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Decreased striatal dopamine receptor binding in primary focal dystonia: a D2 or D3 defect?

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Abstract

Dystonia is an involuntary movement disorder characterized by repetitive patterned or sustained muscle contractions causing twisting or abnormal postures. Several lines of evidence suggest that abnormalities of dopaminergic pathways contribute to the pathophysiology of dystonia. In particular dysfunction of D2-like receptors that mediate function of the indirect pathway in the basal ganglia may play a key role. We have demonstrated with positron emission tomography (PET) that patients with primary focal cranial or hand dystonia have reduced putamenal specific binding of [^{18}F]spiperone a non-selective D2-like radioligand with nearly equal affinity for serotonergic 5-HT(2A) sites. We then repeated the study with [^{18}F]N-methyl-benperidol (NMB), a more selective D2-like receptor radioligand with minimal affinity for 5-HT(2A). Surprisingly, there was no decrease in NMB binding in the putamen of subjects with dystonia. Our findings excluded reductions of putamenal uptake greater than 20% with 95% confidence intervals. Following analysis of the *in vitro* selectivity of NMB and spiperone demonstrated that NMB was highly selective for D2 receptors relative to D3 receptors (200-fold difference in affinity), whereas spiperone has similar affinity for all three of the D2-like receptor subtypes. These findings coupled with other literature suggest that a defect in D3, rather than D2, receptor expression may be associated with primary focal dystonia.

Keywords

dystonia; dopamine; NMB; D2-like dopamine receptors; D3 dopamine receptor; PET

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Introduction

Dystonia is an involuntary movement disorder characterized by repetitive patterned or sustained muscle contractions causing twisting or abnormal postures.1 Several lines of evidence suggest that abnormalities of dopaminergic pathways contribute to the pathophysiology of dystonia. Dystonia can be the initial manifestation in some patients with Parkinson disease (PD) which respond to L-dopa.2,3 Patients with PD can develop dystonia as a side effect of L-dopa treatment.4 Hereditary forms of dystonia due to abnormal synthesis of dopamine respond to treatment with L-dopa.5 Further, a mutation in the gene coding for D5 receptors might be a susceptibility factor for cervical dystonia.6 Some people with familial dystonia have reduced [^{18}F]dopa uptake in putamen.7 Finally, dopamine release may be impaired in a mouse model of DYT1dystonia8 and is also diminished in a non-human primate model of transient dystonia.9

In particular, dysfunction of D2-like receptors that mediate function of the indirect pathway in the basal ganglia may play a key role.10,11 Exposure to neuroleptics that block D2-like receptors can cause acute dystonia and long term treatment with these drugs can produce tardive dystonia.12–14 Non-manifesting carriers of DYT1 have decreased D2-like binding. 11 Further, non-human primates treated with intra-carotid MPTP developed transient dystonia that corresponded to a decrease in D2-like striatal receptors.15

We demonstrated that patients with primary focal hand or cranial dystonia have a reduction of the putamenal specific binding of [^{18}F]spiperone, a non-selective D2-like radioligand with nearly equal affinity to serotonergic 5-HT(2A) receptors.10,16 In contrast, [^{18}F]N-methyl-benperidol (NMB) is a more specific D2-like receptor ligand with minimal affinity for 5-HT(2A) or D1-like receptors.17

A goal of this study was to determine whether patients with primary focal hand or cranial dystonia have reduced striatal D2-like dopamine receptor binding as measured with the more selective positron emission tomography (PET) radioligand [^{18}F]NMB. This would exclude the possibility that a defect in 5-HT(2A) receptors accounted for the previous findings with [^{18}F]spiperone. Given the unanticipated finding that PET studies with [^{18}F]NMB demonstrated no difference in receptor binding within dystonic patients and controls, we proceeded to investigate the subtype selectivity of NMB and spiperone for dopaminergic receptors.

Methods

Subjects

Twenty four subjects with primary cranial or arm dystonia were included (age: 52±11; 16 women, 8 men). No subject had evidence of a secondary cause of dystonia as assessed by history or physical examination. No one had a history of exposure to dopamine antagonistic or dopaminergic medication (except for a few weeks trial of levodopa at least one month prior to study), reserpine, tetrabenazine. Treatment with botulinum toxin was not excluded. The eleven subjects with a history of botulinum toxin exposure had received the injection within 3 months preceding the study. Twenty-two individuals without any neurologic or psychiatric history served as normal controls (age: 49± 14; 14 women, 8 men). Six subjects in the dystonic and six in the control group reported a history of smoking. All subjects underwent Mini-Mental Status examination and Hamilton Depression Inventory.18,19

These studies were approved by the Washington University Human Research Protection Office and the Radioactive Drug Research Committee. Each participant provided written informed consent.

Radiopharmaceutical preparation

[^{18}F]NMB was synthesized from [^{18}F]fluoride using a three step procedure.20 The radiopharmaceutical had a radiochemical purity exceeding 95% and a specific activity ≥1000 Ci/mmol. The *in vivo* kinetics and radiation dosimetry of [^{18}F]NMB are published elsewhere.17,21

MRI procedure

All subjects had 3-D MPRAGE MRI scans of the brain on a Siemens Magnetom Vision 1.5 T scanner. These scans were used for identification of volumes of interest.

PET procedure

Most PET studies were performed using a Siemens/CTI ECAT 953B scanner; five (normals) were performed using a Siemens/CTI ECAT EXACT HR. All studies were acquired in 3D mode and reconstructed using measured attenuation and model-based scatter correction followed by Gaussian filtering to a common resolution of 16 mm FWHM in all three dimensions.22,23 We have compared high-count FDG images using a Hoffman 3D phantom on both scanners which were similarly processed and observed no differences.

Subjects were placed within the scanner and attenuation scans were obtained as described elsewhere.24 A maximum of 8 mCi of [^{18}F]NMB was injected over 20 seconds followed by 10 second saline flush. Emission scans were started with the injection and continued for 2 hours. The initial 4 frames were 2 minutes with the remaining frames each lasting 5 minutes.

Data analysis

A blinded observer outlined volumes of interest (VOIs) for the left and right caudate, putamen and cerebellum by following the anatomical outlines on the T1-weighted MRI. All dynamic PET frames were aligned to correct for movement during the scans for each individual. A composite PET was co-registered to Talairach25 atlas with the help of the corresponding MPRAGE.26,27 We used a graphical analysis method with the cerebellum as the reference region to calculate the binding potential (BP). BP is an index of specific binding, as validated for this particular radioligand.28 The BP for each voxel was calculated and the average BP for each VOI was computed.29 The left and right regional values were averaged for each participant.

Statistical analysis

The mean age of the dystonic subjects was not significantly different from the controls ($p > 0.5$). Nevertheless, since age may be a factor in D2-like uptake in striatum, we corrected the data for age, as done by others.11 Linear regression analysis was used to correlate age with striatal BP values across the normals. We divided the observed BP value in each dystonic subject by the age-predicted normal value. A two tailed unpaired t-test was applied to compare the BP between normal controls and individuals with focal dystonia.11

In Vitro Radioligand binding studies

A filtration binding assay was used to characterize the binding properties of spiperone and NMB at human D2, D3 and D4 dopamine receptors. Competition curves were performed using [^{125}I]-IABN30 and human D2, D3 or D4 dopamine receptors stably expressed in HEK 293 cells. The binding buffer contained 50 mM Tris–HCl, 10 mM EDTA, and 150 mM NaCl at pH 7.4. Nonspecific binding was defined using 4 μM (+)-butaclamol. For competition experiments the radioligand concentration was approximately equal to the K_d value and the concentration of the competitive inhibitor ranged over 5 orders of magnitude. Binding was terminated by the addition of cold wash buffer (10 mM Tris-HCl/150 mM

NaCl, pH 7.4) and filtration over a glass-fiber filter (Schleicher and Schuell No. 32). Filters were washed with 10 ml of cold buffer and the radioactivity was quantitated. A Packard Cobra gamma counter was used for ^{125}I-labeled radioligands (efficiency = 75%). The protein concentration was determined using a BCA reagent (Pierce) with bovine serum albumin as the protein standard.

Data from competitive inhibition experiments was modeled using nonlinear regression analysis31 to determine the concentration of inhibitor that inhibits 50% of the specific binding of the radioligand ($\mathbf{IC_{50}}$ value). Competition curves were modeled for a single site using the following equation:

$$\mathbf{B=B_o/(1+(L/IC_{50}))+B_{ns}}$$

where **B** is the amount of ligand bound to tissue, $\mathbf{B_o}$ is the amount of ligand bound in the absence of competitive inhibitor, **L** is the concentration of the competitive inhibitor, $\mathbf{B_{ns}}$ is the nonspecific binding of the radioligand (defined using a high concentration of a structurally dissimilar competitive inhibitor) and $\mathbf{IC_{50}}$ is the concentration of competitive inhibitor that inhibits 50% of the total specific binding. Data from competition dose response curves were analyzed using Table Curve program (Jandel). IC_{50} values were converted to equilibrium dissociation constants (**Ki values**) using the Cheng and Prusoff correction.32

Results

PET Imaging studies

Normals and patients with dystonia were not significantly different in age. All participants scored less than 10 on Hamilton Depression Inventory and greater than 26 on the Mini-Mental Status examination. Additional details are presented in Table 1. The mean BP's for caudate and for putamen were not significantly different comparing the dystonia group and the normals regardless of age correction (Table 2). The 95% confidence intervals for the difference between the means made it highly unlikely that striatal NMB specific binding was decreased more than 20%.

In vitro receptor affinity studies

In vitro radioligand binding studies were performed to compare the affinity of NMB and spiperone for D2, D3 and D4 receptors which comprise the D2-like family of dopamine receptors. Figure 1 shows representative competition curves for the binding of these compounds at human D2 and D3 receptors and Table 3 lists the affinity of these ligands for binding to D2, D3 and D4 receptors. Spiperone was found to exhibit approximately 5-fold greater selectivity for D2 compared to D3 receptors and 7-fold greater selectivity for D2 compared to D4 receptor subtype. In contrast, NMB was found to be almost 200-fold selective for D2 relative to D3 receptors but only 8-fold selective compared to D4 dopamine receptor subtype.

Discussion

Previous studies report decreased striatal D2-like radioligand uptake in various forms of primary dystonia. These include hand or cranial dystonias, cervical dystonia, DYT1, DYT 6 and DYT11 dystonias regardless of clinical manifestation.11,33–36 In the present study, we found no significant differences between caudate or putamenal [^{18}F]NMB binding in patients with adult-onset primary focal arm or cranial dystonias compared to normals. This conclusion applies to the findings whether or not corrected for age. Several explanations

may account for this apparent discrepancy. The simplest issue is whether the current study had sufficient power to detect the magnitude of differences found in other studies. This study was designed to have 90% power to detect a 20% difference in BP between healthy control subjects and those with cranial or arm dystonia (95% confidence intervals). For this reason, it is unlikely that insufficient power is what led to a failure to detect a difference in uptake in the healthy and diseased.

A more plausible explanation for the discrepancy between our study and previous studies is the improved selectivity of the radioligand [^{18}F]NMB. Earlier imaging protocols used either [^{18}F]spiperone10, [^{11}C]raclopride11 or [^{123}I]-iodobenzamide.36 All of these radioligands have lower receptor specificity compared to NMB. The least specific of these ligands is [^{18}F]spiperone which binds to both D2-like and 5-HT(2A) receptors.16 Although [^{11}C]raclopride has much lower 5-HT(2A) affinity than spiperone, it binds with nearly equal affinity to D2 and D3 receptors.37 IBZM is known to have only 12% of raclopride's affinity to D2-like receptors.37 The specificity analysis in this paper demonstrates that NMB has 200-fold higher affinity for D2 compared to D3 receptors. The D2 specificity of NMB is thus superior to raclopride, which has only an 11-fold greater affinity for binding to D2 receptors38, as well as spiperone, which has only about a 5-fold higher affinity for D2 receptor binding. The high selectivity of NMB for D2 versus D3 and D4 receptors may shed light on the discrepancy between the results of the current study and previous investigations that employed less specific radioligands for PET imaging, including our own study involving [^{18}F]spiperone.

One explanation for the discrepancy between the spiperone and the NMB study could be spiperone's high affinity for 5HT(2A). However, such interpretation would dismiss the evidence of dopaminergic involvement in various primary and secondary dystonias.2–4,11,33,35,36,39 Further, raclopride studies although mainly performed in genetic forms showed a reduced uptake making serotonergic binding a less likely explanation for the observed discrepancies.33

An alternative explanation for the findings is that there are differences in the levels of endogenous striatal dopamine between dystonics and normals. Due to its relatively low receptor binding affinity, raclopride may be displaced or blocked by endogenous dopamine, whereas NMB with its higher D2 affinity is far less susceptible to this effect.17 However, this explanation is less likely, since studies suggest that striatal dopamine is reduced rather than increased in primary dystonia40, and in a non-human primate model of dystonia.9 Future studies of patients with focal dystonia designed to measure endogenous striatal dopamine, such as PET measurement of amphetamine induced [^{11}C]raclopride displacement, would offer more conclusive information regarding the role of endogenous dopamine on D2-like receptor binding.

The more likely explanation for the divergent findings using the various D2-like selective radiopharmaceuticals is that primary dystonia is associated not with reduced striatal D2 receptors, but instead with a decrease in D3 or D4 binding sites. The literature supporting the presence of low D4 receptors in striatum is limited41, while the presence of D3 receptors in the striatum at a concentration below D2 receptors is supported by a number of studies.42,43 In this interpretation, if only D3 binding were decreased, the highly D2 selective radioligand NMB would not be expected to demonstrate reduced striatal binding, since it has relatively low affinity for the affected D3 receptor sites. Evaluation of this hypothesis must await the development of D3 and D4-selective radiopharmaceutical for PET imaging.

Possible role of D3 receptors in the pathophysiology of dystonia

The classic basal ganglia model describes cortical projections to putamen, which in turn project to the internal segment of the pallidum (GPi) via two major pathways: the direct pathway and the indirect pathway via globus pallidus pars externa (GPe) and subthalamic nucleus. GPi output neurons target thalamus which then projects to the cortical areas.[44–46] Some proposed models of dystonia suggest that dysfunction of indirect pathways leads to a loss of inhibition of unwanted muscle activity surrounding a selected movement. This is consistent with clinical observations that a selected movement may begin nearly normally while excessive muscle activity quickly impairs the intended function.[47]

D1-like and D2-like receptors are mostly segregated to different medium spiny neurons (MSN) with D2-like receptors that predominantly localize to and inhibit the striatopallidal neurons of the indirect pathway that project to GPe, whereas D1-like receptors localize to and facilitate the neurons of the direct pathway that project from striatum to GPi.[46,48,49] Hence, any abnormality in D2-like receptors could lead to dysfunction of the indirect pathway and contribute to the pathophysiology of dystonia. Indeed, D2-like receptors act presynaptically to reduce striatal GABA release. It has been shown in a DYT1 mouse model that there is disinhibition of striatal GABAergic synaptic activity in cells containing D2, GABA and enkephalin receptors projecting to GPe, suggestive of D2 receptor dysfunction in the indirect pathway.[50] Interestingly, the agonist was a D2-like ligand. However, the findings were attributed to D2 receptors assuming there were no D3 receptors present in the striatum.

Alternatively, abnormal plasticity with reduced cortical inhibition has been regarded a key factor in the pathophysiology of dystonia.[51–53] There is mounting evidence suggesting that striatal synaptic plasticity plays a pivotal role in procedural and sensorimotor learning,[54–56] which could explain abnormal plasticity at basal ganglia, in addition to, cortical levels in dystonia.[57] Several observations may provide insights into how dopamine plays a central role in mediating such synaptic plasticity. Striatal MSNs receive excitatory glutamatergic cortical and thalamic input and nigral dopaminergic afferents.[48] Dopamine receptors are strategically positioned on the neck of the dendritic spines of MSNs close to the glutamatergic cortical synapses at the spine heads.[58] In addition all striatal interneurons express dopamine receptors and can be modulated by dopamine.[59–61] In fact numerous studies suggest that corticostriatal long term potentiation and depression, which are forms of plasticity, are regulated and likely induced by dopamine.[62,63]

While the main focus has been on D2 receptor, D3 receptors have not received much attention given the low level of their expression in the striatum. Interestingly, an L-type calcium channel agonist provoked dystonia in mice via affecting the D3 and possibly D1-like receptors, while D2 receptor did not seem to play a role.[64] Indeed, D3 receptor could be a major contributor to dopaminergic synaptic modulations: A small subpopulation of striatal MSN contain both D1 and D2-like receptors[65–66] and D3 receptor may interact with other receptors at pre-synaptic sites contributing to auto-regulation.[67–68] In addition, recent studies indicate that D1 and D3 receptors can form heterodimers capable of enhancing D1 receptor mediated activity.[69,70] Finally, D3 receptor affects the extracellular dopamine level most likely via regulation of DAT expression.[71] These features could allow D3 receptor to contribute to striatal neuroplasticity despite its relative low density compared to D2 and D1 receptors. Hence, a disturbance in D3 receptors might have far reaching effects on interactions of the various components of the basal ganglia dopaminergic system. Of course, evaluation of a possible D3 or even D4 abnormality in dystonia requires *in vivo* measurements with a D3 or D4-specific radioligands. Most importantly such studies need to be conducted in various forms of dystonia to prove commonality.

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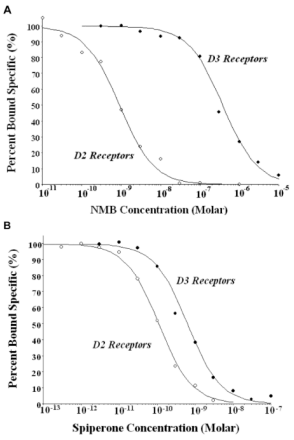

Figure 1. Competitive radioligand binding of NMB and spiperone to human D2 and D3 dopamine receptor subtypes *in vitro*

Competitive radioligand binding studies were performed to compare the binding affinity and selectivity of spiperone and NMB for human D2 (○) and D3 (●) dopamine receptors. Competition experiments were performed using ^{125}I-IABN and human dopamine receptors expressed in stably transfected HEK-293 cells. Representative competition curves obtained using NMB (**A**) and spiperone (**B**) are shown.

Table 1

Characteristics of participants with focal hand or cranial dystonia

Subjects with dystonia (total)	24
Mean age (years)	52±11
Cranial dystonia	12
Hand dystonia	12
Blepharospasm alone	8
Pure writing cramp	4
Right handedness	20
Median duration of dystonia (years)	5
Ever treated with botulinum toxin	11

Table 2

Mean NMB binding potential in patients with dystonia and normal subjects

		Normals	*Dystonics*
caudate	Age correction	**2.42 ± 0.22**	**2.47 ± 0.28**
	No age correction	**2.35 ± 0.33**	**2.47 ± 0.35**
putamen	Age correction	**3.12 ±0.26**	**3.18 ± 0.32**
	No age correction	**3.14 ±0.35**	**3.18 ± 0.42**

A two tailed unpaired t-test was applied to compare the binding potential between normal controls and individuals with focal dystonia. No significant difference (confidence interval 95%) was detected in the PET measured NMB binding potential with or without age correction. The BP values are mean± standard deviation.

Table 3

Comparison of the affinity of spiperone and NMB for human D2-Like dopamine receptors

	Ki values (nMolar)	
Receptors	spiperone	NMB
D2 receptors	0.06 ± 0.01 (18)	0.58 ± 0.21 (3)
D3 receptors	0.33 ± 0.02 (21)	114 ± 27 (4)
D4 receptors	0.45 ± 0.01 (4)	4.9 ± 0.26 (4)

The affinity (Ki value) for the binding to human D2 and D3 dopamine receptors was obtained using competitive radioligand binding experiments with ^{125}I-IABN. The Ki values are the mean ± the S.E.M. and the number in the parentheses is the number of independent experiments (n). Dopamine receptors were expressed in stably transfected HEK 293 cells.