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Research paper

Chemoreflex and baroreflex alterations in Parkinsonism induced by 6-OHDA in unanesthetized rats

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HIGHLIGHTS

- Chemoreflex is up-regulated in 6-OHDA injured rats.
- Baroreflex is altered in unanesthetized rats submitted to Parkinsonism.
- Supersensitivity denervation may explain cardiovascular reflexes alterations in Parkinsonism.

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ABSTRACT

Parkinson's disease (PD) is mainly characterized by motor signals. However, non-motor signals also affect and decrease the quality of life of PD patients. Among these non-motor signs are cardiovascular disorders as orthostatic hypotension, postprandial hypotension and cardiac arrhythmias, which may be due to the involvement of both central nervous system and peripheral autonomic nervous system. In the present study we investigated the cardiovascular function, evaluating cardiovascular reflexes (chemoreflex and baroreflex), in an animal model of Parkinsonism induced by bilateral infusion of the toxin 6-hydroxydopamine (6-OHDA), in the substantia nigra pars compacta (SNpc). The results showed that the animals induced to Parkinsonism had lower arterial pressure (AP) and heart rate HR) compared to control animals. We showed that after activation of the baroreceptors by phenylephrine (Phe) and sodium nitroprusside (SNP), the baroreflex sensitivity index was not changed between the groups. However, there was a greater increase in the AP when stimulated with Phe and greater tachycardia when stimulated with SNP in 6-OHDA animals. After activation of the peripheral chemoreceptors through KCN injection (cytotoxic hypoxia), there was a higher increase in pressor and bradycardic response in injured animals with bilateral 6-OHDA. These changes in the cardiovascular reflexes may be important adjustments mechanisms to maintain the cerebral blood flow in those animals, and may be a result of denervation supersensitivity to catecholamines in autonomic targets.

1. Introduction

In Parkinson's disease (PD), clinically characterized by motor symptoms such as bradykinesia, rigidity, tremor and postural instability [24], it is also observed non-motor symptoms that impair significantly quality of life of patients [5,19]. Among these symptoms, cardiovascular disorders such as orthostatic hypotension (OH), postprandial hypotension, cardiac arrhythmias, *livedo reticularis* and edema position dependent members, as disturbances in pulmonary ventilation may be present in PD patients [4,20,31,32]. It may involve changes in the central nervous system (CNS), mainly characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compact (SNpc) associated with cytoplasmic inclusions termed Lewy bodies [24]; as in peripheral pathways of the autonomic nervous system (ANS) [1,10,14].

The activity of ANS, that allows the organism to adjust the circulation and ventilation to maintain the oxygen supply to the tissues, seems to be altered in PD [13]. Recent data showed that animals induced to Parkinsonism with bilateral 6-hydroxydopamine (6-OHDA) injury in the substantia nigra pars compact (SNpc) presented lower blood pressure than their controls. Analysis

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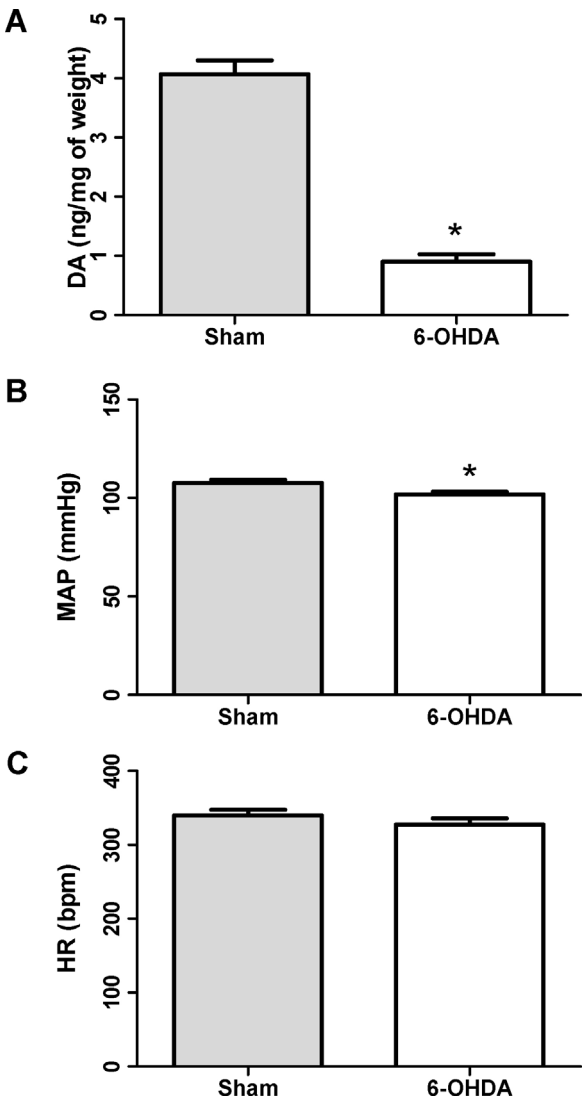

Fig. 1. (A) Dopamine concentrations in the striatum of rats after 7 days of saline (sham) or 6-OHDA microinfusion in the substancia nigra. (B) and (C) Analysis of cardiovascular parameters at baseline, mean arterial pressure (MAP) and heart rate (HR), in non-anesthetized rats after 7 days of bilateral intranigral infusion of saline (sham) or 6-OHDA. The values are expressed as mean ± SEM (n = 16/group, $^*p < 0.05$).

of blood pressure variability also showed lower sympathetic modulation. Thus, it is possible that these changes also commit reflex functions for maintaining blood flow [3].

Previous data have shown that the baroreflex is impaired in patients with PD [20]. However, no alterations in baroreflex sensitivity have been also observed in those patients [17]. Data of our group showed that 6-OHDA-lesioned rats presented increases in both the up and down gain of spontaneous baroreflex [3]; also the phasic alterations during head up tilt on cardiovascular variables seems to be detrimental [27], however, the baroreflex sensitivity using pharmacological stimulus in 6-OHDA model of Parkinsonism was not published yet.

The literature also showed that PD patients present disturbances in breathing pattern and pulmonary ventilation [26]. Data from animal studies demonstrate a differential modulation of the phrenic and hypoglossal neural output with increased chemical drive when dopaminergic pathways were impaired by 6-OHDA, suggesting that such a mechanism may contribute to respiratory insufficiency in Parkinson's disease [4]. On the other hand, the chemosensitivity to hypoxia has not been evaluated in Parkinsonism.

Changes in cardiovascular reflexes in Parkinsonism have been discussed by some authors [13,17], however, there is still no absolute conclusion on the functioning of peripheral sensors after the central involvement caused by the pathology, mainly on cardiovascular responses after hypoxia. Also, no study evaluated the chemoreflex changes in 6-OHDA model of Parkinsonism. We utilized the bilateral infusion of 6-OHDA, a method already used by our group and others to induce Parkinsonism [3,11,27,28,30].

2. Material and methods

2.1. Animals

Adult male Wistar rats were used (n = 32, 16 controls and 16 6-OHDA treated rats), weighing between 280 and 320 g, from the Central Animal care facility at the State University of Londrina. The animals were housed individually in Perspex cages in a room with a 12:12-h light/dark cycle. Food and water were freely available at all times, except during the experiments. All the experimental protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals and the Ethical Principles for Animal Experimentation established by the Brazilian Committee for Animal Experimentation (COBEA). This study was approved by the Ethics Committee on Animal Use of the State University of Londrina (Process number: 1288.2012.55; date: 3/ 29/2012).

2.2. Stereotaxic surgery

2.2.1. Lesion of SNpc with 6-OHDA

The animals were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg) and placed in a stereotaxic apparatus (David Kopf, model 957L) for bilateral 6-OHDA microinfusion (6-OHDA—6 mg/mL; Sigma Chemical Co., USA, dissolved in sterile saline supplemented with 2% ascorbic acid) in the SNpc, using the following stereotaxic coordinates determined from bregma: —5.0 mm from the bregma; latero–lateral (LL) ±2.1 mm from the midline; dorsoventral (DV),—8.0 mm from the skull according to Paxinos and Watson (1986). Then, perforations were made in the skulls of the animals with a drill low speed, allowing microinfusion the neurotoxin directly into SNpc. The microinfusion was carried out with the aid of a needle (30 gauges) attached to a polyethylene tube adapted to a 10 mL microsyringe (Hamilton, USA), which in turn was embedded in an infusion pump (Harvard Apparatus, USA). After the infusion (3 min), the needle remained in place for 2 min to prevent reflux. The false group operated (sham) underwent the same surgical procedure but only the vehicle was infused [2]. As a prophylactic measure, after surgery the animals receive 80,000 IU of Veterinary pentabiotic (Fontoura-Wyeth, Brazil) applied in 0.2 mL (im).

2.3. Femoral artery and vein catheterization

On the 6th day after surgery, the animals were submitted to a new surgery under anesthesia (ketamine hydrochloride—100 mg/kg and xylazine hydrochloride—20 mg/kg) for chronic femoral artery (for recording cardiovascular parameters) and vein (for infusion drugs) catheterization. Catheters were made of polyethylene PE-segments 10 (4–5 cm) welded PE-50 polyethylene segments (12–13 cm) (Clay Adams, USA), previously filled with saline and an anticoagulant (15 U/ml heparin in saline) and blocked with metal pin. After implantation of catheters, these are externalized in the dorsal region of the animal and attached to the skin with surgical suture.