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EFFECT OF NEUROTOXIC LESION OF PEDUNCULOPONTINE NUCLEUS IN NIGRAL AND STRIATAL REDOX BALANCE AND MOTOR PERFORMANCE IN RATS

J. JIMENEZ-MARTIN,[a,b] L. BLANCO-LEZCANO,[a,c*] M. E. GONZÁLEZ-FRAGUELA,[a] M.-L. DÍAZ-HUNG,[a] T. SERRANO-SÁNCHEZ,[a] J. L. ALMENARES[b] AND L. FRANCIS-TURNER[c,a]

[a] *Experimental Neurophysiology Department, International Center of Neurological Restoration, Avenue 25 No. 15805 e/ 158 and 160. Playa, Havana CP 11300, Cuba*

[b] *Neuro Investigation Department, Cuban Neuroscience Center, 190th Street e/ Ave. 25 and Avenue 27. Playa, Havana CP 11300, Cuba*

[c] *Faculty of Sciences, Tolima University, 42nd Street, Barrio Santa Elena, Parte Alta, CP 730001, Colombia*

Abstract—Early degeneration of pedunculopontine nucleus (PPN) is considered part of changes that characterize premotor stages of Parkinson's disease (PD). In this paper, the effects of unilateral neurotoxic lesion of the PPN in motor execution and in the development of oxidative stress events in striatal and nigral tissues in rats were evaluated. The motor performance was assessed using the beam test (BT) and the cylinder test (CT). Nigral and striatal redox balance, was studied by means of biochemical indicators such as malondialdehyde (MDA), nitric oxide (NO) and the catalase enzymatic activity (CAT EA). Lesioned rats showed fine motor dysfunction expressed both as an increase in the length ($p < 0.001$) and deviation ($p < 0.001$) of the traveled path and also in the time spent ($p < 0.01$) in the circular small beam (CBS) ($p < 0.01$) in comparison with control groups. In addition, the lesioned rats group presented a right asymmetry index greater than 0.5 which is consistent with a significant increase in the percentage of use of the right forelimb (ipsilateral to the lesion), compared with the control group ($p < 0.05$). Biochemical studies revealed that after 48-h PPN neurotoxic injury, the CAT EA showed a significant increase in the *subtantia nigra pars compacta* (SNpc) ($p < 0.05$). This significant increase of CAT EA persisted in the nigral tissue ($p < 0.001$) and reached the striatal tissue ($p < 0.001$) seven days after PPN injury. Also at seven days post-injury PPN, increased concentrations of MDA ($p < 0.01$) and a tendency to decrease in the concentrations of NO in both structures (SNpc and *striatum*) were found. The events associated with the generation of free radicals at nigral and striatal levels, can be part of the physiological mechanisms underlying motor dysfunction in rats with unilateral PPN neurotoxic lesion.

Key words: pedunculopontine nucleus, *substantia nigra compacta*, *striatum*, oxidative stress.

INTRODUCTION

Several decades of neuropathological and imaging studies have provided valuable evidence about the changes in cholinergic neurotransmission associated with a dopaminergic dysfunction in Parkinson's disease (PD) (Bohnen and Albin, 2011; Knaryan et al., 2011). In this context, the pedunculopontine nucleus (PPN) represents one of the most important sources of cholinergic projections in the brain and in turn it is the origin of the only cholinergic projection that receives the *susbtantia nigra pars compacta* (SNpc) (Bohnen and Albin, 2011).

The anatomical location of the PPN and its morphofunctional relationships with basal ganglia, thalamus and spinal cord, points to PPN, as a relay nucleus and essential interface in motor control (Jenkinson et al., 2009; Mazzonne et al., 2011; Holmstrand and Sesack, 2011). Multiple investigations support that early degeneration of the PPN, together with lower modulating influence of cholinergic efferents in the SNpc, may be part of changes that characterize the premotor stages of the PD (Bohnen and Albin, 2011; Müller and Bohnen, 2013).

To mimic the preclinical stage of PD and to test the efficacy of different therapeutic strategies it is required an animal model of partial lesion progresses to total or near total degeneration of the SNpc (Sgadò et al., 2011). This ideal model must correlate with the dopaminergic neuronal degeneration underlying behavioral deficits. Some attempts aimed in this direction, have employed schemes based on the gradual administration of 6-hydroxydopamine (6-OHDA) in concentrations and/or volumes below the internationally standardized to obtain a model of complete lesion of the SNpc, with a

*Correspondence to: L. Blanco-Lezcano, Experimental Neurophysiology Department, International Center of Neurological Restoration, Avenue 25 No. 15805 e/ 158 and 160. Playa, Havana CP 11300, Cuba. Tel: +53-7-271-5914; fax: +53-7-273-6028.
E-mail address: lisette.blanco@infomed.sld.cu (L. Blanco-Lezcano).

Abbreviations: 6-OHDA, 6-hydroxydopamine; ANOVA, analysis of variance; BSA, bovine serum albumin; BT, beam test; CAT, catalase enzyme; CE, corticospinal pathway; CSB, circular small beam; CT, cylinder test; EA, enzyme activity; iNOS, inducible NO synthase; i.p., intraperitoneal; MDA, malondialdehyde; NMDA, N-methyl-D-aspartate; NO, nitric oxide; PBS, phosphate-buffered saline; PD, Parkinson's disease; PPN, pedunculopontine nucleus; RNS, reactive nitrogen species; ROS, reactive oxygen species; SCP, superior cerebellar peduncle; SMART, Spontaneous Motor Activity Recording Tracking; SNpc, *substantia nigra pars compacta*; ST, *striatum*.

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single intracerebral injection of the neurotoxin (Ungerstedt, 1968; Ungerstedt and Arbuthnott, 1970; Truong et al., 2006). These experimental approaches have faced obstacles such as compensatory processes described in the model of hemiparkinsonism by intracerebral administration of 6-OHDA (Zigmond et al., 1990).

The effect of the lesion of the PPN on the electrical activity of different basal ganglia has been studied to investigate the role of an early dysfunction of PPN in the pathophysiology of PD (Breit et al., 2005). The literature refers that healthy rats undergoing excitotoxic lesion of the PPN show changes in the rate of discharge of action potentials in the SNpc, in the subthalamic nucleus (STN) and in the medial *globus pallidus* (GPm), similar to changes seen in hemiparkinsonism rats (Breit et al., 2005).

In the same direction, the PPN unilateral excitotoxic injury in non-human primates induces a parkinsonian syndrome characterized by flexed trunk posture and severe contralateral hypokinesia (Kojima et al., 1997). It has been noted that the PPN lesion can reduce its excitatory influence on the network of interneurons of the spinal cord through its descending projections. Alternatively, the interruption of the upward projections from the PPN to the thalamus or basal ganglia may directly or indirectly influence the thalamocortical activity (Kojima et al., 1997).

Additionally, it has been demonstrated that the modification of cholinergic synapses may increase the vulnerability of nigral dopaminergic neurons to oxidative stress events which activate the programed cell death (De Sarno et al., 2003). In this regard, it is known that oxidative stress is involved in the pathogenic mechanisms of a wide range of neurological diseases including PD (López-Lozano and Alvarez-Santullano, 2004; Zhou et al., 2008). Free radicals affect vital processes for cell survival as the electron transport chain and ATP synthesis and cause structural damage to cell membranes, DNA and proteins (Berg et al., 2011; Kumar et al., 2012; Pomierny-Chamioło et al., 2013). At the same time the excessive generation of reactive nitrogen species (RNS), including nitric oxide (NO) contributes to cell death in neurodegenerative diseases (Nakamura and Lipton, 2011).

Different stable compounds such as malondialdehyde (MDA) and glutathione; and the activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) can be used as oxidative stress indicators (González et al., 2007; Melo et al., 2011; Díaz-Hung et al., 2014).

In spite of the current knowledge regarding the participation of PPN dysfunction in the premotor phase of PD, the impact of unilateral neurotoxic lesion PPN on the oxidant homeostasis nigral and striatal levels as well as the motor performance in rats is unknown . The aim of this study was to determine the effect of the neurotoxic lesion of PPN, on motor activity and nigrostriatal redox homeostasis in rats.

EXPERIMENTAL PROCEDURES

Animals

Male, Wistar rats weighing 200–350 g, from the Centre for the Production of Laboratory Animals (CENPALAB), Mayabeque, Cuba were housed 5 per cage under a temperature of 22–24 °C, with a relative humidity of 60 ± 5% and a light–darkness cycle of 12–12 h. Water and food were provided *ad libitum*. All experimental procedures complied with the ethical principles for animal research established by Clark et al. (1998) and the Canadian Council for Animal Care (Olferd et al., 1997a,b).

Surgical procedure

Rats were anesthetized by means of intraperitoneal (i.p.) injection of chloral hydrate (420-mg/kg weight) and placed in a stereotactic frame for rodent surgery (David Kopf Instruments, Tujunga, USA). An incision was made in the midline, retiring the periosteal connective tissue, and cleaning with hydrogen peroxide. Then, taking as reference the Bregma point (Paxinos and Watson, 1998), were determined the right PPN coordinates (mm): AP: −7.80; ML: 1.60, DV: 7.60. PPN neurotoxic lesion was performed by injecting 0.5 μL of a solution of N-methyl-D-aspartate (NMDA) (Sigma, St. Louis, MO, USA) a glutamate receptor agonist with the same name (0.1 M in physiological saline solution; pH 7.4) at a rate of 0.1 μL/min, by means of a 1 μL Hamilton syringe. The syringe needle was left in place at the end of each injection for an additional 10 min and then withdrawn slowly to prevent reflux of the solution. After surgery, rats were given subcutaneous administration of 0.05-mg/kg of analgesic (buprenorphine hydrochloride, Temgesic, Reckitt Benckiser, Hull, UK) prophylactically to control pain; and were kept warm in an incubator until fully recovered from anesthesia.

Sham lesion of the PPN. The procedure was similar to the NMDA lesion of the PPN but 0.5 μL of physiological saline solution was injected instead of NMDA.

Behavioral studies

The study of sensorimotor dysfunction associated with neurotoxic injury of PPN was performed using two behavioral tests that assess the spontaneous activity of the animals: the beam test (BT) (Allbutt and Henderson, 2007) and the cylinder test (CT) (Schallert et al., 2000).

Seven days after PPN lesion, both behavioral tests were performed. After this moment, on four consecutive days, BT during the first two days and the CT the remaining two days, were carried out. Behavioral studies were conducted under appropriate conditions of silence and lighting.

BT. Rats were placed in the half point of a beam (60 cm long) connecting two platforms (60 cm height above the support surface) and they were allowed to go freely to anyone of them. A little square air bed was collocated immediately under the beam and platform in order to protect the animals from fall injuries. In this test the motor task difficulty was increased gradually by using beams of a rectangular or circular traverse section placed in the following order: rectangular large [2.5 cm wide, (RLB)], circular large [2.5 cm diameter, (CLB)],