Nicotinic acetylcholine receptor density in cognitively intact subjects at an early stage of Parkinson’s disease

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We investigated in vivo brain nicotinic acetylcholine receptor (nAChR) distribution in cognitively intact subjects with Parkinson’s disease (PD) at an early stage of the disease. Fourteen patients and 13 healthy subjects were imaged with single photon emission computed tomography and the radiotracer [123I]ido-3-[2(5)-2-azetidinylmethoxy]pyridine ([123I]I5IA). Patients were selected according to several criteria, including short duration of motor signs (<7 years) and normal scores at an extensive neuropsychological evaluation. In PD patients, nAChR density was significantly higher in the putamen, the insular cortex and the supplementary motor area and lower in the caudate nucleus, the orbitofrontal cortex, and the middle temporal gyrus. Disease duration positively correlated with nAChR density in the putamen ipsilateral (ρ = 0.56, p < 0.05) but not contralateral (ρ = 0.49, p = 0.07) to the clinically most affected hemibody. We observed, for the first time in vivo, higher nAChR density in brain regions of the motor and limbic basal ganglia circuits of subjects with PD. Our findings support the notion of an up-regulated cholinergic activity at the striatal and possibly cortical level in cognitively intact PD patients at an early stage of disease.

Keywords: 5IA-SPECT, nicotinic receptors, Parkinson disease, cognitive decline, dopamine acetylcholine

INTRODUCTION

At an early motor stage, Parkinson’s disease (PD) is predominantly characterized by a progressive loss of the nigrostriatal dopaminergic neurons leading to a severe state of dopamine depletion. In addition to the decline in dopaminergic function, other neurotransmitter systems are involved in PD, including the nicotinic cholinergic system (Jellinger, 1991; Posadas et al., 2013). Indeed, anti-cholinergics were the first widely accepted treatment for parkinsonism. In 1867, Ordien first reported their antiparkinsonian effect, which Charcot had discovered fortuitously when administering tinctures of deadly nightshade (Atropa belladonna) for excessive salivation in parkinsonian patients (Lang and Blair, 1889).

The two primary sources of acetylcholine (ACh) in the brain include local interneurons that are interspersed among their cellular targets and projection neurons that innervate distal areas. Most brain regions, including the pedunculopontine and laterodorsal tegmental areas, belong to the latter category, whereas the former includes the striatum and nucleus accumbens. ACh signals through two classes of receptors localized both pre- and postsynaptically: metabotropic muscarinic acetylcholine receptors (mAChRs) and ionotropic nicotinic acetylcholine receptors (nAChRs). Presynaptic mAChRs (M2, M4) act as inhibitory autoreceptors on the cholinergic terminals, with M4 predominant in striatum. Postsynaptic mAChRs can be either inhibitory (M2, M4) or excitatory (M1, M3, M5). Although the actual mechanism of action in PD is not known, clinically available anti-cholinergics (e.g., trihexyphenidyl, benzhexol, etc.) act mainly as competitive antagonists of mAChRs (Bross, 1999). The nAChRs are pentameric ligand-gated ion channels composed of α-subunits (homomeric receptors) or of α- (α2–α7) and β-subunits (β2–β4) (heteromeric receptors) (reviewed in Gotti and Clementi, 2004). Presynaptic nAChRs induce release of a number of neurotransmitters, including dopamine. Postsynaptic nAChRs depolarize neurons, increase their firing rate, and can contribute to long-term potentiation (reviewed in Picciotto et al., 2012).

The striatum is a nodal structure of the basal ganglia circuits and one of the brain areas with the highest concentration of markers of cholinergic transmission. Large aspiny cholinergic interneurons (ChIs) constitute 2% of the entire striatal neuronal population but exert a powerful influence on its output, which is mediated by the medium spiny neurons (MSNs). Dopamine depletion elicits an increased excitability of ChIs, mainly due to the removal of D2-mediated inhibitory control (Maurice et al., 2004). In addition, rhythmic firing of the ChIs and breakdown of autoinhibition of ACh release by M4 results in the unregulated...
release of ACh, which selectively increases excitability of MSNs, particularly those of the indirect pathway (Aosaki et al., 1995; Kreitzer and Malenka, 2007). Increased cholinergic activity in subjects with PD was not confirmed by anatomopathological studies that documented mainly an extensive nAChR reduction in the brain of patients with PD (Perry et al., 1989; Rinne et al., 1991; Aubert et al., 1992). In vivo studies in non-demented PD subjects are instead limited and controversial. Molecular imaging using either \( ^{123} \text{I} \)-FA-SPECT or positron emission tomography (PET) demonstrated variably reduced nAChRs density in cortical areas only (i.e., frontal and parietal lobes), amygdala (Fujita et al., 2006; Oishi et al., 2007; Meyer et al., 2009) or in the striatum and substantia nigra (Kas et al., 2009).

In the present study, we investigated by means of \( ^{5} \text{[123]I} \)-iodo-3-[2(S)-2-azetidinylmethoxy]pyridine (\( ^{123} \text{I} \)-SIA), a specific \( \alpha_{4}\beta_{2} \) nAChR ligand, and \( ^{123} \text{I} \)-SPECT in patients with PD specifically selected for a short disease duration, the capability to be withdrawn from all dopaminergic medications for 3 days, and normal scores from extensive neuropsychological evaluation.

MATERIALS AND METHODS

Healthy subjects were enrolled at the Yale University School of Medicine with the approval of the Yale Human Investigation Committee, the West Haven Veterans Administration Human Subjects Subcommittee, the Radiation Safety Committee, and the Food and Drug Administration. \( ^{123} \text{I} \)-SIA-SPECT in patients with PD was performed at the University Hospital of Würzburg and approved by the University Hospital of Würzburg and the German Federal Office for Radiation Protection (Bundesamt für Strahlenschutz, Salzgitter, Germany). All participants gave written informed consent.

SUBJECTS

The study involved 14 subjects with PD (8 males; median age: 64 years, range: 52–78 years; recruited at Saarland University) and a control group of 13 neurologically intact adults (5 males; median age: 64 years, range: 52–78 years; recruited at Saarland University). The diagnosis of PD was made according to the UK Parkinson Disease Brain Bank criteria and patients evalu-
intravenously over 60 min. The above described radiosynthesis provided \[^{123}\text{I}]5IA\) in form of carrier-free (n.c.a.) tracer, with the highest possible specific activity. The approximate administered mass in an injectable solution with 185 MBq of \[^{123}\text{I}]5IA\) was \(<0.001\) nmol (\(<1\) pmol).

Cerebral SPECT imaging was acquired with a dual-head gamma camera (E.Cam Duet, Siemens Medical Solutions, Hoffman Estates, IL, USA) equipped with medium energy collimators. At 2 and 4 h after injection of \[^{123}\text{I}]5IA\), 120 views (40 s per view) were acquired over a 360° circular orbit, and reconstructed into a \(128 \times 128\) matrix with a pixel size of 3.9 mm and slice thickness of 3.9 mm. Imaging at 4 h after injection was chosen in accordance with previous kinetic modeling data in healthy volunteers (Oishi et al., 2007; Cosgrove et al., 2012) and for practical reasons concerning scanning the patients. Reconstruction was performed with filtered back-projection with a Butterworth filter (order 8, cutoff 0.4) followed by attenuation correction according to the Chang method (Chang, 1978), with an attenuation coefficient of 0.11/cm to generate the transversal slices.

For further data analysis, the reconstructed transverse sections were transferred to a Hermes workstation (Hermes Medical Solutions, Stockholm, Sweden). Brain regions were analyzed using the brain analysis program BRASS (version 3.5, Hermes Medical Solutions, Stockholm, Sweden). Each image volume was recorded to match to the built-in ECD template using an affine transform (nine parameters). Manual fitting was necessary, because of the low background uptake resulting in insufficient contrast for automatic delineation of the brain contour. The ECD template consisted of a three dimensional region of interest (ROI) map of 46 predefined brain regions. The mean count per voxel was determined for each region in both hemispheres. The normalized data were calculated as the ratio of mean count per voxel to mean count per voxel of the global \[^{123}\text{I}]5IA\) brain uptake (\(=\) average of all 46 measured brain regions) for each region and each subject. We selected as a reference the whole brain uptake as the most conservative approach (Terrière et al., 2010). We also performed a statistical parametric mapping (SPM version 8, Wellcome Department of Cognitive Neurology, UCL, London, UK) analysis. This method allows exploratory voxel-by-voxel group comparisons throughout the entire brain volume without requiring an \textit{a priori} hypothesis. The template for SPM analysis was provided by Oishi et al. (2007). Of relevance to this manuscript, we performed a voxel-based analysis applying the proportional scaling global mean, thresholded at \(p < 0.05\) and corrected for Family wise-error (FWE). Brain regions (approximate Brodmann areas) were estimated based on the methods of Talairach and Tournoix (1988) after adjustment (www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html) for differences between MNI and Talairach coordinates.

**STATISTICAL ANALYSIS**

Normality of data distribution was tested by the Shapiro–Wilks test. Gender distribution among groups was tested with FIGURE 1 | Binding values of nAChRs of the caudate nucleus and putamen of PD patients and HC. Compared to controls, nAChRs density was bilaterally lower in the caudate nucleus \((p < 0.01,\) Wilcoxon rank-sum test) and higher in the putamen of PD patients \((p < 0.001,\) Wilcoxon rank-sum test). No significant difference was found when comparing ipsilateral and contralateral (or left and right) side, both in patients and controls. Contralateral refers to the side opposite to the clinically most affected hemibody. Right is conventionally contralateral for HC.
Table 1 | Binding values of nAChRs

|       | PD     | HC     | p Value |
|-------|--------|--------|---------|
| Putamen C | 1.36±0.10 | 1.08±0.10 | <0.0001 |
| Putamen I | 1.37±0.13 | 1.09±0.09 | <0.0001 |
| Caudate C | 0.86±0.12 | 1.04±0.15 | <0.01   |
| Caudate I | 0.81±0.20 | 1.11±0.21 | <0.01   |
| Thalamic C | 1.68±0.23 | 1.67±0.18 | 0.46    |
| Thalamic I | 1.62±0.24 | 1.68±0.20 | 1.00    |
| Sensorimotor L | 1.07±0.07 | 1.05±0.09 | 0.40    |
| Sensorimotor R | 1.02±0.07 | 1.02±0.08 | 0.88    |
| Frontal lobe L | 0.93±0.06 | 0.95±0.08 | 0.19    |
| Frontal lobe R | 0.92±0.06 | 0.95±0.09 | 0.11    |
| Orbitofrontal L | 0.85±0.08 | 1.00±0.11 | <0.001  |
| Orbitofrontal R | 0.83±0.10 | 1.10±0.10 | <0.001  |
| Temporal lobe L | 0.97±0.05 | 0.96±0.08 | 0.73    |
| Temporal lobe R | 0.94±0.04 | 0.96±0.08 | 0.43    |
| Parieto-temporal L | 1.00±0.04 | 0.90±0.07 | 0.16    |
| Parieto-temporal R | 0.94±0.04 | 0.96±0.08 | 0.43    |
| Insular cortex L | 1.13±0.09 | 0.95±0.09 | <0.001  |
| Insular cortex R | 1.13±0.10 | 0.91±0.07 | <0.001  |
| Gyrus cinguli L | 0.85±0.07 | 0.88±0.10 | 0.16    |
| Gyrus cinguli R | 0.86±0.09 | 0.90±0.10 | 0.40    |
| Occipital L | 0.89±0.05 | 0.86±0.08 | 0.05    |
| Occipital R | 0.87±0.05 | 0.85±0.07 | 0.38    |
| Cerebellum cortex L | 1.01±0.06 | 0.98±0.01 | 0.22    |
| Cerebellum cortex R | 1.01±0.06 | 0.98±0.01 | 0.20    |
| Cerebellum white matter L | 1.12±0.07 | 1.07±0.04 | 0.17    |
| Cerebellum white matter R | 1.16±0.07 | 1.10±0.04 | 0.05    |
| Pons and midbrain | 1.33±0.13 | 1.34±0.09 | 0.69    |

For the striatum and thalamus we listed the binding values of contralateral (C) and ipsilateral (I) with respect to the clinically most affected hemibody. For healthy controls, left hemibody refers conventionally to ipsilateral. Other brain regions are listed as left (L) and right (R). p Value refers to Wilcoxon rank-sum test.

Table 2 | Brain regions of significantly correlation between the voxel-by-voxel [123]IβA distribution in the PD group as compared with the control group in statistical parametric mapping (SPM8) analyses.

| Region – Brodmann area | Coordinate (Talairach) | Z score |
|-------------------------|------------------------|---------|
| INCREASED               |                        |         |
| R precentral gyrus – BA6 | 24, −18, 68            | 5.22    |
| R middle frontal gyrus – BA6 | 32, −1, 57           | 5.12    |

DECREASED

| Region – Brodmann area | Coordinate (Talairach) | Z score |
|-------------------------|------------------------|---------|
| R caudate nucleus       | 4, 7, 12               | 4.82    |
| L middle frontal gyrus – BA11 | −28, 41, −9         | 5.41    |
| L middle temporal gyrus – BA21 | −64, −12, −7     | 6.00    |
| −56, −2, −15            | 4.84                  |
| −53, −22, −13           | 5.97                  |
We also enrolled patients able to stop their dopaminergic therapy in the motor or limbic circuits at an early stage of PD, even in cognitively intact PD patients. An association between anti-cholinergic drug use and cognitive decline in PD has been documented (Ehrt et al., 2010). It should be noted, however, that clinically available anti-cholinergics (e.g., trihexyphenidyl, benztropine, etc.) mainly act as competitive antagonists at mAChRs. A stepwise executive dysfunction has been described in cognitively intact PD patients (Taylor et al., 1986) who suffer damage to the frontal lobes and/or fibers connecting the frontal lobes with the head of the caudate during electrode implantation for deep brain stimulation (Okun et al., 2012). The role of [123I]SIAA as a screening tool for identifying patients at risk for surgery-related cognitive decline should be further investigated.

No previous studies have described higher nAChR binding levels in PD patients compared to controls. Discrepancies might be related to the relatively short disease duration and the early disease stage of patients enrolled in this study. In addition, we carefully excluded patients with cognitive problems by means of an extensive neuropsychological evaluation (Mitsis et al., 2009b). We also enrolled patients able to stop their dopaminergic therapy for 3 days to limit a possible acute effect of dopaminergic drugs on nAChRs binding measurement. Indeed, L-DOPA treatment significantly decreased in vitro [123I]SIAA binding in the striatum, but not in cerebral cortex in normal squirrel monkeys (Quik et al., 2003). Similarly, a high daily dose of dopamine agonist showed a significant negative correlation with density of nAChRs in the cerebellum, temporal, parietal, and occipital cortices (Oishi et al., 2007). In previous studies, dopaminergic drugs were not suspended (Fujita et al., 2006; Meyer et al., 2009) or stopped for 12 h only (Kas et al., 2009), despite the long half-life of some dopaminergic drugs (e.g., ergot derivatives) (Oishi et al., 2007). Still, even a drug-withdrawal of 72 h, as in our study, might not be sufficient in avoiding an acute drug effect on [123I]SIAA binding, especially when patients are taking long-lasting dopaminergic drugs (e.g., dopamine agonists). A study on drug naïve patients is warranted, also to avoid a putative chronic effect of dopaminergic drugs on the striatal cholinergic system.

The majority of anatomopathological studies (Perry et al., 1989; Rinne et al., 1991; Aubert et al., 1992), but not all (Lange et al., 1993), reported a loss of nAChR agonist binding in the striatum of PD patients. Post-mortem findings are however not directly comparable with our results as they cannot detach possible compensatory changes early at a disease stage. In many cases, it is also unclear whether these studies have included demented patients (Rinne et al., 1991; Court et al., 2000).

Few PET studies with [11C]methyl-4-piperidinyl propionate acetylcholinesterase ([11C]PMP) (Gilman et al., 2010) but not others (Shinotoh et al., 1999; Bohnen et al., 2006, 2010) described a reduced striatal cholinergic activity in patients with PD. Indeed, in a large cohort of non-demented PD patients, cholinergic projection alterations, investigated by means of [11C]PMP and PET, were highly heterogeneous with over 65 out of 101 subjects with PD showing neocortical and thalamic acetylcholinesterase activity within the normal range (Bohnen et al., 2012). It is worth mentioning that [11C]PMP and PET does not allow accurate measurements of brain areas with high acetylcholinesterase activity levels, such as the striatum.

Last but not least, the uptake of [123I]iodobenzoatesamic acid (IBVM), an in vivo marker of the vesicular ACh transporter binding, was reduced only in parietal and occipital cortex (Kuhl et al., 1996) but not in the basal ganglia of non-demented PD patients.

Such a great variability in cholinergic activity in PD patients deserves further studies as it might unmask endogenous...
neuroprotective or compensatory mechanisms (Quik et al., 2012, 2013) and overall help profiling the disease changes at an early stage of the disease. In particular, there is increasing evidence that nicotine and other drugs that act at nAChRs may be beneficial in the management of PD. Several studies in animals have shown that nicotine administration enhances dopaminergic integrity in the striatum, especially when administered before/during but not after nigrostriatal damage (Huang et al., 2011). Indeed, a cholinergic loss does not parallel dopaminergic state in PD patients as measured by means of [18F]-DOPA and PET (Kas et al., 2009) or with markers of disease severity (i.e., UPDRS-III), disease duration, and daily dose of l-DOPA and dopamine agonists (i.e., LEDDs) (our study, Bohnen et al., 2006; Oishi et al., 2007; Kas et al., 2009).

Finally, several limitations of this study must be acknowledged. In particular: (1) there is no region devoid of nAChRs and therefore we could not measure accurately non-specific binding and then calculate a specific binding for the striatal area; (2) the low probability of tonically active neurons of the primate's striatum.

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REFERENCES

Aosaki, T., Kimura, M., and Graybiel, A. M. (1995). In vivo mapping of cholinergic terminals in normal aging, Alzheimer’s disease and in relation to neuroleptic medication. Neurology 49, 79–87. doi:10.1212/wnl.49.1.79

Chang, L. T. (1978). A method for attenuation correction in radionuclide computed tomography. IEEE Trans. Nucl. Sci. 25, 638–643. doi:10.1109/TNS.1978.4329385

Court, J. A., Piggott, M. A., Lloyd, S., Cookson, N., Ballard, C. G., McKeith, I. G., et al. (2000). Nicotine binding in human striatum: elevation in schizophrenia and reductions in dementia with Lewy bodies, Parkinson’s disease and Alzheimer’s disease and in relation to neuroleptic medication. Neuroscience 98, 79–87. doi:10.1016/S0306-4522(00)00071-3

Ehrt, U., Broich, K., Larsen, J. P., Ballard, C., and Aarsland, D. (2010). Use of drugs with anticholinergic effect and impact on cognition in Parkinson’s disease: a cohort study. J. Neurol. Neurosurg. Psychiatry 81, 160–165. doi:10.1136/jnnp.2009.186239

Fujita, M., Ichise, M., Zogghi, S. B., Liow, J. S., Ghose, S., Vines, D. C., et al. (2006). Widespread decrease of nicotinic acetylcholine receptors in Parkinson’s disease. Ann. Neurol. 59, 174–177. doi:10.1002/ana.20688

Gilman, S., Koepp, R. A., Nan, B., Wang, C. N., Wang, X., Junck, L. et al. (2010). Cerebral cortical and subcortical cholinergic deficits in parkinsonian syndromes. Neurology 74, 1416–1423. doi:10.1212/WNL.0b013e3181c4a55

Gotti, C., and Clementi, F. (2004). Neuronal nicotinic receptors from structure to pathology. Prog. Neurobiol. 74, 363–396. doi:10.1016/j.pneurobio.2004.09.006

Huang, L. Z., Grady, S. R., and Quik, M. (2011). Nicotine reduces L-DOPA-induced dyskinesias by acting at β2* nicotinic receptors. J. Pharmacol. Exp. Ther. 338, 932–941. doi:10.1124/jpet.111.182949

Isaias, I. U., Bentii, R., Cilia, R., Canesi, M., Marotta, G., Gerundini, P., et al. (2007). [123I]FP-CIT striatal binding in early Parkinson’s disease patients with tremor vs. akinetic-rigid onset. Neuroreport 18, 1499–1502. doi:10.1097/01.WNR.0b013e328e6f9b

Jellinger, K. A. (1991). Pathology of Parkinson’s disease. Changes other than the nigrostriatal pathway. Mol. Chem. Neurother. 14, 153–197. doi:10.1007/BF03159935

Kas, A., Bottlaender, M., Gallezot, J. D., Vidalet, M., Villafane, G., Grégoire, M. C., et al. (2009). Decrease of nicotinic receptors in the nigrostriatal system in Parkinson’s disease. J. Cereb. Blood Flow Metab. 29, 1601–1608. doi:10.1038/jcbfm.2009.77

Kreutz, A. C., and Malenka, R. C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson’s disease’s model. Nature 445, 643–647. doi:10.1038/nature05506

Kuhl, D. E., Minoshima, S., Fessler, J. A., Frey, K. A., Foster, N. L., Figuro, E. P., et al. (1996). In vivo mapping of cholinergic terminals in normal aging, Alzheimer’s disease, and Parkinson’s disease. Ann. Neurol. 40, 399–410. doi:10.1002/ana.410040009

Lang, A. E., and Blair, R. D. G. (1999). “Anticholinergic drugs and amantadine in the treatment of Parkinson’s disease,” in Drugs for the Treatment of Parkinson’s Disease, 1234–1252.
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