Journal of Biological Research-Thessaloniki **9**: 35 – 43, 2008
J. Biol. Res.-Thessalon. is available online at http://www.jbr.gr
Indexed in: WoS (Web of Science, ISI Thomson), CAS (Chemical Abstracts Service) and DOAJ (Directory of Open Access Journals)

Alterations in the density of gabaergic BDZ, mu opioid and muscarinic receptors of the pedunculopontine nucleus in a 6-hydroxydopamine hemiparkinsonian rat model

LISETTE BLANCO LEZCANO[1*], LOURDES DEL C. LORIGADOS PEDRE[1], LISIS MARTÍNEZ MARTÍ[1], NANCY PAVÓN FUENTES[1], TERESA SERRANO SÁNCHEZ[1], MA. ELENA GONZÁLEZ FRAGUELA[1] and LUISA ROCHA ARRIETA[2]

[1] *International Center of Neurological Restoration (CIREN)*, *Ave. 25, No. 15805 e/158 and 160*, *Playa*, *11300*, *Havana*, *Cuba*
[2] *Department of Pharmacobiology*, *Center for Research and Advanced Studies*, *México DF, México*

Received: 4 September 2007 Accepted after revision: 31 January 2008

Although there is evidence suggesting a relationship between parkinsonism and modifications to gabaergic, cholinergic and opioid activities in the basal ganglia and related structures, so far studies are not conclusive, and the molecular mechanisms underlying these changes remain unknown. Here, we studied the changes in population density of benzodiazepine (BDZ), muscarinic cholinergic and mu opioid receptors in the pedunculopontine nucleus (PPN) from hemiparkinsonian rats using three experimental groups; i) untreated, ii) treated with 6-hydroxydopamine (6-OHDA) and iii) treated with physiologic saline solution. One month after inducing lesion on the *substantia nigra pars compacta* (SNc), all rats were sacrificed by decapitation and frozen coronal sections of the samples, representative of the PPN, were obtained and studied by autoradiography using ^{3}H-flunitrazepam, [^{3}HTyr-D-Ala-(Nme)Phe-Gly-ol] (^{3}H-DAMGO) and [^{3}H]Quinuclidinylbenzylate (QNB) for BDZ, mu and muscarinic receptors, respectively. The optical density (OD) was measured in PPN ipsilateral to the SNc lesion, using the OD readings of the tritium standards to determine tissue radioactivity values for the accompanying tissue sections and to convert them to fmol mg^{-1} protein. All receptors showed statistically significant variations among the experimental groups. The densities of BDZ and mu opioid receptors were down regulated ($p < 0.05$), while the density of muscarinic receptors was upregulated in the PPN from the SNc lesioned group ($p < 0.05$). These results prove that there are changes in the densities of BDZ, mu opioid and muscarinic receptors in the PPN upon intracerebral administration of 6-OHDA. These changes may take part in one of the steps of the sequence of molecular and neurochemical events underlying the imbalance between "direct" and "indirect" pathway of the basal ganglia, which is typically seen in parkinsonism. Additionally, these results reinforce the importance of the mesopontine tegmentum in the physiopathology of Parkinson's disease.

Key words: PPN, BDZ, mu opioid receptor, muscarinic receptors, autoradiography.

INTRODUCTION

The basal ganglia have the richest array of neurotransmitters and receptors of any region of the brain (McGeer & McGeer, 1993). The major part of the neurochemical studies carried out on experimental models of Parkinson's disease (PD) has focused on the dopaminergic system (Mayeux, 2003). The study of other neurotransmitters, such as amino acids, opioid peptides and acetylcholine, is largely depended on the experimental design centered on the determination of the effects of dopaminergic drugs on these molecules (Calon *et al.*, 2003; Steiniger & Kretschmer, 2003). Additionally, although the populations of

* Corresponding author: tel.: +53 7 271 6841, fax: +53 7 273 2420, e-mail: lisette.blanco@infomed.sld.cu

dopaminergic receptors has been extensively studied in models of Parkinsonism, less is know about the behavior of receptors for other neurotransmitters in PD.

Benzodiazepine (BDZ) receptor is part of a macromolecular complex known as the $GABA_A$ receptor, which includes a selective chloride ion channel and binding sites for different molecules, including benzodiazepines (Olsen & DeLorey, 1999; Simeone *et al.*, 2003). Benzodiazepinic drugs act by improving gabaergic transmissions through an increase in the opening frequency of the chloride ion channel and consequently, a decrease in neuronal excitability (Olsen & DeLorey, 1999; Ondo *et al.*, 2003).

A number of studies suggest that the chronic stimulation of dopaminergic receptors results in an increased regulation of the $GABA_A$/BDZ complex (Calon *et al.*, 1999), and this mechanism has been postulated to be involved in the dyskinesia induced by L-DOPA in an experimental primate model of Parkinsonism, but not in humans (Calon & Di Paolo, 2002).

On the other hand, there are reports in the literature on changes in the peptidergic neurotransmission of the basal ganglia related to nigral degeneration (Fernández *et al.*, 1994). Many neuropeptides are localized in the circuits of the basal ganglia (Parent & Hazrati, 1995; Akil *et al.*, 1998). The "direct pathways" of the motor circuits co-express, together with γ-aminobutyric acid (GABA), peptides such as substance P and dynorphins, whereas the "indirect pathways" co-express GABA and enkephalins (Groenewen, 2003). From the point of view of their anatomical localization, the mu (μ), delta (δ) and kappa ($\varkappa$) opioid receptors are found in the *substantia nigra pars compacta* (SNc), the *globus pallidus* (GP), the *striatum* and the cortex (Minami, 2004).

In connection with muscarinic cholinergic receptors (mAChRs), five subtypes (M_1-M_5) are described in literature (Cauldfield & Birdsall, 1998). All mAChRs are seven transmembrane metabotropic receptors (Taylor & Heller, 1999). The muscarinic receptors regulate kinase and phosphatase activities either through the heterotrimeric G protein activation and subsequent second-messenger production or through the direct activation of small-molecular weight G proteins (Felder *et al.*, 2000). Activation of the cholinergic neurons in the pedunculopontine nucleus (PPN), which provides a major cholinergic input to the ventral tegmental area (VTA), excites dopaminergic neurons and induces a prolonged dopamine increase in the nucleus accumbes (Zhou *et al.*, 2003; Winn, 2006).

Currently, the role of the PPN on the physiopathology of PD is the subject of some debate (Erro & Giménez-Amaya, 1999; Lee *et al.*, 2000; Mena-Segovia *et al.*, 2004; Winn, 2006). From the neurochemical point of view, the PPN is regarded as a heterogeneous structure (García-Rill, 1991). This nucleus sends a cholinergic and glutamatergic projection to the SNc and the subthalamic nucleus (STN) (Mena-Segovia *et al.*, 2004). Simultaneously, the PPN receives dopaminergic, glutamatergic and gabaergic afferences from the SNc, the STN and the output nuclei of the basal ganglia, *substantia nigra pars reticulata* (SNr) and the internal segment of the GP, respectively (Semba & Fibiger, 1992). The pontine cells express cholinergic: muscarinic and nicotinic receptors (Mamiya *et al.*, 2005), $GABA_A$ gabaergic receptors (Nandi *et al.*, 2002a; Pal & Mallick, 2004), as well as receptors to different neurotrophins such as fibroblast-derived growth factor (García-Rill, 1991).

Recently, it has been suggested that the PPN could be a therapeutic target to improve gait in certain parkinsonian patients and also, that this nucleus may be a good target for deep brain stimulation techniques (Gómez-Gallego *et al.*, 2007; Stefani *et al.*, 2007). For these reasons, it is currently interesting to study the molecular changes in PPN associated with the nigral denervation.

The purpose of the present paper was to study the changes in the density of the mu opioid, BDZ and muscarinic receptors in the PPN in a rat model of hemiparkinsonism induced by the intracerebral injection of 6-hydroxydopamine (6-OHDA).

MATERIALS AND METHODS

The animals used were adult male Wistar rats, with a body weight range of 200 to 250 g, obtained from the Center for the Production of Laboratory Animals (CENPALAB, Havana, Cuba). Three animals were kept per cage throughout the experiments, with a photoperiod of 12 hours of light alternating with 12 hours of darkness, and water and feed were provided *ad libitum*. The experimental work respected and followed the Practical Guidelines for the use of laboratory animals.

SNc lesion

The rats were anaesthetized by intraperitoneal administration (i.p.) of chloral hydrate (420 mg kg^{-1}), after which they were placed in a stereotaxic surgery device for rodents (David Kopf Instruments, USA). They

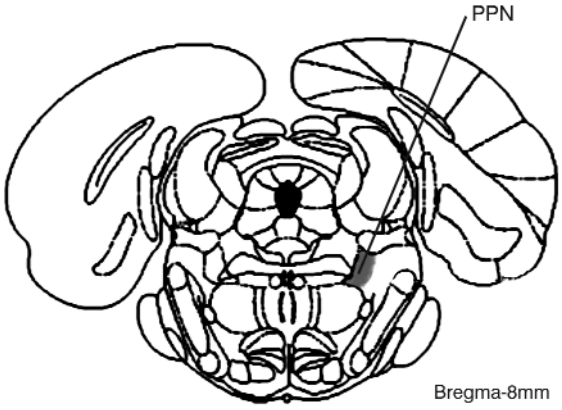

FIG. 1. Diagram showing the localization of the pedunculopontine nucleus (PPN) in a coronal section. The area sampled for optical density measurement is shaded in grey.

received a 3 μl injection of a solution of 6-OHDA [8 μg per 3 μl of physiological saline solution (0.9% NaCl) and 0.5 mg ml^{-1} ascorbic acid] at a rate of 1 μl per min, on the right SNc, using the following coordinates, according to the atlas of Paxinos and Watson (Paxinos & Watson, 1998): AP = –4.4 mm; L = 1.2 mm; DV = 7.8 mm (referents to Bregma).

One month after the SNc lesion, the rotatory activity induced by D-amphetamine (5 mg kg^{-1}, i.p.) was analyzed. This variable was studied for 90 min, using an electronic multicounter (LE 3806, PanLab, Barcelona, Spain) coupled to sensors to detect the direction of rotation (LE 902, PanLab, Barcelona, Spain). This study only included animals displaying more than 7 complete turns per minute ipsilateral to the SNc lesion, which corresponds to a dopaminergic denervation equal to or higher than 90%. A control group of sham operated animals was produced by administering physiological saline solution under the same conditions.

The animals were distributed in three experimental groups: non-treated rats (n = 9), rats with SNc lesions (n = 7) and sham-operated rats (n = 5).

Upon concluding the study of rotatory activity, the rats were anaesthetized by applying chloral hydrate (480 mg kg^{-1}, i.p.) and sacrificed by decapitation. Brains were extracted, frozen in dry ice, and then stored at –80°C until further processing.

For the autoradiography studies, a series of six coronal sections (20 μm) were obtained using the coordinates of the PPN (mm), which first appeared on the rostrocaudal coordinate –7.3, disappearing on coordinate –8.8 (referents to Bregma) (Fig. 1).

Autoradiography for gabaergic BDZ, mu opioid and cholinergic muscarinic receptors

The autoradiography experiments were carried out on adjacent sections, and the experimental conditions are summarized in Table 1. The brain sections were initially washed to remove endogenous ligands. Then, they were incubated in a solution containing the specific ^{3}H-ligand. Binding obtained in the presence of a non-labeled ligand was considered to be nonspecific. Values of the specific binding reported in the present study were obtained from the difference between nonspecific and total binding. Finally, incubation was terminated with two consecutive washes (1 min each) in buffer and a distilled water rinse (2 sec) at 4°C. The sections were then quickly dried under a gentle stream of cold air.

The slides were arrayed in X-ray cassettes together with tritium standards (Amersham), and apposed to a ^{3}H-sensitive film (Kodak MR) at room temperature. The films were developed using Kodak D19 developer and fixer at room temperature. Optical den-

Table 1. Conditions for autoradiography experiments

Binding	**Ligand (nM) S.A.**	**Buffer pH 7.6**	**Incubation**	**Exposition**	**Non-labeled ligand**
BDZ gabaergic receptors	^{3}H flunitrazepan (2.08 nM) 88 Ci $mmol^{-1}$	Tris HCl (170 mM)	45 min 4°C	2 weeks RT	Diazepam (1 mM)
Mu opioid receptors	^{3}H-DAMGO (2 nM) 55 Ci $mmol^{-1}$	Tris HCl (50 mM)	60 min RT	10 weeks RT	Naloxone (2 mM)
Muscarinic cholinergic receptors	^{3}H-QNB (1.23 nM) 43.9 Ci $mmol^{-1}$	PBS (50 mM)	2 hrs 22°C	3 weeks 4°C	Atropine (1 mM)

S.A.: specific activity; RT: room temperature; ^{3}H-DAMGO: [^{3}HTyr-D-Ala-(Nme)Phe-Gly-ol]; QNB: [^{3}H]Quinuclidinylbenzilate

sity (OD) of the right PPN zone was determined using a video-computer enhancement program (JAVA Jandel Video Analysis Software). For each sample, 10 optical density readings were taken from at least three sections, and were averaged. The optical density readings of the standards were used to determine tissue radioactivity values (dpm mm^{-2}) for the accompanying tissue sections. Then, dpm mm^{-2} values were converted to fmol mg^{-1} protein by dividing dpm by the specific activity of specific ^{3}H-ligand (Table 1) by 2.22. The value 2.22 represents the conversion factor from Ci to dpm.

Data analysis

Compliance of the data to a normal distribution was checked with the Kolmogorov-Smirnov test. Receptor density of the different experimental groups was compared using a single classification analysis of variance, followed by a separate Tukey test for each receptor. Significance level for statistical tests was set to 0.05. The Statistica CSS profesional software, version 6.1, was used for the statistical analyses.

RESULTS

BDZ gabaergic receptor density

The comparison between experimental groups for BDZ receptor density in the PPN, revealed statistically significant differences ($F_{(2, 18)} = 8.54$, $p < 0.05$). These differences were caused by a statistically important decrease (~ 30%) of this variable in the group of rats with SNc injuries, compared to the remaining control groups (Fig. 2A).

Mu opioid receptor density

The comparison between experimental groups for mu opioid receptor density showed a statistically significant decrease (~ 15%) in the group of rats with SNc injuries in contrast with the control groups ($F_{(2, 14)} = 6.02$, $p < 0.05$) (Fig. 3A).

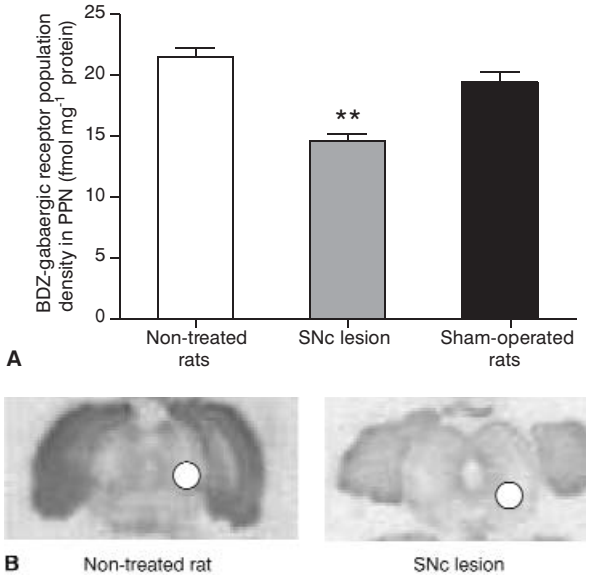

Fig. 2. A. Comparison of the BDZ-gabaergic receptor population density in the pedunculopontine nucleus between experimental groups ($F_{(2, 18)} = 8.54$, $p < 0.05$). Experimental groups: Non-treated rats (n = 9), *substantia nigra compacta*-lesioned (SNc lesion) rats (n = 7), sham-operated rats (n = 5). The asterisks (**) represent statistical significant differences between lesioned and control groups ($p < 0.01$). The bars represent mean ± s.e.m. B. Autoradiographs of the PPN coronal section from non-treated and *substantia nigra* lesioned rats. The zone inside the circle corresponds with the zone where the optical density was measured.

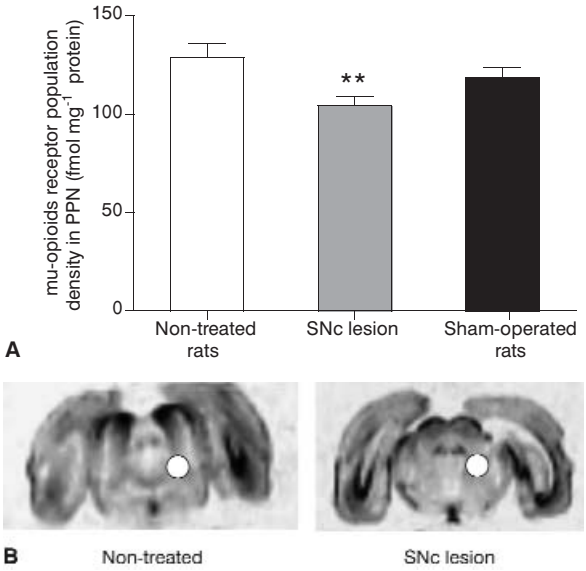

Fig. 3. A. Comparison of the *mu*-opioids receptor population density in the pedunculopontine nucleus between experimental groups ($F_{(2, 14)} = 6.02$, $p < 0.05$). Experimental groups: Non-treated rats (n = 9), *substantia nigra compacta*-lesioned (SNc lesion) rats (n = 7), sham-operated rats (n = 5). The asterisks (**) represent statistical significant differences between lesioned and control groups ($p < 0.01$). The bars represent mean ± s.e.m. B. Autoradiographs of the PPN coronal section from non-treated and *substantia nigra* lesioned rats. The zone inside of the circle corresponds with the zone where the optical density was measured.

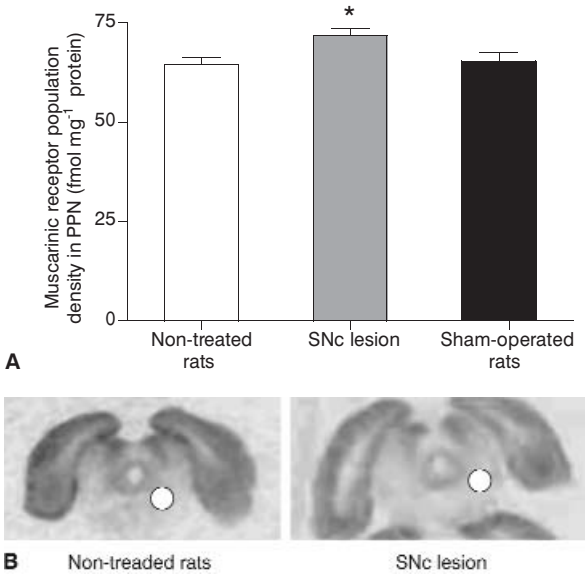

Fig. 4. A. Comparison of the muscarinic cholinergic receptor populations density in the pedunculopontine nucleus between experimental groups ($F_{(2, 13)} = 3.93$, $p < 0.05$). Experimental groups: Non-treated rats (n = 9), *substantia nigra compacta*-lesioned (SNc lesion) rats (n = 7), sham-operated rats (n = 5). The asterisk (*) represent statistical significant differences between lesioned and control groups ($p < 0.05$). The bars represent mean ± s.e.m. B. Autoradiographs of the PPN coronal section from non-treated and *substantia nigra* lesioned rats. The zone inside of the circle corresponds with the zone where the optical density was measured.

Cholinergic muscarinic receptor density

The comparison between experimental groups for muscarinic receptor density showed a statistically significant increase (~ 10%) in the groups of rats with SNc injuries in contraposition to the control groups ($F_{(2, 13)} = 3.93, p < 0.05$) (Fig. 4A).

The Figures 2B, 3B and 4B show the zone where the optical density was recorded in each coronal section for posterior analysis.

DISCUSSION

BDZ gabaergic receptor density

Although the PPN is anatomically located outside the basal ganglia, it is connected to them through reciprocal connections involving several nuclei (Erro & Giménez-Amaya, 1999). The PPN is a point of convergence for projections of varying nature and origin, including glutamatergic, gabaergic and dopaminergic projections from the STN, SNr and/or Gpi, and the SNc, respectively (Semba & Fibiger, 1992; Bevan & Bolam, 1995).

In physiological conditions, the density of the gabaergic receptors present in the PPN is under the control of the gabaergic projection from the Gpi/SNr complex (entopeduncular nucleus in rodents) (Groenewen, 2003). The activity of this projection is controlled by the input from direct and indirect pathway of the basal ganglia motor circuit (Winn, 2006).

Although there are reports on the activity of the gabaergic $GABA_A$ and $GABA_B$ receptors in the context of parkinsonism, there is very little information available on the changes of the BDZ site of the $GABA_A$ receptor in the PPN in the experimental models of this disorder.

The decrease in density of the BDZ gabaergic receptors in the PPN ipsilateral to the 6-OHDA lesions showed in the present study, suggests that there are changes in the gabaergic activity in the PPN under conditions of parkinsonism.

Already published results showed a significant increase in the release of GABA in the PPN of hemiparkinsonian rats (Blanco-Lezcano *et al.*, 2005). Our present study suggests a postsynaptic $GABA_A$/benzodiazepine receptor complex change due to an adaptive response following chronic over activity on the nigropontine pathways. This change would be an expression of the mechanism of synaptic plasticity at this level (Viallet & Witjas, 2002).

Other investigators have pointed out that the microinjection of agonist drugs for $GABA_A$ receptors, such as bicuculine in the PPN, attenuates the akinesia and other parkinsonian symptoms in models of parkinsonism in non-human primates based on the systemic administration of 1-methyl 4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) (Nandi *et al.*, 2002b). Bicuculine is a $GABA_A$ antagonist that decreases the frequency and average time for the opening of the chloride ion channel (Olsen & DeLorey, 1999). This drug competes with GABA for one or several receptor binding sites, and it has been postulated that one of these sites may be the BDZ site of the $GABA_A$ receptor (Olsen & DeLorey, 1999).

Other studies deal with the role of the BDZ receptors in the *striatum*, showing an increase of the density of these receptor populations in the putamen of brains from deceased parkinsonian patients (Calon *et al.*, 2003). This finding underscores the relationship between the degeneration of the nigrostriatal dopaminergic route and the state of the populations of gabaergic receptors (Sun *et al.*, 2004).

The changes in density of the BDZ gabaergic receptors in the PPN represent a link in the chain of molecular and neurochemical events that characterize the imbalance between the two projection routes for the passage of motor information from the cortex, passing through the nuclei in the basal ganglia and the thalamus, and back to the motor cortex, which is typical of parkinsonism.

Mu opioid receptor density

The results showed that there was a decrease in the density of the mu opioid receptors in the PPN ipsilateral to the 6-OHDA injections. This is particularly significant, considering the fact that no peptidergic afferences reaching the PPN have been described, although, on the other hand, the PPN has an important representation of peptidergic receptors (Winn, 2006).

It is known that the interactions between opioid peptides and their cognate receptors do not always follow the classical synaptic pathway of the release from a pre-synaptic terminal and binding to receptors in post-synaptic terminals (Bach-y-Rita, 1993; Akil *et al.*, 1998). It is also known that peptides can follow alternative transmission routes, such as diffusion to receptors located in structures, which do not necessarily receive the corresponding afferent innervations (Bach-y-Rita, 1993). These are some of the reasons why peptides are classified as neuromodulators rather than classical neurotransmitters (Akil *et al.*, 1998).

There is no previous report about the changes in opioid receptor density in PPN in parkinsonian conditions. In other structures such as the *striatum*, the literature suggests a close interaction between the dopaminergic and opioid activity, which is altered in the parkinsonism (Samadi *et al.*, 2003). The mu opioid receptors are located joint to the NMDA receptor in the corticostriatal terminals (Akil *et al*., 1998). The simultaneous activation of both receptors has opposite effects on the entry of calcium to the cells. This evidence has suggested that the mu opioid receptors modulate the glutamate release in the corticostriatal synapse (Samadi *et al.*, 2003). This interaction is permanently modified under parkinsonian conditions, in the *striatum* and probably in other nuclei where it is also present.

In the PD and its experimental models, the dopaminergic innervations that in physiological conditions reach the PPN, are dramatically compromised (Hamani *et al*., 2007). The decrease of the dopaminergic tone in the PPN level could reinforce the loss of interaction between the opioid and dopaminergic systems in this structure. Thus, we can hypothesize that the decrease in the mu opioid receptor density in the PPN of hemiparkinsonian rats, detected in the present work, may be a response to the loss of the interaction mentioned above.

There are significant changes in peptidergic activity during parkinsonism (Fernández *et al.*, 1994). The studies using binding assays have revealed marked regional differences regarding the number of neuropeptide receptors found in the brain of deceased parkinsonian patients, as compared to healthy persons (Fernández *et al.*, 1994).

The interactions between the different receptors are often established through the molecules responsible for intracellular signaling, such as G protein (Agnati *et al.*, 2003; di Michelle *et al.*, 2003). Several types and subtypes of G protein coupled receptors form heterodimeric complexes with this protein that afterwards modulate the activity of other receptors of the ionic channels superfamily or specific for kinase-type enzymes (Agnati *et al.*, 2003). These interactions do take place between gabaergic and opioid receptors, and have been studied in models of epilepsy (Rocha *et al.*, 1993).

By taking into account that we did not found significant differences between the non treated and the sham operated rats, we can suppose that the changes found in the 6-OHDA lesioned rats are associated with the nigral denervation.

Cholinergic muscarinic receptor density

Our results indicate a significant increase in the muscarinic cholinergic receptor density in the PPN ipsilateral to the SNc lesion. It is well known that the muscarinic receptors in the PPN have characteristics of autoreceptors because they are located in the cholinergic cells from the self nucleus (Vilaro *et al.*, 1994). Some investigators have described in the PPN from non human primates, the presence of subtypes M_2, M_3 and M_4 muscarinic receptors, which participate in feed back mechanisms controlling the acetylcholine synthesis rate and release by pontine cells (Grofova & Zhou, 1998; Waelbroeck, 2003). The activation of the pontine cholinergic neurons provide a cholinergic input that reaches the dopaminergic neurons located in the SNc and in the VTA (Bolam *et al.,* 1991; Lee *et al.*, 2000).

On the other hand, the cholinergic system presents neuroplastic changes under parkinsonian conditions. The increase in the number and size of cholinergic terminals in the SNc has been published (Anglade *et al.*, 1995). This finding suggests that neuroplasticity mechanisms are extended to the pontine-nigra synapse. We can hypothesize that the changes in density of the muscarinic receptor in the PPN of hemiparkinsonian rats may be the result of these mechanisms.

The activation of subtypes of muscarinic receptors M_2 and M_4 with presynaptic location, decreases the glutamatergic transmission in different structures such as hippocampus and *striatum* (Scanzianni *et al.*, 1995; Rawls & McGinty, 1998). It is possible that in the PPN from hemiparkinsonian rats, the activity of the muscarinic receptors also modulates the glutamatergic neurotransmission, if we take into account that this nucleus sends a cholinergic and glutamatergic projection to different nuclei (Matsumura, 2005). This modulatory effect would be very important in the 6-OHDA model, since this model presents an increase of the glutamatergic activity associated to the lack of inhibition of the "indirect pathway" of the motor circuit (Obeso *et al.*, 2000).

Since there are not previous publications concerning the changes in the density of the muscarinic receptor population in the PPN from hemiparkinsonian rats, we consider that the increase detected in the present study, may help understand the complex interactions between the pontine cholinergic projection and its target nuclei: the thalamus and the SNc.

The increase detected could be related with the changes in a particular subtype of muscarinic receptors located in the PPN of rodents. Nevertheless, the radioactive ligand employed in the present study does not differentiate among them. Due to this reason, it will be interesting to continue this study in the future, with selective markers for the different muscarinic receptor subtypes.

Concluding remarks

It has only been ten years since PPN was first considered as a key factor for understanding the physiopathology of PD. The changes in this structure regarding the populations of mu opioid, BDZ and muscarinic receptors in the model of 6-OHDA, confirm the dysfunctional nature of these neurotransmission systems during PD, providing evidence that underscores the importance of the mesopontine segment in the function of the basal ganglia.

REFERENCES

Agnati LF, Ferré S, Lluis C, Franco R, Fuxe K, 2003. Molecular mechanisms and therapeutical implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal GABA neurons. *Pharmacological reviews*, 55: 509-550.

Akil H, Owens C, Gutstein H, Taylor L, Curran E, Watson S, 1998. Endogenous opioids: overview and current issues. *Drug and alcohol dependence*, 51: 127-140.

Anglade P, Tsuji S, Javoy-Agid F, Agid Y, Hirsch EC, 1995. Plasticity of nerve afferents to nigrostriatal neurons in Parkinson's disease. *Annals of neurology*, 37: 265-272.

Bach-y-Rita P, 1993. Neurotransmission in the brain by diffusion through the extracellular fluid: a review. *Neuroreport*, 4: 343-350.

Bevan MD, Bolam JP, 1995. Cholinergic, GABAergic and glutamate-enriched inputs from the mesopontine tegmentum to the subthalamic nucleus in the rat. *The journal of neuroscience*, 15: 7105-7120.

Blanco-Lezcano L, Rocha L, Alvarez L, Martínez-Martín L, Pavón N, González-Fraguela ME, Bauza Calderin Y, Coro Y, 2005. Efecto de la lesión de la *substantia nigra pars compacta* sobre la liberación de glutamato y gaba en el núcleo pedunculopontino. *Revista neurología*, 40: 23-29.

Bolam JP, Francis CM, Henderson Z, 1991. Cholinergic input to dopaminergic neurons in the *substantia nigra*: a double inmunocytochemical study. *Neuroscience*, 41: 483-494.

Calon F, Di Paolo T, 2002. Levodopa response motor complications-GABA receptors and preproenkephalin expression in human brain. *Parkinsonism and related disorders*, 8: 449-454.

Calon F, Morissette M, Goulet M, Grondin R, Blanchet PJ, Bédard PJ, Di Paolo T, 1999. Chronic D1 and D2 dopaminomimetic treatment of MPTP-denervated monkeys: effects on basal ganglia $GABA_A$/benzodiazepine receptor complex and GABA content. *Neurochemistry international*, 35: 81-91.

Calon F, Morissette M, Rajput AH, Hornykiewicz O, Bédard PJ, Di Paolo T, 2003. Changes of GABA receptors and dopamine turnover in the postmortem brains of parkinsonians with levodopa-induced motor complications. *Movement disorders*, 18: 241-253.

Cauldfield MP, Birdsall NJM, 1998. International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacological reviews*, 50: 279-290.

di Michelle F, Longone P, Romeo E, Lucchetti S, Brusa L, Pierantozzi M, Bassi A, Bernardi G, Stanzione P, 2003. Decreased plasma and cerebrospinal fluid content of neuroactive steroids in Parkinson's disease. *Neurological sciences*, 24: 172-173.

Erro E, Giménez-Amaya J, 1999. Pedunculopontine tegmental nucleus: Anatomy, functional considerations and physiopathological implications. *Anales del sistema sanitario de Navarra*, 22: 189-201.

Felder CC, Bymaster FP, Ward J, DeLapp N, 2000. Therapeutic opportunities for muscarinic receptors in the central nervous system. *Journal of medicinal chemistry*, 43: 4333-4353.

Fernandez A, de Ceballos ML, Jenner P, Marsden CD, 1994. Neurotensin, substance P, delta and mu opioid receptors are decreased in basal ganglia of Parkinson's disease patients. *Neuroscience*, 61: 73-79.

García-Rill E, 1991. The pedunculopontine nucleus. *Progress in neurobiology*, 36: 363-389.

Gómez-Gallego M, Fernández-Villalba E, Fernández-Barreiro A, Herrero MT, 2007. Changes in the neuronal activity in the pedunculopontine nucleus in chronic MPTP-treated primates: an *in situ* hybridization study of cytochrome oxidase subunit I, choline-acetyl transferase and substance P mRNA expression. *Journal of neural transmission*, 114: 319-326.

Groenewegen HJ, 2003. The basal ganglia and motor control. *Neural plasticity*, 10: 107-120.

Grofova I, Zhou M, 1998. Nigral innervation of cholinergic and glutamatergic cells in the rat mesopontine tegmentum: light and electron microscopic anterograde tracing and immunohistochemical studies. *The journal of comparative neurology*, 395: 359-379.

Hamani C, Stone S, Laxton A, Lozano AM, 2007. The pedunculopontine nucleus and movement disorders: Anatomy and role for deep brain stimulation. *Parkinsonism and related disorders*, 13: S276-S280.

Lee MS, Rinne JO, Marsden CD, 2000. The pedunculopontine nucleus: Its role in the genesis of movement disorders. *Yonsei medical journal*, 41: 167-184.

Mamiya N, Buchanan R, Wallace T, Skinner RD, García-Rill E, 2005. Nicotine suppresses the P13 auditory evoked potential by acting on the pedunculopontine nucleus in the rat. *Experimental brain research*, 164: 109-119.

Matsumura M, 2005. The pedunculopontine tegmental nucleus and experimental parkinsonism. *Journal of neurology*, 252: IV/5-IV/12.

Mayeux R, 2003. Epidemiology of neurodegeneration. *Annual review of neuroscience*, 26: 81-104.

McGeer E, McGeer PL, 1993. Neurotransmitter and their receptors in the basal ganglia. In: Narabayashi H, Nagatsu T, Yanagisawa N, Mizuno Y, eds. *Basic neurochemistry*, *molecular*, *cellular and medical aspects.* Lippicott Williams & Wilkins, Philadelphia: 335-339.

Mena-Segovia J, Bolam JP, Magill PJ, 2004. Pedunculopontine nucleus and basal ganglia: distant relatives or part of the same family? *Trends in neurosciences*, 27: 585-588.

Minami M, 2004. Molecular pharmacology of opioid receptors. *Nippon yakurigaku zasshi*, 123: 95-104.

Nandi D, Stein JF, Aziz TZ, 2002a. Exploration of the role of the upper brainstem in motor control. *Stereotactic and functional neurosurgery*, 78: 158-167.

Nandi D, Aziz T, Giladi N, Winter J, Stein J, 2002b. Reversal of akinesia in experimental parkinsonism by GABA antagonist microinjections in the pedunculopontine nucleus. *Brain*, 125: 2418-2430.

Obeso JA, Rodríguez-Oroz MC, Rodríguez M, Lanciego JL, Artieda J, Gonzalo N, Olanow WC, 2000. Pathophysiology of the basal ganglia in Parkinson's disease. *Trends in neurosciences*, 23: S8-S19.

Olsen WR, DeLorey TM, 1999. GABA and glycine. In: Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD, eds. *Basic neurochemistry*, *molecular*, *cellular and medical aspects*. Lippicott Williams & Wilkins, Philadelphia: 335-339.

Ondo WG, Hunter C, 2003. Flumazenil, a GABA antagonist, may improve features of Parkinson's disease. *Movement disorders*, 18: 683-685.

Pal D, Mallick BN, 2004. GABA in pedunculo pontine tegmentum regulates spontaneous rapid eye movement sleep by acting on $GABA_A$ receptors in freely moving rats. *Neuroscience letters*, 365: 200-204.

Parent A, Hazrati LN, 1995. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamocortical loop. *Brain research. Brain research reviews*, 20: 91-127.

Paxinos G, Watson C, 1998. *The rat brain in stereotaxic coordinates*. Academic Press, New York.

Rawls SM, McGinty J, 1998. Muscarinic receptors regulate extracellular glutamate levels in the rat *striatum*: An *in vivo* microdialysis study. *The journal of pharmacology and experimental therapeutics*, 286: 91-98.

Rocha L, Tatsukawa K, Chugani HT, Engel J Jr, 1993. Benzodiazepine receptor binding following chronic treatment with naloxone, morphine and met-enkephalin in normal rats. *Brain research*, 612: 247-252.

Samadi P, Grégoire L, Bédard PJ, 2003. Opioid antagonists increase the dyskinetic response to dopaminergic agents in parkinsonian monkeys: interaction between dopamine and opioid systems. *Neuropharmacology*, 45: 954-963.

Scanziani M, Gahwiler BH, Thompson SM, 1995. Presynaptic inhibition of excitatory synaptic transmission by muscarinic and metabotropic glutamate receptor activation in the hippocampus: are Ca^{2+} channels involved? *Neuropharmacology*, 34: 1549-1557.

Semba K, Fibiger HC, 1992. Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: a retro- and antero-grade transport and immunohistochemical study. *The journal of comparative neurology*, 323: 387-410.

Simeone TA, Donevan SD, Rho JM, 2003. Molecular biology and ontogeny of γ-aminobutyric acid (GABA)

receptors in the mammalian central nervous system. *Journal of child neurology*, 18: 39-48.

Stefani A, Lozano AM, Peppe A, Stanzione P, Galati S, Tropepi D, Pierantozzi M, Brusa L, Scarnati E, Mazzone P, 2007. Bilateral deep brain stimulation of the pedunculopontine and subthalamic nuclei in severe Parkinson's disease. *Brain*, 130: 1596-1607.

Steiniger B, Kretschmer BD, 2003. Glutamate and GABA modulate dopamine in the pedunulopontine tegmental nucleus. *Experimental brain research*, 149: 422-430.

Sun N, Kong L, Wang X, Holmes C, Gao Q, Zhang GR, Pfeilschifter J, Goldstein DS, Geller AI, 2004. Coexpression of tyrosine hydroxylase, GTP cyclohydrolase I, aromatic amino acid decarboxylase, and vesicular monoamine transporter 2 from a helper virus-free herpes simplex virus type 1 vector supports high-level, long-term biochemical and behavioural correction of a rat model of Parkinson's disease. *Human gene theraphy*, 15: 1177-1196.

Taylor P, Heller J, 1999. Acetylcholine. In: Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD, eds. *Basic neurochemistry*, *molecular*, *cellular and medical aspects*. Lippicott Williams & Wilkins, Philadelphia: 213-243.

Viallet F, Witjas T, 2002. Neuroplasticity and Parkinson's disease. *Revue neurologique*, 158: 42-48.

Vilaro MT, Palacios JM, Mengod G, 1994. Multiplicity of muscarinic autoreceptor subtypes? comparison of the distribution of cholinergic cells and cells containing mRNA for five subtypes of muscarinic receptors in the rat brain. *Molecular brain research*, 21: 30-46.

Waelbroeck M, 2003. Allosteric drugs acting at muscarinic acetylcholine receptors. *Neurochemical research*, 28: 419-422.

Winn P, 2006. How best to consider the structure and function of the pedunculopontine tegmental nucleus: evidence from animal studies. *Journal of the neurological sciences*, 248: 234-250.

Zhou FM, Wilson C, Dani JA, 2003. Muscarinic and nicotinic cholinergic mechanisms in the mesostriatal dopamine systems. *The neuroscientist*, 9: 23-36.