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Widespread Decrease of Nicotinic Acetylcholine Receptors in Parkinson's Disease

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Abstract

Nicotinic acetylcholine receptors (nAChRs) have close interactions with the dopaminergic system and play critical roles in cognitive function. nAChRs were imaged in 10 non-demented Parkinson's disease (PD) patients and 15 age-matched healthy subjects using a single photon emission computed tomography ligand [¹²³I]5-iodo-3-[2(S)-2-azetidylmethoxy]pyridine. Using an arterial input function, we measured the total distribution volume (V ; specific plus non-displaceable) as well as the delivery (K_1). PD showed a widespread significant decrease ($\sim 10\%$) of V in both cortical and subcortical regions without a significant change in K_1 . These results indicate the importance of extending the study to demented patients.

In addition to the well documented loss of dopaminergic neurons, a number of animal and clinical studies have shown that nicotinic acetylcholine receptors (nAChRs) play critical roles in Parkinson's disease (PD). nAChR activation stimulates dopamine release in the striatum¹ and an agonist at nAChRs showed synergistic therapeutic effects with L-DOPA in a monkey model of PD². Epidemiological studies showed that cigarette smoking protects against PD³. Both animal and human studies have shown that nAChR is one of the central components in cognitive function⁴, and a substantial number of PD patients become demented. Finally, most postmortem studies showed widespread decrease of nAChRs both in striatum and cerebral cortices of PD patients^{5,8}. However, as in most other postmortem studies, many of these lacked critical clinical information such as the presence of dementia and a history of cigarette smoking. Therefore, it is critical to image nAChRs in living PD patients whose clinical information is available to study changes and to explore possible therapeutic intervention at these receptors. However, to our knowledge, such a study has not been published.

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Recently 3-[2(*S*)-2-azetidylmethoxy]pyridine (A-85380), has been developed⁹, which has high affinity to the predominant type of nAChRs in the brain composed of $\alpha 4$ and $\beta 2$ subunits¹⁰. A few analogs of A-85380 including [¹²³I]5-iodo-3-[2(*S*)-2-azetidylmethoxy]pyridine (5-I-A-85380), have been radiolabeled and successfully used in humans^{11, 12}. An ex vivo study in non-human primate has shown that radiolabeled metabolites of [¹²³I] 5-I-A-85380 do not cross the blood brain barrier¹³. 5-I-A-85380 labels several $\beta 2$ -containing nAChRs, including $\alpha 4\beta 2$ (the most predominant nicotinic receptor in human brain), $\alpha 3\beta 2$ and $\alpha 6\beta 2$ containing subtypes¹⁴.

The purpose of the present study was to perform a pilot study of nAChR imaging in early to moderate stage PD patients using [¹²³I]5-I-A-85380.

Subjects and Methods

Subjects

The study was approved by National Institute of Neurological Disorders and Stroke (NINDS) and National Institute of Mental Health institutional review boards. Patients were recruited from NINDS clinics. Control subjects were healthy volunteers recruited from community via advertisement who did not have a history or signs of neurological disorders. After complete description of the study to the subjects, written informed consent was obtained. Sample demographics and clinical characteristics are shown in Table 1. For all participants, the absence of axial focal lesions was confirmed by a neuroradiologist using a non-contrast MRI. All patients and healthy subjects had not smoked cigarettes for at least last 5 years. None of the patients had used cholinergic or anticholinergic medications within 60 days of the single-photon emission computed tomography (SPECT) scan. On the day of the SPECT scans, all patients continued dopaminergic medications including carbidopa-levodopa. There was no significant difference in age between PD patients and healthy subjects.

Clinical ratings

Motor function was evaluated by Unified Parkinson's Disease Rating Scale and Hoehn & Yahr staging. Cognitive function was measured with Mini Mental Status Exam. and Mattis Dementia Rating Scale.

Single photon emission tomography

[¹²³I]5-I-A-85380 was prepared as described previously¹². SPECT data were acquired using a triple-headed camera with low-energy high-resolution parallel whole collimators (Trionix XLT-20, Triad, Twinsburg, OH, USA). Initially a transmission scan was obtained using a ¹⁵³Gd line source. Subsequently [¹²³I]5-I-A-85380 was administered intravenously as a bolus (injection dose, healthy: 486 ± 79 , PD: 506 ± 75 MBq, no significant difference, with these and subsequent data expressed as mean $\pm$ SD). SPECT data were acquired at 0 – 40, 115 – 135 and 210 – 230 min. Arterial samples were obtained every 15 s for the first 2 min, and at 3, 4, 5, 10, 30, 80, 120 and 180 min.

Plasma analysis

Plasma [¹²³I]5-I-A-85380 concentration and the free fraction (f_1) were determined as described previously¹².

Image analysis

SPECT projection data were reconstructed on a 64×64 matrix with pixel size of $4.48 \times 4.48 \times 4.48$ mm in the x, y, and z axis, respectively, with correction for attenuation and scattered radiation¹⁵. Parametric images of the delivery of the radioligand (K_1) and total distribution

volume (V) were created using a multilinear algorithm^{16, 17} implemented in PMOD 2.55 (<http://www.pmod.com/technologies/index.html>). Plasma free [¹²³I]5-I-A-85380 levels were used to calculate V instead of using plasma total (free plus protein bound) [¹²³I]5-I-A-85380 because patients were on medication and concomitant medication may change plasma protein binding of [¹²³I]5-I-A-85380. Parametric images were spatially normalized to a standard anatomic orientation (Montreal Neurological Institute (MNI) space) based on K_1 images and using Statistical Parametric Mapping version '02 (SPM2, <http://www.fil.ion.ucl.ac.uk/spm/>). Spatially normalized K_1 and V images were smoothed with 10 mm full-width at half-maximum. To confirm the magnitude of changes in V , volume of interest (VOI) data were obtained from brain regions in MNI space listed on Table 2.

Statistical analysis

SPM2¹⁸ was used. Two-sample t-test was applied to compare K_1 and V between patients and healthy subjects, and simple regression analysis was applied to study relationship between V and clinical ratings. Gray matter threshold was set at 20% for V and 80% for K_1 images, respectively. Because each pixel had a measured value of K_1 or V , global normalization was not applied. No sphericity correction was applied by assuming replication over groups. False discovery rate $p < 0.05$ and cluster-level corrected $p < 0.05$ were considered as significant.

A two-sample t-test was applied to compare plasma free fraction of [¹²³I]5-I-A-85380 between groups.

Results

Patients were in the early to moderate stages of PD and were not demented (Table 1). Patients tended to show lower f_1 values than healthy subjects ($p = 0.09$, Table 2).

An SPM t-test showed a significant decrease of V in many brain regions with the greatest T values of 4.96 (Figure). The decrease measured by the VOI was 15% in thalamus while occipital and frontal cortices showed only 3% and 5% decreases, respectively, where many voxels did not reach significance. Decreases in parietal and temporal cortices were 8–9% (Table 2). SPM did not detect a significant increase of V in any brain region in patients. There was neither a significant increase or a decrease of K_1 in any region. There was no significant regression between any clinical rating scores and V .

Discussion

In the present study, we detected a significant and widespread decrease of nAChRs in early to moderately affected, non-demented PD patients by applying accurate quantification with the measurement of arterial input function and the plasma free fraction (f_1) of [¹²³I]5-I-A-85380 in each subject. Such measurements made the outcome of imaging studies free from intersubject and between group differences in the metabolism and the protein binding of the imaging agent. Because patients tended to show lower f_1 values, if total plasma parent had been used instead of free [¹²³I]5-I-A-85380, the decrease in V would have been overestimated. Further, for accurate measurement, scatter correction¹⁵ and a pixel-based modeling that minimizes noise-induced biases^{16, 17} were applied.

There are three possible reasons that the decreases in V detected in this study were smaller than those reported at postmortem (in most studies, $> 30\%$). First, because there is no large region devoid of nAChRs, it was not possible to measure nondisplaceable radioactivity, which could then have been used to calculate specific binding of [¹²³I]5-I-A-85380. Since V is a summation of specific and nondisplaceable distribution volumes, a decrease of specific binding was underestimated. Second, in the present pilot study, only non-demented PD patients were

enrolled whereas postmortem studies found larger decreases in nAChRs in demented patients^{5, 7}. Therefore, postmortem studies including demented patients showed greater decrease in nAChR than in the present study. The lack of significant regression between clinical ratings and V may also be explained by a fairly uniform population of non-demented patients. Third, while Bmax is measured in postmortem studies, Bmax/Kd plus nondisplaceable activity is measured by *in vivo* imaging studies including the current one. If there were a decrease in Kd measured *in vivo* in addition to a decrease in Bmax, a decrease in Bmax/Kd would not be as great as that in Bmax. In fact, a postmortem study reported nonsignificant but substantial 10 – 40% decrease in Kd in both cortical and subcortical regions⁶.

There are a couple of factors that may confound interpretation of the results of the current study. All patients were taking L-DOPA containing medications. L-DOPA treatment significantly decreased *in vitro* [¹²⁵I]5-I-A-85380 binding in the striatum but not in cerebral cortex in normal squirrel monkeys¹⁹. However, in the same study, L-DOPA treatment did not decrease [¹²⁵I]5-I-A-85380 binding in the same regions in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated animals whose dopaminergic terminals were almost completely destroyed. Therefore, the widespread decreases in nAChRs found in this study are more likely to be the result of PD pathology than L-DOPA treatment. Brain atrophy can cause widespread decrease in nAChRs detected in SPECT. However, a voxel-based morphometric (VBM) study on non-demented patients did not detect a widespread decrease in gray matter volume²⁰. By taking together the factors described above, non-demented patients with Parkinson's disease did show a widespread decrease of Bmax/Kd in $\beta 2$ -containing nAChRs both in cortices and subcortical regions. Because postmortem studies have shown greater decreases in nAChRs in demented patients, it would be interesting to extend the study to include such patients.

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References

1. Rapier C, Lunt GG, Wonnacott S. Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: concentration dependence and repetitive stimulation. *J. Neurochem* 1988;50:1123–1130. [PubMed: 3346670]
2. Schneider JS, Pope-Coleman A, Van Velson M, et al. Effects of SIB-1508Y, a novel neuronal nicotinic acetylcholine receptor agonist, on motor behavior in parkinsonian monkeys. *Mov. Disord* 1998;13:637–642. [PubMed: 9686767]
3. Tanner CM, Goldman SM, Aston DA, et al. Smoking and Parkinson's disease in twins. *Neurology* 2002;58:581–588. [PubMed: 11865136]
4. Picciotto MR, Zoli M. Nicotinic receptors in aging and dementia. *J. Neurobiol* 2002;53:641–655. [PubMed: 12436427]
5. Rinne JO, Myllykyla T, Lonnberg P, Marjamaki P. A postmortem study of brain nicotinic receptors in Parkinson's and Alzheimer's disease. *Brain Res* 1991;547:167–170. [PubMed: 1860068]
6. Aubert I, Araujo DM, Cecyre D, et al. Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. *J. Neurochem* 1992;58:529–541. [PubMed: 1729398]
7. Lange KW, Wells FR, Jenner P, Marsden CD. Altered muscarinic and nicotinic receptor densities in cortical and subcortical brain regions in Parkinson's disease. *J. Neurochem* 1993;60:197–203. [PubMed: 8417140]
8. Quik M, Bordia T, Forno L, McIntosh JM. Loss of alpha-conotoxinMII- and A85380-sensitive nicotinic receptors in Parkinson's disease striatum. *J. Neurochem* 2004;88:668–679. [PubMed: 14720216]
9. Abreo MA, Lin NH, Garvey DS, et al. Novel 3-Pyridyl ethers with subnanomolar affinity for central neuronal nicotinic acetylcholine receptors. *J. Med. Chem* 1996;39:817–825. [PubMed: 8632405]

10. Mukhin AG, Gundisch D, Horti AG, et al. 5-Iodo-A-85380, an $\alpha 4\beta 2$ subtype-selective ligand for nicotinic acetylcholine receptors. *Mol. Pharmacol* 2000;57:642–649. [PubMed: 10692507]
11. Sihver W, Nordberg A, Langstrom B, et al. Development of ligands for in vivo imaging of cerebral nicotinic receptors. *Behav. Brain Res* 2000;113:143–157. [PubMed: 10942041]
12. Fujita M, Ichise M, van Dyck CH, et al. Quantification of nicotinic acetylcholine receptors in human brain using [^{123}I]5-I-A-85380 SPET. *Eur. J. Nucl. Med* 2003;30:1620–1629.
13. Baldwin RM, Zoghbi SS, Staley JK, et al. Chemical composition of ^{123}I -5-IA in baboon brain after intravenous administration. *J. Nucl. Med* 2002;43:45P.
14. Kulak JM, Sum J, Musachio JL, et al. 5-Iodo-A-85380 binds to alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors (nAChRs) as well as alpha4beta2* subtypes. *J. Neurochem* 2002;81:403–406. [PubMed: 12064487]
15. Iida H, Narita Y, Kado H, et al. Effects of scatter and attenuation correction on quantitative assessment of regional cerebral blood flow with SPECT. *J. Nucl. Med* 1998;39:181–189. [PubMed: 9443759]
16. Ichise M, Toyama H, Innis RB, Carson RE. Strategies to improve neuroreceptor parameter estimation by linear regression analysis. *J. Cereb. Blood Flow Metab* 2002;22:1271–1281. [PubMed: 12368666]
17. Ichise M, Fujita M, Zoghbi SS, et al. Parametric imaging of distribution volume and tracer delivery by noise-resistant linear regression analysis: application to [^{123}I]5-I-A-85380 SPECT imaging of $\alpha 4\beta 2$ nicotinic acetylcholine receptors in human. *NeuroImage* 2004;22:T180–181.
18. Friston KJ, Holmes AP, Worsley KJ, et al. Statistical parametric maps in functional imaging: a general linear approach. *Hum. Brain Mapping* 1995;2:189–210.
19. Quik M, Bordia T, Okihara M, et al. L-DOPA treatment modulates nicotinic receptors in monkey striatum. *Mol. Pharmacol* 2003;64:619–628. [PubMed: 12920198]
20. Burton EJ, McKeith IG, Burn DJ, et al. Cerebral atrophy in Parkinson's disease with and without dementia: a comparison with Alzheimer's disease, dementia with Lewy bodies and controls. *Brain* 2004;127:791–800. [PubMed: 14749292]

1. .

Brain areas with a significant decrease of [^{123}I]5-I-A-85380 distribution volume (V) in Parkinson's disease patients detected with a two sample t-test in SPM2. Areas with a significant decrease are displayed in the glass brain (left) and on transverse and coronal slices through thalamus of an MR image of a control subject (middle). Corresponding MR images without superimposition are shown on the right. Highlighted areas showed p less than 0.05 false discovery rate, which was corrected for multiple comparisons. The area also showed cluster-level corrected $p < 0.001$. The color bar shows T values with the maximum value of 4.96. Note that the glass brain view displays decreases in the entire brain superimposed to anterior-posterior (left top) or top-bottom (left bottom) views while the MR images with the superimposition display decreases on single slices.

Table 1
Sample Demographics and Clinical Characteristics

Characteristics	Healthy	Parkinson
N	15	10
Age	59 ± 5	56 ± 3
Gender	10F/5M	4F/6M
Cigarette smoking	No	No
Cholinergic medication	No	No
Dopaminergic medication	No	Yes
[¹²³ I]5-I-A-85380 dose (MBq)	486 ± 79	506 ± 75
Hoehn & Yahr		2.5 ± 0.4
Total Unified Parkinson's Disease Rating Scale		46 ± 5 [*]
Mini Mental Status Exam.		≥ 27
Mattis Dementia Rating Scale		135 ± 4 [†]

Mean ± SD values are shown on the table. F, female; M, male.

^{*} N = 9,

[†] N = 8.

Table 2
Plasma free fraction and total distribution volume of [123 I]5-I-A-85380

	Healthy	Parkinson
Plasma free fraction (%)	48.1 $\pm$ 4.4	45.3 $\pm$ 3.1
Total distribution volume (mL/cm ³)		
Thalamus	71.9 $\pm$ 13.5	61.2 $\pm$ 10.7
Caudate	41.9 $\pm$ 6.2	36.9 $\pm$ 4.3
Putamen	44.4 $\pm$ 6.7	40.2 $\pm$ 5.6
Pons	43.3 $\pm$ 8.1	39.0 $\pm$ 8.1
Frontal cortex	24.9 $\pm$ 3.1	23.7 $\pm$ 3.1
Parietal cortex	26.9 $\pm$ 3.5	24.4 $\pm$ 3.0
Temporal cortex	30.7 $\pm$ 3.9	28.1 $\pm$ 4.0
Occipital cortex	27.1 $\pm$ 3.6	26.3 $\pm$ 4.2
Cerebellum	33.8 $\pm$ 6.2	29.2 $\pm$ 5.9*

* One patient was excluded whose cerebellum was partially out of field of view.