Neurobiology of Aging 61 (2018) 82–92

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Altered mGluR5 binding potential and glutamine concentration in the 6-OHDA rat model of acute Parkinson's disease and levodopa-induced dyskinesia

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ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form 24 May 2017
Accepted 8 September 2017
Available online 21 September 2017

Keywords:
Small-animal PET
Parkinson's disease
Metabotropic glutamate receptor type 5
[18F]FPEB
6-OHDA rat model

ABSTRACT

Several lines of evidence point to alterations in glutamatergic signaling in Parkinson's disease (PD) and levodopa-induced dyskinesia (LID), involving the metabotropic glutamate receptor type 5 (mGluR5). Using small-animal positron emission tomography (PET) with [18F]FPEB and proton magnetic resonance spectroscopy, we investigated cerebral changes in the mGluR5 and glutamate/glutamine availability in vivo in PD rats and following onset of LIDs. In parallel, behavioral tests were performed. Comparing PD to control rats, mGluR5 binding potential was decreased in a cluster comprising the bilateral caudate-putamen (CP), ipsilateral motor cortex and somatosensory cortex, and the contralateral somatosensory cortex and parietal association cortex, with the most pronounced reduction in the ipsilateral CP. mGluR5 binding potentials were not significantly altered upon levodopa (L-DOPA) treatment. However, following L-DOPA, an increase in relative mGluR5 uptake was present in the contralateral motor cortex and somatosensory cortex. Glutamate and glutamine concentrations did not differ between control and untreated PD rats or between hemispheres. Though, glutamine levels were higher in the contralateral CP of saline- and L-DOPA-treated rats as compared to the ipsilateral side. Relative mGluR5 uptake in the CP of levodopa-treated rats was also found positively correlated with abnormal involuntary movement scores. Conclusively, mGluR5 availability and glutamine concentrations in the CP are involved in PD, whereas mGluR5 availability in cortical regions may be involved in LID pathology.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder, characterized by a loss of dopaminergic neurons in the substantia nigra pars compacta (SN_{pc}) (Ma et al., 1997; Niethammer et al., 2013). Chronic L-3,4-dihydroxyphenylalanine (levodopa) therapy has been considered the gold standard for its treatment. However, it does not arrest dopaminergic neuronal degeneration and is associated with detrimental side effects, such as levodopa-induced dyskinesia (LID) (Fahn, 1974; Obeso et al., 1989; Ziv et al., 1997). LIDs are caused by a complex pattern of changes in the basal ganglia and cause characteristic chorea and dystonia (Duvoisin, 1974; Marsden and Parkes, 1976). Previous preclinical research and clinical research have shown an upregulation of postsynaptic dopamine receptors upon PD progression, which lowers the threshold for the dyskinetic effect of levodopa, whereas altered neuroplasticity, caused by the therapy itself, leads to increasing severity of dyskinesia upon chronic treatment (Cenci and Lundblad, 2006; Jenner, 2000). Furthermore, several groups suggested an overactive glutamate transmission in the basal ganglia to be a key factor in the maladaptive plasticity of PD and LID (Bezard et al., 2001; Chase and Oh, 2000).

At present, postponing the onset and reducing the severity of LID are still considerable issues in research. Among several non-dopaminergic strategies, metabotropic glutamate receptors (mGluRs) have recently been investigated as a novel target for the treatment of PD with and without LID. Of the 8 receptor subtypes, the metabotropic glutamate receptor type 5 (mGluR5) has received increasing attention. mGluR5 is expressed postsynaptically in the

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0197-4580/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.neurobiolaging.2017.09.006

striatopallidal synaptic cleft, where it is coupled with G-proteins to stimulate downstream modulators, including adenylyl cyclase, phospholipase C-β, and mitogen-activated protein kinase (Conn and Pin, 1997; Jong et al., 2009). Besides the striatum, high densities of mGluR5 can also be found on neurons of the hippocampus, cerebral cortex, and nucleus accumbens, as well as on immune cells such as astrocytes and microglia (Abe et al., 1992; Byrnes et al., 2009; Shigemoto et al., 1993). mGluR5 has been reported to play a causal role in the development of PD-related motor and cognitive dysfunctions and has neuroprotective properties in animal models (Battaglia et al., 2004; Breysse et al., 2003; Conn et al., 2005; Morin et al., 2014; Ossowska et al., 2007). In early-stage PD rats, decreased mGluR5 expression was reported while, in later stages of the disease, an upregulation was noted in the caudate-putamen (CP), similarly to observations upon LID development (Jenkins et al., 2015; Ouattara et al., 2010, 2011; Samadi et al., 2008). Nonetheless the efficacy of several mGluR5 antagonists in phase II clinical trials remains inconclusive, demanding a more detailed understanding of the role of mGluR5 in PD and LID pathology (Tison et al., 2016; Trenkwalder et al., 2016). To our knowledge, no data are available correlating receptor and ligand (glutamate) status in vivo to disease severity, in a longitudinal design. In this manuscript, we therefore wish to investigate, for the first time, mGluR5 and glutamate/glutamine levels in vivo in the well-known 6-hydroxydopamine (6-OHDA) PD model, upon development of LID. To do this, we employed 3-(18)F-fluoro-5-(2-pyridinylethynyl) benzonitrile ([^{18}F]FPEB) micro positron emission tomography (microPET) and proton magnetic resonance spectroscopy (^{1}H-MRS), in relation to behavioral measures.

2. Materials and methods

2.1. 6-OHDA animal model

All animal experiments were performed according to the European Communities Council Directive of November 24, 1986 (86/609/EEC) and approved by the local Animal Ethics Committee of the KU Leuven. Experiments were conducted on 30 female Wistar rats (on average 8 weeks old; body weight range at the start of the experiment 193.6 ± 11.7 g). Animals had free access to pellet food and tap water and were under a 12-hour light/dark cycle. Stereotactic 6-OHDA injections into the SN_{pc} were executed in accordance to the protocol described by Van der Perren et al. (2015). In short, animals were injected with 4 μL containing either 6-OHDA (n = 20; 24 μg dissolved in 4 μL of 0.05% ascorbate saline) or ascorbate saline (n = 10), using following coordinates for the SN_{pc}: anteroposterior (AP) = −5.3, lateral (LAT) = −2.0, and dorsoventral (DV) = −7.2. All rats were allowed to recover from surgery for 21 days, before the start of the experiment. A detailed timeline of the experiment is shown in Fig. 1. 6-OHDA- and saline-injected rats will be mentioned hereafter as PD and sham groups, respectively.

2.2. Levodopa treatment

To assess the effects of LID on mGluR5 and glutamate/glutamine levels, a subset of PD rats (n = 10) received levodopa (L-DOPA) therapy (6 mg/kg, intraperitoneal [i.p.], L-DOPA methyl ester; Sigma-Aldrich AB, Saint Louis, MO, USA) combined with a peripheral DOPA decarboxylase inhibitor, benserazide (12 mg/kg, i.p., benserazide HCl; Sigma) twice daily for 2 weeks. L-DOPA and benserazide were dissolved in physiological saline (2.0 mL/kg, i.p.) and administered in a single injection. Chronic treatment with this L-DOPA and benserazide dose has previously shown to cause a gradual development of LID-like movements in 6-OHDA-lesioned rats (Putterman et al., 2007). Control treatment (n = 10) consisted of injections with physiological 0.9% sterile saline (2.0 mL/kg, i.p.). L-DOPA- and saline-treated rats will be noted hereafter as L-DOPA and saline groups, respectively.

2.3. Small-animal PET imaging

mGluR5 imaging was performed using the radioligand [^{18}F]FPEB, which was synthesized on-site using the nitro-precursor obtained from ABX (Advanced Biochemical Compounds, Radeberg, Germany). To optimize synthesis, a chromafix anion exchange column conditioned with oxalate was used, with kryptofix in combination with the weaker base potassium oxalate for elution of the [^{18}F]-fluoride from the cartridge. PET experiments were performed on a lutetium oxyorthosilicate detector–based small-animal tomograph (FOCUS-220; Siemens/Concorde Microsystems, Knoxville, TN, USA). This tomograph has a 1.35 mm full-width at half-maximum transaxial resolution. Data were

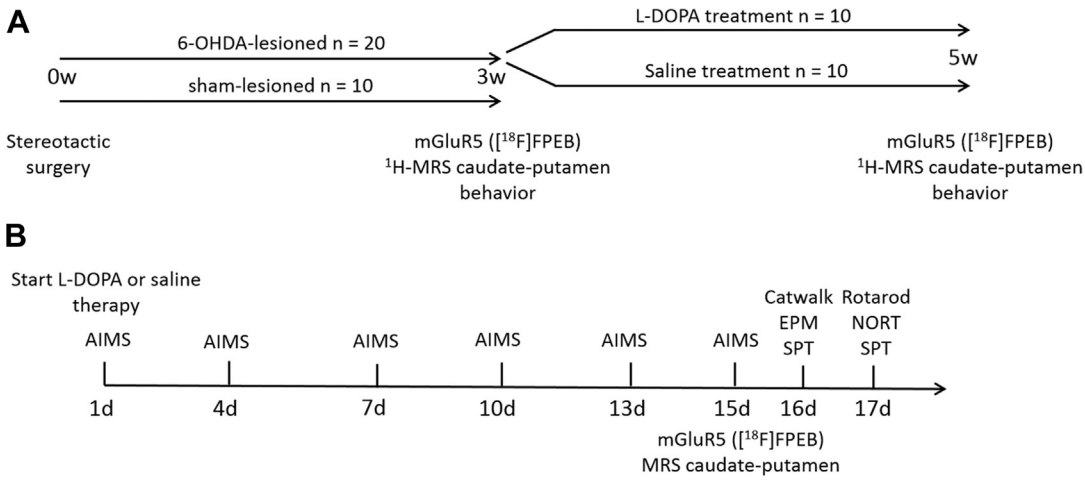

Fig. 1. Experiment timeline (A and B). (A) Experiment timeline indicating functional imaging (PET), glutamate/glutamine quantification (MRS), and behavioral tests conducted in 6-OHDA-lesioned rats and upon development of levodopa-induced dyskinesia. (B) A detailed description of time points at which behavioral tests were conducted during saline and L-DOPA treatment. Abbreviations: 6-OHDA, 6-hydroxydopamine; AIMS, abnormal involuntary movement score; d, day; EPM, elevated plus maze; L-DOPA, levodopa; MRS, magnetic resonance spectroscopy; NORT, novel object recognition test; PET, positron emission tomography; SPT, sucrose preference test; w, week.