NeuroImage 157 (2017) 209–218

Contents lists available at ScienceDirect

NeuroImage

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

# Altered adenosine 2A and dopamine D2 receptor availability in the 6-hydroxydopamine-treated rats with and without levodopa-induced dyskinesia

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ARTICLE INFO

*Keywords:*
PET
Parkinson's disease
Dopamine D2 receptor
Adenosine 2A receptor
[$^{11}$C]preladenant
6-OHDA-lesioned rats

ABSTRACT

Several lines of evidence imply alterations in adenosine signaling in Parkinson's disease (PD). Here, we investigated cerebral changes in adenosine 2A receptor ($A_{2A}R$) availability in 6-hydroxydopamine (6-OHDA)-lesioned rats with and without levodopa-induced dyskinesia (LID) using positron-emission tomography (PET) with [$^{11}$C]preladenant. In parallel dopamine type 2 receptor ($D_2R$) imaging with [$^{11}$C]raclopride PET and behavioral tests for motor and cognitive function were performed.

*Methods:* Parametric $A_{2A}R$ and $D_2R$ binding potential ($BP_{ND}$) images were reconstructed using reference tissue models with midbrain and cerebellum as reference tissue, respectively. All images were anatomically standardized to Paxinos space and analyzed using volume-of-interest (VOI) and voxel-based approaches. The behavioral alternations were assessed with the open field test, Y-maze, novel object recognition test, cylinder test, and abnormal involuntary movement (AIM) score. In total, 28 female Wistar rats were included.

*Results:* On the behavioral level, 6-OHDA-lesioned rats showed asymmetry in forepaw use and deficits in spatial memory and explorative behavior as compared to the sham-operated animals. 15-Days of levodopa (L-DOPA) treatment induced dyskinesia but did not alleviate motor deficits in PD rats. Intranigral 6-OHDA injection significantly increased $D_2R$ binding in the lesioned striatum ($BP_{ND}$: 2.69 ± 0.40 6-OHDA vs. 2.31 ± 0.18 sham, + 16.6%; p = 0.03), whereas L-DOPA treatment did not affect the $D_2R$ binding in the ipsilateral striatum of the PD rats. In addition, intranigral 6-OHDA injection tended to decrease the $A_{2A}R$ availability in the lesioned striatum. The decrease became significant when data were normalized to the non-affected side ($BP_{ND}$: 4.32 ± 0.41 6-OHDA vs. 4.58 ± 0.89 sham; NS, ratio: 0.94 ± 0.03 6-OHDA vs. 1.00 ± 0.02 sham; −6.1%; p = 0.01). L-DOPA treatment significantly increased $A_{2A}R$ binding in the affected striatum ($BP_{ND}$: 6.02 ± 0.91 L-DOPA vs. 4.90 ± 0.76 saline; + 23.4%; p = 0.02). In PD rats with LID, positive correlations were found between $D_2R$ and $A_{2A}R$ $BP_{ND}$ values in the ipsilateral striatum (r = 0.88, $p_{peak} = 8.56.10^{-4}$ uncorr), and between AIM score and the $D_2R$ $BP_{ND}$ in the contralateral striatum (r = 0.98; $p_{peak} = 9.55.10^{-5}$ uncorr).

*Conclusion:* $A_{2A}R$ availability changed in drug-naïve and in L-DOPA-treated PD rats. The observed correlations of striatal $D_2R$ availability with $A_{2A}R$ availability and with AIM score may provide new knowledge on striatal physiology and new possibilities to further unravel the functions of these targets in the pathophysiology of PD.

## Introduction

Parkinson's disease (PD) is a progressive degenerative disorder caused by the selective neurodegeneration of dopamine neurons in the substantia nigra pars compacta (SNpc). This neurodegenerative pattern leads to a striatal deficit in dopamine that underlies motor symptoms of PD, such as resting tremor, rigidity and bradykinesia (Samii et al., 2004). The current treatment for PD is aimed at restoring the striatal dopaminergic signaling using levodopa (L-DOPA) or dopamine receptor agonists. However, symptomatic or protective pharmacotherapeutic benefits with minimal

*Abbreviations:* $A_{2A}R$, Adenosine 2A receptor; AIM, abnormal involuntary movement; ANOVA, analysis of variance; $BP_{ND}$, non-displaceable binding potential; CNS, central nervous system; $D_2R$, dopamine type 2 receptor; L-DOPA, levodopa; LID, levodopa-induced dyskinesia; MRTM, Ichise's multilinear reference tissue model; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; PET, positron-emission tomography; SNpc, substantia nigra pars compacta; SPM, statistical parametric mapping; SRTM, simplified reference tissue model; SD, standard deviation; VOI, volume-of-interest

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http://dx.doi.org/10.1016/j.neuroimage.2017.05.066
Received 7 December 2016; Accepted 31 May 2017
Available online 02 June 2017

side effects remain limited, fueling the need for non-dopaminergic approaches.

Adenosine is an important modulator of neuronal activity in the brain. The brain adenosine effects are induced by four receptor subtypes: $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ (Fredholm et al., 2001). Of these, adenosine 2A receptors ($A_{2A}$Rs) are especially enriched in the basal ganglia, of which the striatum contains the highest density on medium-sized spiny neurons of the indirect output pathway (Ongini and Fredholm, 1996; Schiffmann and Vanderhaeghen, 1993). Striatal $A_{2A}$Rs interact with dopamine D2 receptors ($D_2$Rs) at the plasma membrane and the second messenger level, thus playing a role in the regulation of dopamine transmission (Fredholm and Svenningsson, 2003; Fuxe et al., 2007; Canals et al., 2003; Ciruela et al., 2004; Hillion et al., 2002). Therefore, the $A_{2A}$R has been proposed as a potential non-dopaminergic drug target for PD. In PD patients, altered $A_{2A}$R density, functionality and expression have been reported in brain sections of basal ganglia nuclei post-mortem (Hurley et al., 2000; Varani et al., 2010). Especially, dyskinesia development upon long-term L-DOPA therapy was reported to be linked with increased $A_{2A}$R expression in the lateral putamen ex vivo (Calon et al., 2004) and in the striatum of a small number of patients in vivo upon positron-emission tomography (PET) imaging (Ramlackhansingh et al., 2011; Mishina et al., 2011). The efficacy of several $A_{2A}$R antagonists in phase I/II clinical trials, however, remains inconclusive (Kalia et al., 2013), necessitating for more detailed understanding of the role of $A_{2A}$Rs in PD pathology.

In vivo imaging of the $A_{2A}$R has recently become feasible using quantitative PET due to the development of $A_{2A}$R selective radioligands, such as [$^{11}$C]preladenant (Zhou et al., 2014). So far only Bhattacharjee and coworkers briefly reported on in vivo $A_{2A}$R changes in a PD model (Bhattacharjee et al., 2011). They demonstrated increased $A_{2A}$R-tracer uptake in the ipsilateral striatum of hemiparkinsonian rats, but did not assess the effects of chronic L-DOPA treatment, nor studied the impact of $A_{2A}$R changes on behavioral outcome. Also the interaction between $A_{2A}$R and $D_2$R at the different stages of PD development and treatment remains unknown.

The objective of this study was thus to assess the effects of 6-hydroxydopamine (6-OHDA)-induced striatal lesions and L-DOPA treatment on striatal $A_{2A}$R availability, and its interaction with $D_2$R, using [$^{11}$C]preladenant- and [$^{11}$C]raclopride-PET. A secondary aim was to study the relationship between $A_{2A}$R-$D_2$R availability and PD symptoms in 6-OHDA lesioned rats with and without L-DOPA-induced dyskinesia (LID).

## Materials and methods

### *Animals*

Experiments were performed on 28 female Wistar rats (HsdCpb:WU, 245 ± 20 g at the day of PET scanning). All animals were housed in groups in Makrolon cages (38 × 26 × 24 cm$^3$) with a 12-h light-on period. Animals had ad libitum access to laboratory chow (RMH-B, The Netherlands) and tap water. The research protocol was approved by the Animal Care and Use Committee of the University of Groningen (DEC6689E) and was performed according to guidelines from the European Communities Council (decree 86/609/EEC). The study design is schematically presented in Fig. 1.

### *Nigrostriatal 6-OHDA lesion*

Stereotactic lesioning of the SNpc was performed under ketamine (60 mg/kg IP) and medetomidine (0.4 mg/kg IP) anesthesia. After 7 days of habituation, a stereotactic head frame (KOPF®, California, U.S.A) was used to drill a unilateral hole over the rats' right SNpc. The 6-OHDA (n = 21) and sham (n = 7) injections were done using 24 μg of 6-OHDA dissolved in 4 μL of 0.9% sterile NaCl containing 0.02% ascorbic acid or 0.9% sterile NaCl, respectively. The following coordinate was used: anterio-posterior − 5.3 mm; lateral + 2.0 mm and dorsoventral − 7.2 mm, relative to bregma, as described previously (Casteels et al., 2008). The 6-OHDA and 0.9% sterile NaCl solutions were injected over 8 min with an infusion rate of 0.5 μL/min, followed by 5 min of equilibrium before retracting the needle. All rats were allowed to a 21-day recovery period, before the start of the experiment.

### *Drug treatment*

To investigate the effects of LID on $A_{2A}$R and $D_2$R availability, a subset of 6-OHDA rats (n = 8) were admitted to drug treatment on day 21. L-DOPA (L-3,4-dihydroxyphenylalanine) and benserazide-HCl (a peripheral DOPA-decarboxylase inhibitor) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Both compounds were dissolved upon sonication (BRANSONIC, Shelton, U.S.A) in 0.9% saline and administered IP at 2.0 mL/kg body weight. L-DOPA (6 mg/kg) and benserazide-HCl (6 mg/kg) were given twice a day for 15 days. Chronic treatment with these doses gradually induces dyskinesia in 6-OHDA-lesioned rats (Putterman et al., 2007). Control treatment (n = 6) was done with 0.9% sterile NaCl (2.0 mL/kg IP).

### *Behavioral testing*

Motor and cognitive functions were assessed in all animals 1–2 days prior to PET imaging, i.e. at day 21 and 22 for the sham- and 6-OHDA-lesioned rats (group 1 and 2) and at day 35 and 36 for the 6-OHDA-lesioned rats on saline or L-DOPA treatment (group 3 and 4). All rats were tested during the 12 h light-on period using the cylinder test and open field test for motor assessment, and using the novel object recognition test and Y-maze test for assessment of cognitive capability. Animals subjected to L-DOPA or saline treatment were tested for LID using abnormal involuntary movement (AIM) recording. A description of behavioral testing procedures in detail can be found in the Supplementary material.

### *Radiotracer preparation and PET imaging*

One day after behavioral testing, animals were subjected to PET imaging. Brain $A_{2A}$R imaging was done using the high affinity radioligand [$^{11}$C]preladenant (2-(2-furanyl)-7-[2-[4-[4-(2-[$^{11}$C]methoxyethoxy)phenyl]-1-piperazinyl]ethyl]7H-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine-5-amine; rat Ki = 2.5 nM) (Neustadt et al., 2007). Radiolabeling was done by methylation of the O-desmethyl-preladenant (in-house prepared precursor) with [$^{11}$C]methyl iodide, as previously described (Zhou et al., 2014). Brain $D_2$R imaging was performed using the radioligand [$^{11}$C]raclopride (Ki = 1.9 nM) (Seeman et al., 1989). Radiolabeling was done by alkylation of S-(+)-O-demethylraclopride (ABX, Radeberg, Germany) using [$^{11}$C] methyl iodide. The radiochemical purity of both radioligands was > 98%; specific activity at the end of synthesis ranged 64–637 GBq/μmol.

Prior to PET imaging, anesthesia was induced to all rats with isoflurane in medical air (induction: 5%, maintenance: 1.5–2.5%). All rats were kept on electronic heating pads to maintain their body temperature during the study. Their tail veins were catheterized for injection of one of the radioligands ([$^{11}$C]preladenant 48.2 ± 26.6 MBq (2.6 ± 1.3 nmol/kg) and [$^{11}$C]raclopride 32.5 ± 17.7 MBq (0.8 ± 0.6 nmol/kg); no significant differences in injected mass between groups; total volume approximately 0.5 mL). The injected mass resulted in receptor occupancy of < 5% for both $A_{2A}$R and $D_2$R (Zhou et al., 2017; Boyson et al., 1986). [$^{11}$C]Preladenant and [$^{11}$C] raclopride PET data were acquired on the same day with an alternating order (~ 4-h interval between 1st and 2nd scan). Animals were allowed to wake up after the first scan. PET imaging was done on a Focus220 tomograph (Preclinical Solutions, Siemens Healthcare Molecular Imaging, U.S.A Inc.), which has a maximum transaxial resolution of 1.5 mm. All acquisitions were obtained dynamically for 60 min. The