Published in final edited form as:

Schizophr Res. 2015 October ; 168(0): 373–376. doi:10.1016/j.schres.2015.06.027.

ANTIPSYCHOTIC BINDING TO THE DOPAMINE-3 RECEPTOR IN HUMANS: A PET STUDY WITH [¹¹C]-(+)-PHNO

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Abstract

Background—All currently available antipsychotic medications bind to both the dopamine-2 (D2) and dopamine-3 (D3) receptors *in vitro*. However, there is conflicting evidence from *in vivo* studies about whether or not antipsychotic medications bind to the D3 receptor (D3R). The purpose of this study was to determine whether acute doses of risperidone bind to the D3R in humans.

Methods—We performed PET scans on an mCT scanner with [¹¹C]-(+)-PHNO injected as a bolus, before and after a 2mg oral dose of risperidone in five medication free subjects with schizophrenia. Subjects were scanned for 120 minutes and underwent an MRI scan for region of interest delineation and coregistration. Cerebellum was used as a reference region. Simplified reference tissue modeling (SRTM) was used to calculate BP_{ND}.

Results—We observed binding to the D3R receptor by risperidone as evidenced by observable occupancy in regions in which the [¹¹C]-(+)-PHNO signal is almost exclusively from the D3R (i.e., substantia nigra/ventral tegmental area). Using a regression model to estimate D2R:D3R selectivity, we observed a D2R:D3R selectivity of 2.1 for risperidone.

Conclusion—Our preliminary results provide further support that acute doses of antipsychotic medications bind to the D3R and provide additional support for the further development of this receptor as a treatment target in schizophrenia.

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Contributors

All authors contributed to the study design and implementation. Authors RRG and MS managed the literature searches and analyses. All authors contributed to interpreting the analyses. Author RRG wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest

RRG is disclosing that he receives research support from Otsuka and Genentech. JAL serves on the advisory board of Intra-Cellular Therapies. He receives grant support from Allon, Biomarin, Eli Lilly, F. Hoffman–La Roche, Genentech, GlaxoSmithKline, Merck, Novartis, Pfizer, Psychogenics, Sepracor (Sunovion), and Targacept and holds a patent from Repligen. MS has consulted for Amgen and has received research support from Pierre Fabre. AA has received research support from Pierre Fabre and Forest, and has been a consultant for or on the scientific advisory board of Roche, Pfizer, Takeda, Otsuka, Amgen and Shire.

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Keywords

risperidone; dopamine-3; PET; PHNO; schizophrenia; occupancy

1. Introduction

While extensive preclinical data suggest that antipsychotic medications display similar affinities to D3Rs and D2Rs (Sokoloff et al., 2006), D3R binding levels by antipsychotics were called into question by two recent studies using [^{11}C]-(+)-PHNO, a relatively recently developed D3R-preferring radioligand (Ginovart et al., 2007; Wilson et al., 2005b), in populations of chronic (Graff-Guerrero et al., 2009) and drug-naïve (Mizrahi et al., 2011) individuals with SCZ which showed that, after several weeks of treatment with antipsychotic medications, no binding in D3R-rich regions was observed.

These data are surprising given the *in vitro* data which suggest that antipsychotic medications bind to both D2Rs and D3Rs. In light of these data, we performed PET imaging in baboons with [^{11}C]-(+)-PHNO to examine the binding to D2Rs and D3Rs by single, intravenous doses of clozapine (0.5534 mg/kg) and haloperidol (0.0109 mg/kg) and estimated D2R:D3R selectivity as 5.25 for clozapine and 2.38 for haloperidol, which are similar to *in vitro* estimates (Girgis et al., 2011).

Our objective in this study was to examine the binding of a single dose of risperidone to the D3R in patients with SCZ in order to determine whether our acute dose findings in non-human primates extend to humans.

2. Patients and Methods

2.1 Subjects

The study was approved by the Institutional Review Board of the New York State Psychiatric Institute. Subjects were medically healthy, psychiatrically stable (i.e., not an acute safety risk, able to tolerate a medication free interval), non-substance using adult (ages 18–60) patients with SCZ or schizoaffective disorder. Diagnoses were confirmed with the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1995) or Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994). Subjects were eligible if they had a Clinical Global Impression Score-Severity of 5 or less (Guy, 1976), weighed between 44 and 115kg, and were antipsychotic free for at least 21 days. Subjects were recruited from the Lieber Schizophrenia Research Clinic at Columbia University and the New York State Psychiatric Institute. All participants provided written informed consent after receiving oral and written descriptions of the study procedures and aims. Nine subjects were enrolled.

2.2 PET Procedures

PET studies were performed on a Siemens mCT hybrid PET/CT scanner (Siemens, Knoxville TN). [^{11}C]-(+)-PHNO was prepared by N-acylation of the despropyl precursor with [^{11}C]-propionyl chloride followed by reduction of the resulting amide with lithium aluminum hydride and purification by reverse-phase high performance liquid chromatography (HPLC) (Wilson et al., 2005a). An intravenous catheter was inserted in the

antecubital vein for radiotracer administration. Head movement was prevented with a polyurethane head immobilizer system molded around the subject's head (Soule Medical, Tampa, FL). A 7 second CT scan was performed for attenuation correction immediately prior to [^{11}C]-(+)-PHNO administration (maximum injected dose 15mCi). Following tracer administration, emission data were collected for 120 minutes. 3D data were acquired in list mode, rebinned into a sequence of 21 frames of increasing duration (3×20 seconds, 3×1 minute, 3×2 minutes, 2×5 minutes and 10×10 minutes), and, following correction for scatter, random coincidences, dead-time and attenuation, reconstructed by filtered back projection using manufacturer-supplied software. Correction for isotope decay was applied. Reconstructed voxel dimensions were 1 mm $\times$ 1 mm in-plane; slice thickness was 2 mm. Subjects received a second [^{11}C]-(+)-PHNO PET scan 2 hours after receiving 2mg of risperidone PO.

2.3 MRI scans

High-resolution T1-weighted MRI scans (Spoiled Gradient Recall, SPGR) were acquired for each subject for purposes of image coregistration and region of interest delineation.

2.4 Data Analysis

Data Preprocessing—Reconstructed PET data were analyzed with a combination of SPM8 (Wellcome Trust), MEDx (Medical Numerics, Germantown MD) and in house-developed software using the Matlab programming environment (Mathworks, Natick, MA). PET frames were realigned and coregistered to individual subjects' structural MRIs with SPM8. Regions of interest (ROIs) were drawn manually on individual's MRIs in MEDx and transferred to the coregistered PET. Included regions were pre-commissural caudate (pre-ca), post-commissural caudate (post-ca), total caudate (CAD) pre-commissural putamen (pre-pu), post-commissural putamen (post-pu), total putamen (PUT), ventral striatum (VST), GP, thalamus (THA), and a midbrain region encompassing the substantia nigra and the ventral tegmental area (SN/VTA). Cerebellum (CER) was included as a reference tissue as shown previously (Ginovart et al., 2007; Willeit et al., 2006). Operational definitions for all ROIs have been published previously (Abi-Dargham et al., 2000; Martinez et al., 2003). After transfer of ROIs to PET data, the average activity in each ROI in each frame of data was computed and ROI time activity curves were formed.

Kinetic Analysis—All time activity curve data were analyzed using the SRTM model (Lammertsma and Hume, 1996) with cerebellum as reference region to obtain the primary outcome measure, the regional binding potential BP_{ND} . The relative change in BP_{ND} following risperidone, BP_{ND} , was computed as $\text{BP}_{\text{ND}} = [\text{BP}_{\text{ND}} (\text{post-dose}) - \text{BP}_{\text{ND}} (\text{baseline})] / \text{BP}_{\text{ND}} (\text{baseline})$. [^{11}C]-(+)-PHNO BP_{ND} contains a mixture of D2R and D3R binding. Generally, SN/VTA BP_{ND} is thought to be mostly D3 binding whereas dorsal striatum BP_{ND} is thought to be mostly D2 binding, with other regions where BP_{ND} is non-negligible (GP, VST and THA) having mixed D2R/D3R binding (Narendran et al., 2006; Searle et al., 2010b). Complete use of all the data requires an additional regression model to obtain separate estimates of D2R and D3R occupancy (Rabiner et al., 2009). [^{11}C]-(+)-PHNO BP_{ND} were therefore fitted to the nonlinear regression model $\text{BP}_{\text{ND}} = f_{\text{D3}}(\text{D3 occupancy}) + (1 - f_{\text{D3}})(\text{D2 occupancy})$ where f_{D3} is the fraction of the regional baseline

BP_{ND} attributable to D3R receptor binding. In this model, regional f_{D3} is fitted across all sample subjects, whereas occupancy at the D2R and D3R is fitted separately for each subject.

3. Results

Of the nine subjects who consented for this study, one received only a baseline scan due to chemistry failure and was not included in the data analysis, and three others withdrew consent. Two female subjects and 3 male subjects, ages 42 ± 9 yrs of age (range 34 to 55), completed procedures. Ethnicity of subjects was 4 African American and one of more than one race. Two subjects were Hispanic and 3 were non-Hispanic. Baseline psychopathology was assessed with the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987). The average total PANSS score was 56.4 ± 22.4 . All subjects were non-substance using and medication free for at least 3 weeks before the first PET scan. All subjects had previously been treated with at least 2 weeks of antipsychotic medications in their lifetime and before their medication free interval.

Average injected radioactivity was 210 ± 90 MBq at baseline and 228 ± 101 MBq following risperidone. Specific activity was 55 ± 70 MBq/nmol at baseline and 94 ± 122 MBq/nmol following risperidone. Injected mass of [¹¹C]-(+)-PHNO was 1.85 ± 0.22 µg at baseline and 1.98 ± 0.03 µg following risperidone. There were no significant differences between conditions in any of these parameters (two sided paired t test or Wilcoxon nonparametric t test). Table 1 shows SRTM BP_{ND} and BP_{ND} across subjects and brain regions. These results demonstrate robust occupancy of a single 2mg oral dose of risperidone in all examined brain regions, including midbrain in which the [¹¹C]-(+)-PHNO signal is essentially all due to D3R binding (Rabiner et al., 2009; Searle et al., 2010a). Estimated occupancy results from the regression model were 53 ± 10 % at D2R and 24 ± 9 % at D3R, with a D2:D3 selectivity ratio of 2.21 ($R^2=0.79$).

4. Discussion

This preliminary study used [¹¹C]-(+)-PHNO to investigate whether acute doses of risperidone bind to the D3R in individuals with SCZ. The results demonstrate robust occupancy in all regions examined, including regions in which the [¹¹C]-(+)-PHNO signal is nearly all due to D3R binding (SN/MTA). In addition, the regression model estimated a D2R:D3R selectivity ratio of 2.21 which is very similar to published *in vitro* values (Levant, 1997; Schotte et al., 1996). These data suggest that acute doses of risperidone bind to the D3R, as predicted by *in vitro* studies, and agree with our previous report showing D3 occupancy by acute doses of clozapine and haloperidol in non-human primates (Girgis et al., 2011).

Two other studies observed paradoxical increases in [¹¹C]-(+)-PHNO binding in GP and SN/MTA following subchronic administration of various antipsychotic medications including risperidone (Graff-Guerrero et al., 2009; Mizrahi et al., 2011). Taken together, these data suggest that D3R upregulation occurs over the course of treatment, but more definitive evidence would be provided by a study in which the same subjects were imaged following acute and subchronic dosing.

A limitation of this study is the small sample size, but nonetheless, measured occupancy was robust and consistent across subjects (Supplemental Table 1). In addition, this study does not address the contribution of D3R occupancy to the clinical effect of antipsychotic medications nor the relative contributions of D3R and D2R stimulation to psychosis. However, this is first step in establishing that the D3R is engaged by commonly used antipsychotic medications and this was the main goal of this study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to acknowledge the patients who participated in this study.

Funding Body Agreements and Policies

The project described was supported by the National Institute of Health: 1) Center for Research Resources and the National Center for Advancing Translational Sciences, 2KL2RR024157; 2) a NARSAD Young Investigator Award; 3) the Evelyn Toll Family Foundation; 4) Lieber Center for Schizophrenia Research and 5) New York State Office of Mental Hygiene

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Figure 1. BP_{ND} across conditions. Coronal (top) and transverse (bottom) slice levels are indicated by the lines on the sagittal view (bottom right). Data are averaged across the 5 subjects in the study. All data are normalized into the MNI single subject template space and the average BP_{ND} maps are fused with the MRI. The image shows decreased binding in the GP and VST following risperidone.

Table 1

Average $\pm$ SD binding potentials for pre and post-risperidone scans.

Region	Baseline BP _{ND}	Post Risperidone BP _{ND}	- BP _{ND}
AST	1.97 $\pm$ 0.48	1.14 $\pm$ 0.30	41 $\pm$ 12
CAD	1.74 $\pm$ 0.44	0.93 $\pm$ 0.27	46 $\pm$ 11
SN/VTA (midbrain)	0.86 $\pm$ 0.22	0.62 $\pm$ 0.19	28 $\pm$ 10
GP	4.59 $\pm$ 0.88	3.46 $\pm$ 0.87	24 $\pm$ 13
PUT	2.15 $\pm$ 0.49	1.24 $\pm$ 0.30	40 $\pm$ 18
STR	2.08 $\pm$ 0.46	1.27 $\pm$ 0.31	38 $\pm$ 14
THA	0.67 $\pm$ 0.25	0.34 $\pm$ 0.19	51 $\pm$ 10
VST	3.66 $\pm$ 0.58	2.57 $\pm$ 0.44	30 $\pm$ 5

AST, associative striatum; STR, striatum.