprusside (SNP) decreased hypotension, tachycardia and vascular relaxation in 6-OHDA-treated rats. Bilateral intrastriatal 6-OHDA led to massive degeneration of tyrosine hydroxylase (TH)-immunoreactive neurons in the substantia nigra and to reductions in the numbers of A1/C1 and A5 catecholaminergic neurons while sparing A2 neurons within the nucleus of the solitary tract (NTS). 6-OHDA-treated rats also showed decreases in Phox2b-expressing neurons in the NTS and in choline acetyltransferase (ChAT) immunoreactivity in the nucleus ambiguus. Altogether, our data suggest that this model of PD includes neuroanatomical and functional changes that lead to cardiovascular impairment.

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. Clinically, it is well known by its motor symptoms such as bradykinesia, rigidity, tremor and postural instability. However, while the motor symptoms of PD are considered pathological hallmarks of the disease (Fearnley and Lees, 1991), several debilitating symptoms that substantially impair patients' quality of life are related to the non-motor aspects of PD (Wolters, 2009). Some common non-motor symptoms of PD include sleep disturbances, neuropsychiatric and cognitive deficits, sensory dysfunction, and breathing instability, as well as cardiovascular autonomic dysfunction (Bassetti, 2011; Chaudhuri et al., 2011; Dickson et al., 2009; Truong et al., 2008; Tuppy et al., 2015). There is no doubt that the motor symptoms of PD are associated with the loss of a specific group of dopaminergic neurons located in the substantia nigra (SN) and that this underlies the physiopathology of the disease; however, the specific populations of neurons responsible for various non-motor symptoms remain unclear. Orthostatic hypotension (OH), the most common cardiovascular dysfunction in PD, results from the impairment of baroreflex function and cardiac sympathetic innervations (Cai et al., 2005; Tipre and Goldstein, 2005). Baroreflex dysfunction can also be associated with neurodegeneration in important regions of the brainstem. Previous reports show that the brainstems of patients with PD show considerable loss of an important adrenergic region involved in the neural control of circulation, i.e., the C1 region (Gai et al., 1993; Guyenet et al., 2013). However, according to other studies, patients with PD and OH showed marked individual variations in the numbers of catecholaminergic neurons in the C1 region, obscuring the correlations among OH, PD and catecholaminergic neurons (Benarroch et al., 2000). Despite the controversial observations in patients with PD as described above, no previous studies have reported cardiovascular autonomic dysfunc-

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tion in a rat model of PD and its relationship with neurodegeneration in specific areas of the brainstem that are responsible for the neural control of blood pressure (Kuo et al., 2010; Lu et al., 1995; Takatsu et al., 2000). Here, we selected a widely used rat model of PD that is generated by injection of 6-hydroxydopamine (6-OHDA) into the striatum. The neuroanatomical and functional assessment of cardiovascular involvement in the 6-OHDA model of PD may represent an important step for future clarification of the mechanisms underlying the appearance of cardiovascular autonomic dysfunction in PD.

Therefore, it is important to use the 6-OHDA model of PD to evaluate cardiovascular dysfunction, vascular reactivity and neuroanatomical changes in the brainstem regions involved in the neural control of circulation.

2. Results

2.1. Animal model of Parkinson's disease: bilateral intrastriatal injection of 6-OHDA destroyed tyrosine hydroxylase-expressing neurons of the substantia nigra

The 6-OHDA neurotoxic lesion within the nigrostriatal dopaminergic system is one of the most widely used methods to model PD in rodents (McDowell and Chesselet, 2012). In our study, 6-OHDA (24 μg/μL) was injected into the dorsal striatum of rats. The rostral-caudal extent of the lesion was determined by counting the neurons in the SN showing tyrosine hydroxylase immunoreactivity (TH-ir) in every sixth 40-μm brain section from each rat (from 5.32 to 6.04 mm caudal to bregma). Compared to vehicle-injected control rats, 6-OHDA dramatically reduced the number of neurons showing TH-ir in the SN (125 ± 10, vs. vehicle: 907 ± 11 neurons, $p < 0.001$) (Fig. 1A, B and I).

2.2. Intrastriatal injection of 6-OHDA selectively reduces the number of brainstem neurons involved in cardiovascular function

To further assess the effects of bilateral intrastriatal 6-OHDA on neurons involved in blood pressure control, at 61 days after the injections, TH-ir, choline acetyltransferase immunoreactivity (ChAT-ir) and Phox2b immunoreactivity (Phox2b-ir) were examined in the regions A1/C1, A2/C2 and A5, in the dorsal motor nucleus of the vagus nerve (DMV) and in the nucleus ambiguus (NA), as well as in the intermediate and commissural regions of the nucleus of the solitary tract (NTSint and NTSc, respectively). In all regions analyzed, except for the A2/C2 and DMV, significant reductions in immunoreactive neurons were observed. For example, the numbers of neurons with TH-ir that were counted in the A5 and A1/C1 regions were reduced by 60% and 70%, respectively, after 6-OHDA (24 μg) was injected into the striatum (A5: 43 ± 1, vs. vehicle: 107 ± 5 neurons and A1/C1: 123 ± 14, vs. vehicle: 404 ± 2 neurons, $p < 0.001$) (Fig. 1C–F and I). The numbers of cells showing ChAT-ir in the NA were decreased by 30% (191 ± 20, vs. vehicle: 269 ± 13 neurons, $p < 0.05$) (Fig. 2C–E). Nuclei in the NTSint and NTSc with Phox2b-ir were reduced by 48% and 73%, respectively (Fig. 3A–E). Interestingly, the area of the NTS with reduced Phox2b-ir was the site of the first synapse of viscerosensory afferents in the brainstem, including those related to cardiorespiratory afferents (Ergene et al., 1994; Kang et al., 2007; Paton et al., 2001; Takakura et al., 2006). The Phox2b-expressing neurons in the NTS are presumably glutamatergic; they innervate the ventrolateral medulla and are collectively responsible for both the cardiovagal and the sympathetic components of the baroreflex (Chan and Sawchenko, 1994; Kang et al., 2007; Weston et al., 2003) (Fig. 3A–E). We also analyzed TH-ir in A2/C2 and ChAT-ir in the DMV; compared to control rats, intrastriatal 6-OHDA did not change the numbers of neurons showing TH-ir in A2/C2 or showing ChAT-ir in the DMV (Figs. 1G–I, 2A, B and E).

2.3. Intrastriatal injection of 6-OHDA impaired cardiac baroreflex

Analysis of the relationship between mean arterial pressure (MAP) and heart rate (HR) revealed a change in the sensitivity of the cardiac baroreflex in the 6-OHDA model of PD. The baroreflex (elicited by intravenous (IV) injections of either phenylephrine (PE) or sodium nitroprusside (SNP)) was tested 61 days after intrastriatal injection of 6-OHDA (24 μg/μL).

Baseline MAP and HR did not differ between 6-OHDA-lesioned and control rats (113 ± 1.25 vs. vehicle: 110 ± 0.7 mmHg and 328 ± 2.17 vs. vehicle: 333 ± 1.54 bpm, $p > 0.05$). In control animals, injections of PE (0.1–12.8 μg/kg) produced dose-dependent increases and decreases in MAP and HR, respectively, and injections of SNP (6.4 and 51.2 μg/kg) produced dose-dependent decreases and increases in MAP and HR, respectively. For example, PE increased MAP (0.2 μg/kg: Δ = 5.5 ± 1; 1.6 μg/kg: 27.5 ± 1.5; 25.6 μg/kg: 58.8 ± 0.9 mmHg, $p < 0.001$) (Fig. 4A and C) and decreased HR (0.2 μg/kg: Δ = −10.8 ± 0.6; 1.6 μg/kg: −34.5 ± 0.7; 25.6 μg/kg: −148.5 ± 9.9 bpm, $p < 0.001$) (Fig. 4A and D). In contrast, SNP decreased MAP (0.2 μg/kg: Δ = −4.5 ± 0.9; 1.6 μg/kg: −21.8 ± 1; 51.2 μg/kg: −43 ± 4 mmHg, $p < 0.001$) (Fig. 4B and E) and increased HR (0.2 μg/kg: Δ = 14 ± 8; 1.6 μg/kg: 41 ± 14; 51.2 μg/kg: 121.3 ± 15.2 bpm, $p < 0.001$) (Fig. 4B and F). In the 6-OHDA group, dose-dependent effects were observed only for the effects of PE on the MAP. Specifically, PE increased MAP (0.2 μg/kg: Δ = 12 ± 2.7; 6.4 μg/kg: 59.3 ± 8.5; 25.6 μg/kg: 75.5 ± 2.9 mmHg, $p < 0.001$) (Fig. 4A and C). These same doses were not able to decrease HR in a dose-dependent manner (0.2 μg/kg: Δ = −11.5 ± 4.9; 6.4 μg/kg: −45.3 ± 5.1; 25.6 μg/kg: −62 ± 6.1 bpm, $p > 0.05$) (Fig. 4A and D).

We observed similar increases in MAP in the 6-OHDA and vehicle groups (Δ = 7 ± 2.6 to 66.5 ± 6.1, vs. vehicle: 7.3 ± 0.5 to 60.3 ± 1.6 mmHg, $p > 0.05$; Fig. 4A and C). The highest dose of PE (25.6 μg/kg) produced a higher increase in MAP in 6-OHDA compared to the vehicle group (Δ = 75.5 ± 2.9, vs. vehicle: 58.8 ± 0.9 mmHg, $p < 0.001$). However, bradycardia was blunted in 6-OHDA-lesioned rats with the three highest doses of PE (Δ = −45.5 ± 5 to −62 ± 6.0, vs. vehicle: −119.7 ± 10 to −148.5 ± 9.8 bpm, $p < 0.001$; Fig. 4A and D).

In 6-OHDA-lesioned rats, IV injection of SNP (6.4 and 51.2 μg/kg) produced a smaller decrease in MAP (Δ = −18.5 ± 3 and −24.3 ± 0.8 vs. vehicle: −30.5 ± 1.9 to −43.5 ± 3.9 mmHg, $p < 0.01$; Fig. 4B and E), and a smaller increase in HR was observed in response to doses of 1.6–51.2 μg/kg (Δ = 15.8 ± 5 to 49.5 ± 13.6 vs. vehicle: 41 ± 14 to 121.3 ± 15.2 bpm, $p < 0.05$; Fig. 4B and F).

Fig. 5A-B show the baroreflex sensitivity in response to PE and SNP injections based on analysis of the slope of the curves. Reflex bradycardia in response to the PE-induced increase in MAP was reduced in the 6-OHDA group (−0.794 ± 0.159, vs. vehicle: −2.053 ± 0.307 bpm/mmHg, $p < 0.05$), whereas the reflex tachycardia in response to the SNP-induced fall in MAP was similar between the 6-OHDA and control groups (−2.37 ± 0.099, vs. vehicle: −3.034 ± 0.655 bpm/mmHg, $p > 0.05$) (Fig. 5A-B).

2.4. Intrastriatal injection of 6-OHDA does not change the vasoconstrictor effect of PE in denuded mesenteric arteries of rats

PE (10 nmol/L to 0.1 mmol/L) induced a concentration-dependent constrictor effect in resistance mesenteric arteries in both the control and 6-OHDA-injected groups (Fig. 6A). The efficacy of PE was similar between denuded (Fig. 6A) mesenteric arteries (E−) of the 6-OHDA and control groups (Maximum effect (ME): 21.63 ± 3.97 vs. vehicle: 20.30 ± 3.18%, N = 5 artery rings/5 ani-