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Striatal dopamine D1-like receptor binding is unchanged in primary focal dystonia

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Abstract

Background—Multiple studies demonstrate decreases in striatal D2-like (D2, D3) radioligand binding in primary focal dystonias. Although most investigations have focused on D2 specific receptors (D2R), a recent study suggests that the decreased D2-like binding may be due to a D3 specific (D3R) abnormality. However, only limited data exist on the role of D1 specific receptors (D1R) and the D1R-mediated pathways within basal ganglia in dystonia. Metabolic PET data in primary generalized dystonia suggest resting state overactivity in the D1R-mediated "direct" pathway leading to excessive disinhibition of motor cortical areas. This work investigated whether striatal D1-like receptors are affected in primary focal dystonias.

Methods—We measured striatal (caudate and putamen) specific binding of the D1-like radioligand [¹¹C] NNC 112 using PET in 19 patients with primary focal dystonia (cranial, cervical or arm) and 18 controls.

Results—We did not detect a statistically significant difference in striatal D1-like binding between the two groups. This study had 91% power to detect a 20% difference, making a false negative study unlikely.

Conclusions—Since [¹¹C] NNC112 has a high affinity for D1-like receptors, very low affinity for D2-like receptors and minimal sensitivity to endogenous dopamine levels, we conclude that D1-like receptor binding is not impaired in these primary focal dystonias.

Kamun et al.

Page 2

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Introduction

Dystonia is a disabling involuntary movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both.¹ Multiple lines of evidence in human and animal models demonstrate abnormal dopaminergic systems in dystonia. Dystonia can be an initial manifestation in Parkinson disease (PD) that may respond to L-dopa^{2,3} or can be a side effect of L-dopa treatment in PD.⁴ Hereditary dystonias due to defects in dopamine synthesis respond to L-dopa.⁵ D5 specific receptor (D5R) alterations may result from a susceptibility gene for cervical dystonia.⁶ Further, animal models of various genetic dystonias indicate involvement of dopaminergic pathways. The TOR1A mouse model of childhood onset generalized dystonia has impaired dopamine release.⁷ D2 R availability is reduced in the DYT11 mouse model of myoclonus dystonia.⁸

Between the two classes of dopamine receptors (D1-like and D2-like), most studies have investigated the role of D2-like family (D2, D3, D4) receptors. In contrast the role of D1-like family (D1, D5) receptors remains largely unknown.

Molecular neuroimaging has provided important insights into the role of dopaminergic pathways in people with dystonia. Several neuroimaging studies reported decreased D2-like radioligand binding in primary dystonias.^{9,10} Most studies have focused on striatal D2R^{10,11} with the assumption that striatal concentrations of D3R and D4R are negligible. However we recently demonstrated lack of decrease in striatal binding of the highly D2R specific radioligand N-([¹⁸F]Methyl) benperidol [¹⁸F]NMB in adult onset focal dystonia.¹² This finding in conjunction with the recent report that D3R concentration in human caudate and putamen is much higher than previously assumed¹³ suggests that dystonia may be associated with a reduction in striatal D3R. Conversely, there are only limited data on the role of D1R and D1R mediated direct pathway in dystonia. One report did not find any change in striatal D1-like receptors in humans with dopa-responsive dystonia (DRD).¹⁴ However, a metabolic PET study showed resting state overactivity in the lentiform nucleus¹⁵, which may reflect increased input to this region. These data have been interpreted as increased activity in the D1R mediated "direct" pathway from the striatum to the GPi, leading to excessive inhibition of the GPi and excessive disinhibition of motor cortical areas that could contribute to the clinical manifestations of dystonia.

In this study we quantified striatal uptake of the D1-like specific radioligand [¹¹C]NNC 112 in patients with adult onset focal dystonias to further characterize role of D1-like receptors in this patient population.

Methods

Subjects

Nineteen subjects with primary cervical, cranial or arm dystonia (age: 58±10; 9 women and 10 men) were recruited from the Movement Disorder Center at Washington University in St. Louis.¹⁶ Only patients who could hold their head still when lying down were enrolled in the

study. Eighteen healthy controls (age: 56 ± 8 ; 13 women and 5 men) were enrolled as well. All subjects underwent a complete history and neurological examination to exclude secondary dystonias or concomitant neurological disorder. No one had prior exposure to reserpine, tetrabenazine, dopamine antagonists, or amphetamines. Three subjects had exposure to L-dopa for less than two months after the initial diagnosis but not within the last year prior to imaging. All but two dystonic subjects had received botulinum toxin within the last 3 months. Only one dystonic and one healthy control subject reported a history of smoking. All subjects had Mini-Mental Status examinations.¹⁷ These studies were approved by the Washington Human Research Protection Office and the Radioactive Drug and Research Committee. Each participant provided written informed consent.

Radiopharmaceutical preparation

[¹¹C]NNC 112 ([¹¹C](+)-8-chloro-5-(7-benzofuranyl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine) was prepared by [¹¹C]methylation of (+)-*N*-desmethyl NNC 112 (provided by the NIMH Chemical Synthesis and Drug Supply Program) using modification of previously-reported procedures.¹⁸ Carbon-11 was produced as ¹¹CO₂ using the Washington University JSW BC 16/8 cyclotron and the ¹⁴N(p,α)¹¹C nuclear reaction. The ¹¹CO₂ was converted to ¹¹CH₃I using the microprocessor-controlled PETtrace MeI MicroLab (GE Medical Systems, Milwaukee, WI), and immediately used for [¹¹C]methylation of (S)-*O*-desmethy NNC 112. Product [¹¹C] NNC 112 was purified via semipreparative HPLC, and reformulated in a 10% ethanol/ normal saline solution. The radiochemical purity exceeded 95%, and the specific activity exceeded 1200 Ci/mmol (44.4 TBq/mmol), as determined by analytical HPLC. The mass of NNC 112 was 6.5 µg per injected dose.

MRI procedure

All subjects had a brain magnetization-prepared rapid gradient echo (MP-RAGE)¹⁹ on a 3.0T Siemens Trio scanner using a twelve channel head coil. MP-RAGE is a T1-weighted MRI which offers high contrast to noise structural MR images required for defining volumes of interest (VOI).²⁰ A two-tailed unpaired t-test was applied to compare the volumes of the VOIs used for cerebellum, caudate and putamen between two groups with the alpha level of 0.05.

PET procedure

PET studies were performed using a Siemens/CTI ECAT HR plus in 3D mode. We placed a 20 gauge plastic catheter in an antecubital vein for radioligand injection. Each subject was positioned in the PET scanner using cross laser lines, and a polyform mask (Roylan Industries, Menomonee Falls, WI) helped reduce head movement during the scan. We also videotaped the head to detect movement of the head or face during the PET. A rotating [⁶⁸Ge] rod source was used to collect a transmission scan to measure individual attenuation factors. After a bolus (40 sec) intravenous injection of [¹¹C] NNC112 (8–20 mCi; 296–740 MBq), we collected a 90 minute dynamic acquisition in 27 sequential frames (6 frames each 30 sec, 3 frames each 1 min, 2 frames each 2 min, 16 frames each 5 min). The emission scan

was reconstructed with corrections for attenuation, randoms and scatter yielding a transaxial and axial spatial resolution of 4.6 mm and 4.1 mm full width half maximum, respectively.

PET Data Analysis

Data were analyzed in a blinded fashion. Review of the video recordings during the scan did not reveal any visible head motion.

Dynamic PET images were realigned using automatic image registration (AIR),²¹ so that slow head drift was corrected by aligning the frames. The total drift in any axis did not exceed 10 mm. Volumes of interest (VOIs) were manually drawn on the MP-RAGE for the caudate, putamen and cerebellum following the outline of the anatomical structures for each subject individually in the native space. The MP-RAGE from each subject then was co-registered to the aligned PET image. A summed PET image (frames 2–27) was used for this step. These VOIs were transformed into PET coordinates using the transformation matrices for the MRI to PET co-registration.²² Partial volume effects (PVE) correction was performed using a method that estimates the gray-matter activity in each VOI.^{23,24} A decay corrected tissue activity curve for each VOIs was extracted from the PET data and analyzed by Logan graphical method using a cerebellar region as the reference to calculate the non-displaceable binding potential (BP_{ND}).²⁵ We set the start of linearity at 15 min based on numerous animal and human NNC 112 PET data that were acquired preceding this study.

A two-tailed unpaired t-test was applied to compare the regional values of BP_{ND} and age between healthy controls and subjects with focal dystonia. We used ANCOVA to determine the effect of age and sex and their interaction on putamen and caudate BP_{ND} in healthy controls. GPower 3.1 (www.g-power.de) was used for post-hoc analysis of achieved power. This study had 91% power to detect 20% difference between the two groups with an alpha level of 0.05.

Voxelwise whole brain analysis

We used Ichise's multilinear reference tissue model (MRTM) for voxelwise binding potential analysis.²⁶ The PET images were registered to MP-RAGE data using the vector gradient method. Atlas registration was made using 12-degree-of-freedom affine transformation via MP-RAGE using in-house software.²⁷ BP_{ND} map for each subject was then transformed to the atlas space for further analysis. We used SPM8 (<http://www.fil.ion.ucl.ac.uk>) to perform a t-test between controls and dystonic subjects using a Family Wise Error (FWE) rate of 0.05 to correct for multiple comparisons.

Results

Age distribution of healthy controls (M=56, SD=8) did not significantly differ from dystonic subjects (M=58, SD=10), $t(35)=0.57$, $p=0.58$. Similarly, sex distribution did not differ significantly between the two groups $t(35)=1.55$, $p=0.13$. All participants scored higher than 28 in MMSE. The Table has further characteristics of the dystonia group.

Putamenal binding potential (BP_{ND}), a measure of specific binding, did not differ between healthy (M=1.97, SD=0.34) and dystonic subjects (M=1.87, SD=0.35), $t(35)=0.857$,

$p=0.40$. Similarly caudate BP_{ND} did not significantly differ between healthy ($M=1.60$, $SD=0.44$) and dystonic subjects ($M=1.64$, $SD=0.31$), $t(35)=0.364$, $p=0.069$ (Figure). An ANCOVA indicated no main effect of sex ($F(1, 15)=2.07$, $p=0.17$, or age ($F(1, 15)=0.006$, $p=0.94$) on putamenal BP_{ND} but did reveal a significant interaction between sex and age ($F(1, 15)=7.64$, $p=0.01$) in healthy controls. There was no main effect of sex ($F(1, 15)=1.90$, $p=0.19$) or age ($F(1, 15)=0.02$, $p=0.89$) and no interaction between sex and age ($F(1, 15)=3.74$, $p=0.07$) on caudate BP_{ND} in healthy controls.

There was no statistically significant difference between the volumes used for cerebellum, caudate or putamen between the dystonic subjects and healthy controls ($p > 0.91$ for all measures).

The whole brain voxelwise analysis did not identify any statistically significant differences in the NNC112 BP_{ND} between the controls and dystonics.

Discussion

We did not detect a statistically significant difference in striatal (putamen or caudate) uptake of the D1-like radioligand [^{11}C] NNC 112 in people with primary focal dystonia (arm, cervical or cranial). This study was designed with a high power (91%) to detect at least a 20% difference between dystonics and controls making a false negative study unlikely. Since [^{11}C] NNC112 has a high affinity for binding to D1-like receptors with very low affinity to D2-like receptors and is not sensitive to endogenous dopamine levels^{28,29} we conclude that striatal D1-like specific binding is not impaired in primary focal dystonias. Despite strong evidence of association between primary dystonia and a defect in dopaminergic mediated pathways, neither D1-like nor D2R seems to be altered.¹²

Nevertheless, lack of an abnormality in D1-like or D2R binding does not exclude involvement of D1R or D2R mediated pathways in the pathophysiology of primary dystonia. Alternatively changes in cholinergic striatal interneurons could alter dopaminergic mediated basal ganglia circuits.³⁰ Clinical evidence of symptomatic response to anticholinergics for dystonic symptoms supports this hypothesis. DYT1 mouse models have abnormalities in the cholinergic system.^{31–33} Furthermore, striatal cholinergic density may be decreased in cervical dystonia.³⁴ Specific radioligands are available for PET studies of acetylcholine receptors (FP-TZTP for muscarinic type, 2-FA-85380 for nicotinic type) and specific PET radioligands for acetylcholine transporters may soon be available for human studies.³⁵

Furthermore, D3R have not yet been measured in primary focal dystonia and could be involved in dopaminergic synaptic regulation by influencing extracellular dopamine level via regulation of membranous dopamine transporter (DAT), by effects on auto-regulation or by synergistic interaction with D1R.^{36–39} [^{11}C]PHNO is currently used for investigation of D3R mainly in regions with relatively high D3R concentration since this agonist radioligand has poor selectivity for D2R versus D3R dopamine receptors.⁴⁰ Given the lower concentration of D3R compared to D2R in striatum it is not an ideal candidate for examining the role of striatal D3R in focal dystonias. Development of a more selective D3R radioligand will be valuable for such studies.⁴¹

Our voxelwise whole brain exploratory analysis was consistent with VOI analysis and did not reveal any statistically significant differences in striatal BP_{ND} between the dystonics and healthy controls. We did not find any significant differences in cortical BP_{ND} either.

Numerous studies report age dependent reduction of dopamine receptors in humans and other mammals. Autopsy studies investigating the density of striatal D1-like receptors using [³H]SCH 23390 autoradiography showed marked decrease in the first decade of life but no significant reductions beyond age of 40.⁴² Two PET studies using [¹¹C]SCH23390 demonstrated significant correlations between striatal binding and age in people ranging from either 6 to 93 years old ($R^2=0.39$, $p<0.001$)⁴³ or from 22–74 years old ($R^2=0.77$, $p<0.001$).⁴⁴ However such correlations were either not significant or substantially weaker at the age ranges corresponding to the more limited range of our participants. Our study has 90% power to detect a significant correlation between age and D1-like receptor density with $R^2=0.4$ and $p=0.05$. The most likely reason that we did not detect a significant correlation with age may be the lack of a true correlation in this age range as would be anticipated from the previously reported post-mortem study noted above.

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Figure.

There is no statistically significant difference between healthy subjects and those with focal dystonia in [¹¹C]NNC 112 BP_{ND} of caudate or putamen.

Table

Summary of participants' clinical characteristics

Characteristics of participants		
	Control	Dystonic
N	18	19
Mean age (yr)	56±8	58±10
Gender	13 F; 6 M	9 F; 10 M
Smoker	1	1
Right handedness	18	18
Family history of dystonia	N/A	2 (father and son)
Treated with botulinum toxin within the last 3 months	N/A	18
Cranial dystonia	N/A	8 (6 pure blepharospasm)
Hand dystonia	N/A	7
Cervical dystonia	N/A	4
Pure writing cramp	N/A	0
Median duration of dystonia (yr)	N/A	12
MMSE score	28	28