REPORT

Neurochemical changes in the pedunculopontine nucleus of hemiparkinsonian rats and effect of different treatments

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ABSTRACT

The present work focuses on the physiopathology of Parkinson's disease (PD), analyzing the alterations in neurotransmitter profile of the basal ganglia triggered by the degeneration of cells from the substantia nigra compacta (SNpc). For this purpose, the neurochemical, morphological and molecular changes of the pedunculopontine nucleus (PPN) of rats upon the induction of hemiparkinsonism were evaluated with surgical, immunochemical and molecular biology techniques together with cerebral microdialysis and behavioral tests, also examining the effect of systemic treatments with MK-801 or (-) nicotine or the results of subthalamic injury on these changes. The results evidenced an increase in the extracellular concentration (EC) of glutamate (Glu) ($p < 0.001$) and GABA ($p < 0.001$) as well as a higher density of muscarinic receptors, together with a statistically significant decrease in the density of BDZ gabaergic receptors ($p < 0.001$) and mu opioid receptors ($p < 0.01$) in the PPN of hemiparkinsonian rats. Our results also constitute the first published description of cell death in the PPN of hemiparkinsonian rats. All the treatments achieved a statistically significant decrease in the Glu ($p < 0.01$) and GABA ($p < 0.001$) EC in the PPN, with a neuroprotective effect on the dopaminergic cells of the ventral tegmental area and the SNpc itself. The administration of (-) nicotine improved the striatal expression of brain-derived neurotrophic factor ($p < 0.01$). We conclude that the PPN of hemiparkinsonian rats displays neurochemical changes which can be modified and/or reverted by the treatments described in this work. Our results had the added benefit of requiring the local manufacture of cerebral microdialysis cannulae, resulting in significant cost savings.

Keywords: pedunculopontine nucleus, dopamine, nicotine, MK-801

*I*ntroduction

One of the distinctive features of Parkinson's disease is the presence of dopaminergic deficiencies (PD) [1]. Consequently, the neurotransmission and functional relationships between the nuclei of basal ganglia have been extensively studied in literature (BG) [2]. In comparison, changes such as those taking place in the pedunculopontine nucleus (closely related anatomically and functionally to the BG) during PD remain considerably less well-examined. [3]. However, recent advances in the dissection of the anatomic and functional organization of the BG and their relationship to the PPN have revolutionized the concepts regarding the dominant role of the classical motor cortex-striatum-BG-thalamus-motor cortex circuit, acknowledging the PPN as a mayor player in the integration of cortical, thalamic and BG impulses and as a first-order relay between cerebral cortex and spinal chord [4, 5].

The description of the relationship between dopaminergic deficiencies and the pathogenesis of PD constituted the foundation for the first successful pharmacological treatment of this disease through the administration of L-dihydroxyphenylalanine (L-DOPA), a precursor for the biological synthesis of dopamine (DA) [1]. However, since long-term therapy with L-DOPA results in multiple side effects and fluctuations in the motor response [6], a number of different therapeutic alternatives (both surgical and pharmacological) have been employed in an attempt to repair the neurochemical imbalance associated to the degeneration of the nigrostriatal pathway. The use of glutamatergic antagonists to attenuate the increased activity of this neurotransmitter system in PD and, more recently, the utilization of neuroprotective strategies that try to stop or slow down the rate of cell death in nigral cells are among the main pharmacological alternatives. [7].

One such strategy is the use of (-) nicotine, singled out after the startling discovery of lower incidences of PD among smokers. The last decade has witnessed a surge in research examining the possible neuroprotective role of (-) nicotine, preventing cell death in the SNpc [8]. According to literature, the neuroprotective effects of this substance during PD stem mainly from the release of brain-derived neurotrophic factor (BDNF) and striatal DA from cells surviving neurotoxic damage as well as from the intracellular signaling following the stimulation of nicotinic receptors [9-11].

Other neuroprotective strategies have promoted the therapeutic use of N-methyl D-aspartate (NMDA) receptor antagonists such as amantadine and MK-801, which decrease the glutamatergic excitatory drive over the efferent nuclei of the BG and the SNc itself [12]. Although glutamatergic activity seems to play a positive role during early stages of PD by stimulating the compensatory pre-synaptic mechanisms that contribute to maintain the striatum under dopaminergic

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control [13], glutamatergic hyperactivity seems to be involved in the acceleration of the neurodegenerative process in later stages of the disease [14, 15].

As mentioned above, the long-term failures of L-DOPA therapy together with the development of systems for registering the electric activity of deep brain nuclei and the availability of high resolution imaging techniques have also stimulated a revival in the investigation of surgical treatments for PD [16]. One promising avenue is the selective lesion of the subthalamic nucleus (STN), based on its hyperactivity on experimental models of the disease [17, 18].

The development of experimental models for PD has allowed the detailed study of some aspects of the physiopathology and therapy of this disorder [19]. Neurochemically speaking, the dopaminergic system has been comparatively much more studied than other neurotransmission systems such as those based on aminoacids, as illustrated by the glutamic acid (Glu) or gamma-aminobutyric acid (GABA) systems [20].

This work examined the neurochemical, morphological and molecular changes taking place in the PPN of rats suffering from hemiparkinsonism due to the injection of 6-hydroxydopamine (6-OHDA). Additionally, the effect on those changes of the treatment with MK-801, (-) nicotine or with an excitotoxic lesion of the STN was also evaluated, assessing its impact on the motor disorders characteristic of this model.

Results

The present work focused on the physiology of the PPN in rats, characterizing the extracellular concentration of several aminoacidic neurotransmitters, as well as the density of different receptor populations in this anatomical structure (Figure 1A). Additionally, our results confirmed the appearance of biomolecular changes in the PPN of hemiparkinsonian rats (Figure 1B). From a neurochemical point of view, a significant increase in the extracellular concentrations of Glu ($p < 0.001$) and GABA ($p < 0.001$) in this nucleus was detected. Molecularly, there was also a significant increase in the density of cholinergic muscarinic receptors ($p < 0.05$) accompanied by an equally significant decrease in the density of benzodiazepinic gabaergic receptors (BDZ) ($p < 0.001$) and *mu* opioid receptors ($p < 0.01$) (Figure 1B). These changes may constitute an expression of the neuroplastic mechanisms underlying the Parkinsonian condition.

The results also evidenced the presence and development of a cell death process in the PPN ipsilateral to the 6-OHDA injection, which we infer to follow a mainly necrotic course, associated to an increase in subthalamic glutamatergic activity (Figure 1B).

If neuroprotection is regarded as the possibility of reverting existing damage and preventing future injury, our results emphasize the neuroprotective effect of the pharmacological treatments studied in this work. The administration of either MK-801 or (-) nicotine have several common effects: both attenuated the motor disorders of hemiparkinsonian rats, decreasing the degree of asymmetry and motor incapacity in comparison with untreated controls; and in both cases the extracellular concentrations of Glu and GABA in the PPN showed a considerable decrease (Figure 1C). Both pharmacological strategies seem to preserve the neural mechanisms supporting the processing of raw motor information in hemiparkinsonian rats, which in our judgment constitutes a phenomenon of high adaptive value.

The STN lesion also decreased motor asymmetry as well as the extracellular concentration of Glu and GABA at the PPN, which might constitute part of the mechanisms underlying the beneficial effects of this surgical procedure for the treatment of PD (Figure 1C).

In general, these results underscore first, the importance of the manipulation of the glutamatergic and cholinergic systems as a therapeutic strategy in PD, and second, the integrating role of the PPN in relationship to the BG and the motor cortex, supported by the convergence into this nucleus of the inhibitory gabaergic projections and excitatory glutamatergic projections belonging to the "direct route" or "indirect route", respectively, of the motor circuit.

Blocking the NMDA glutamatergic receptors produces a protective effect on the dopaminergic cells of the ventral tegmental area, and may modulate the increase in corticostriatal glutamatergic tone (Figure 1C). An STN lesion would decrease the glutamatergic excitatory drive over the efferent nuclei of the BG (*substantia nigra pars reticulate* SNpr; *globus pallidum* medial, GPm) acting at the last segment of the motor circuit (Figure 1C). The exposure of the hemiparkinsonian rats to (-) nicotine results in a higher striatal expression of BDNF and simultaneously protects the dopaminergic cells of the SNpc (Figure 1C). The dopaminergic contribution of these cells may help to restore the interaction between DA and the glutamatergic and cholinergic systems at the *striatum* as well as with the gabaergic system at the SNpr/GPm complex (Figure 1C).

Importance of this study

The present investigation takes place among current studies on the consequences of the neurochemical imbalance that leads to the death of nigral cells in the operation of the BG, examining potential treatments for addressing this imbalance through either the systemic delivery of neuroprotective drugs or an excitotoxic lesion of the STN. The main findings of this study are coherent with the model schematically shown in figure 1, which constitutes its principal scientific contribution taking into account the anatomical and functional relationships of the PPN with the BG nuclei, its most relevant changes during experimental Parkinsonism and the effect of the applied treatments.

The relevance of neuroprotective strategies in general has increased during the last years due to the potential for attenuation of the progressive course of neurodegenerative diseases, influencing the cellular and molecular substrates that drive the cell death processes of PD and similar disorders. The lesion of the STN as a surgical alternative would have a large impact in the treatment of PD, and therefore studying its repercussion on neurotransmission at the level of other nuclei related to the processing of motor information has a high scientific and clinical value.

From a strictly academic point of view, the findings of the present study provide a deeper knowledge of the functional relationship between the PPN and the nuclei of the BG, showing for the first time the pre-

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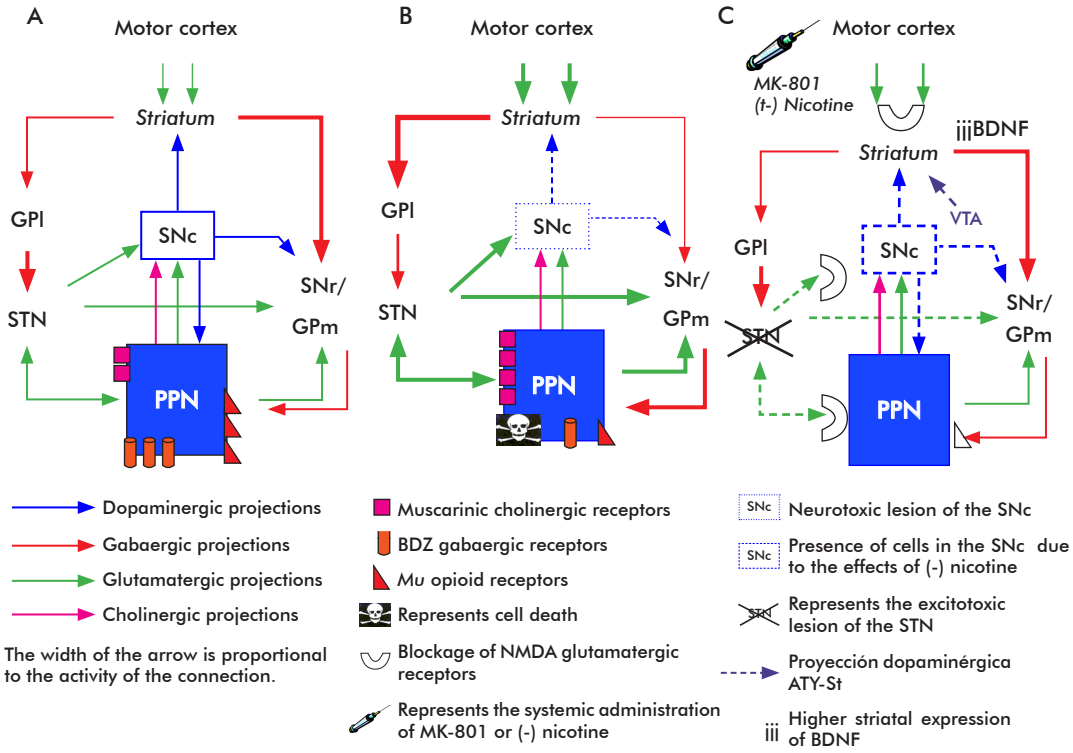

Figure 1. Functional model of the motor circuit at the basal ganglia. A) Healthy condition; B) Parkinsonism; C) After manipulation of the glutamatergic and cholinergic systems. SNc: Substantia nigra compacta; STN: Subthalamic nucleus; PPN: Pedunculopontine nucleus; SNr: substantia nigra reticulata; GPm:Medial Globus pallidum; GPl: Lateral Globus pallidum; BDNF: Brain-derived neurotrophic factor; VTA: Ventral Tegmental Area.

sence of molecular, neurochemical and morphological changes in this structure associated to experimental Parkinsonism. Such is the case, for instance, for the alterations in the extracellular concentrations of aminoacidic neurotransmitters and the cell death processes in this nucleus for the 6-OHDA model. These findings, together with the use of advanced techniques such as cerebral microdialysis for *in vivo* monitoring of the levels of aminoacidic neurotransmitters, underscore the novelty of the present investigation.

Economically, this work was started by implementing the local manufacture of cerebral microdialysis cannulae, at a cost 80 times less than their international market prize. Additionally, manufacturing these cannulae in-house not only resulted in significant cost savings, but also allowed the more intensive application of this technique for the measurement of extracellular neurotransmitter concentrations.

PD is a neurodegenerative disease affecting 1% of the population over 70 years old. In our country, with a life expectancy of 74 years, 15% of the population falls within this age group and this study, therefore, presents a considerable social value due to its examination of the physiopathology of a disorder with a relatively high incidence in our country.

Conclusions

Although cell death at the SNpc and the ensuing dopaminergic deficiency constitute the hallmark of PD, the physiology of other neurotransmitters and the activity of other nuclei in addition to those of the BG are known to be affected by this disorder. PD is currently regarded as a multisystem disease affecting both dopaminergic and non-dopaminergic structures that degenerate progressively. The results of the present work confirm the appearance of a number of molecular and cellular changes in the PPN of hemiparkinsonian rats, underscoring the significant participation of this structure in the degenerative process associated to PD. Additionally, our findings emphasize the protective effect of the examined experimental treatments and support the therapeutic potential of an excitotoxic lesion of the STN for the surgical treatment of this disease.

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