PAPER

$\alpha 4\beta 2$ nicotinic receptor status in Alzheimer's disease using ^{123}I -5IA-85380 single-photon-emission computed tomography

J T O'Brien, S J Colloby, S Pakrasi, E K Perry, S L Pimlott, D J Wyper, I G McKeith, E D Williams

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See end of article for authors' affiliations

Correspondence to: Dr S J Colloby, Institute for Ageing and Health, Newcastle University, Wolfson Research Centre, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, UK; s.j.colloby@ncl.ac.uk

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Background: Loss of the $\alpha 4\beta 2$ nicotinic receptor subtype is found at autopsy in Alzheimer's disease.

Objective: To investigate in vivo changes in this receptor using single-photon-emission CT (SPECT) with ^{123}I -5-iodo-3-[2(S)-2-azetidylmethoxy] pyridine (5IA-85380), a novel nicotinic acetylcholine receptor ligand which binds predominantly to the $\alpha 4\beta 2$ receptor.

Methods: 32 non-smoking subjects (16 with Alzheimer's disease and 16 normal elderly controls) underwent ^{123}I -5IA-85380 and perfusion ($^{99\text{m}}\text{Tc}$ -hexamethylenepropyleneamine oxime (HMPAO)) SPECT scanning. Region of interest analysis was performed with cerebellar normalisation.

Results: Significant bilateral reductions in nicotinic receptor binding were identified in frontal (left, $p=0.004$; right, $p=0.002$), striatal (left, $p=0.004$; right, $p=0.003$), right medial temporal ($p=0.04$) and pons ($p<0.001$) in patients with AD compared to controls. There were no significant correlations with clinical or cognitive measures. The pattern of nicotinic binding significantly differed from that of perfusion in both patients with AD and controls. Both ^{123}I -5IA-85380 and $^{99\text{m}}\text{Tc}$ -HMPAO SPECT imaging demonstrated similar diagnostic performance in correctly classifying controls and patients with AD.

Conclusion: Using ^{123}I -5IA-85380 SPECT we found changes consistent with significant reductions in the nicotinic $\alpha 4\beta 2$ receptor in cortical and striatal brain regions. This method could facilitate diagnosis and may be useful for monitoring progression of the disease and response to treatment in patients with AD and related diseases.

Nicotinic acetylcholine receptors (nAChRs) are known to be associated with important neurophysiological processes such as memory and learning.¹ Reductions in nAChR have been shown in a number of neurodegenerative disorders, including Alzheimer's disease, dementia with Lewy bodies (DLB) and Parkinson's disease.^{2–4} Loss of nAChR binding sites has been identified in frontal⁵ and other cortical areas in brain tissue of patients with AD compared to controls using the nicotinic receptor ligand ^3H -nicotine.⁶ Temporal archicortical regions including the parahippocampal gyrus have also been shown to be affected in the postmortem examination of the brain of patients with AD.³ Nicotinic-agonist (^3H -nicotine, ^3H -epibatidine and ^3H -cytisine) deficits have been demonstrated in the temporal brain cortex in the brain of patients with AD compared to age-matched controls.⁷ nAChRs consist of eight α ($\alpha 2$ – $\alpha 9$) and three β ($\beta 2$ – $\beta 4$) subunits,⁹ of which the most abundant varieties in the mammalian brain are the $\alpha 4\beta 2$ and $\alpha 7$ subtypes.¹

The marker ^{125}I -5-iodo-3-[2(S)-2-azetidylmethoxy] pyridine (5IA-85380) has been used to assess the nicotinic $\alpha 4\beta 2$ subtype receptor status in the postmortem examination of brain tissue of patients with AD, where receptor loss was shown in tissues of the striatum and entorhinal cortex compared to age-matched controls.¹⁰ In addition, a ligand with high affinity to the $\beta 2$ subtype, ^{18}F -2-fluoro-3-[2(S)-2-azetidylmethoxy] pyridine (2FA-85380), revealed a 36% reduction in both the thalamus and occipital cortex in the brains of patients with AD compared to elderly controls.¹¹ Although important in providing information about the mechanism of the receptor–ligand interaction and establishing suitability for specific receptor studies, one limitation of in vitro investigations with tissue postmortem examination is

that the results generally reflect end-stage disease and information regarding early pathological states is limited. By contrast, molecular in vivo imaging can provide data from living patients at various stages of illness and has the potential to investigate clinical correlates and the effects of medication.¹ One previous imaging study using ^{11}C -nicotine positron emission tomography (PET) investigated the nicotinic receptor status in vivo in patients with AD, showing deficits in frontal, temporal and hippocampal regions compared to controls.¹² Imaging studies, to date, are limited by availability of nicotinic subtype-specific ligands, although recently several studies showed that the single-photon-emission CT (SPECT) tracer ^{123}I -5IA-85380 was a suitable agent to quantify and image $\alpha 4\beta 2$ nAChR in normal humans.^{13–15} In this study we investigated, using a semiautomated region of interest (ROI) approach, differences in cortical and subcortical patterns of nAChR in vivo in patients with AD and in elderly normal controls using ^{123}I -5IA-85380 SPECT. ROI was chosen as it is robust, well-validated and can provide important semiquantitative data for this novel ligand. The pattern of nACh receptor status for each group was also compared with their associated regional cerebral blood flow (rCBF) patterns obtained from $^{99\text{m}}\text{Tc}$ -hexamethylenepropyleneamine oxime (HMPAO) SPECT imaging.

Abbreviations: CAMCOG, Cambridge Cognitive Examination; DLB, dementia with Lewy bodies; HMPAO, hexamethylenepropyleneamine oxime; MMSE, Mini-Mental State Examination; nAChR, nicotinic acetylcholine receptor; NPI, Neuropsychiatric Inventory; PET, positron emission tomography; rCBF, regional cerebral blood flow; ROI, region of interest; SPECT, single-photon-emission; 5IA-85380, 5-iodo-3-[2(S)-2-azetidylmethoxy] pyridine

METHODS

Subjects

We studied 32 non-smoking (for >10 years) subjects (16 with AD and 16 normal elderly controls). Patients with AD, who had been referred to local old age psychiatry services, were recruited from a community-dwelling population. Normal controls were recruited from among friends and spouses of patients included in this and other research studies. All subjects underwent ^{123}I -5IA-85380 and $^{99\text{m}}\text{Tc}$ -HMPAO SPECT scanning. The study was approved by the Newcastle, North Tyneside and Northumberland local research ethics committee and the UK Department of Health's Administration of Radioactive Substances Advisory Committee in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All participants gave informed consent and, in addition, for patients, the nearest relative provided written assent.

Assessments and diagnosis

Subjects underwent detailed physical, neurological and neuropsychiatric examinations, which included history, mental state and physical examination and, for patients with AD, a standard blood screen with thyroid function tests, B12, folate and syphilis serology and CT scan. Standardised schedules administered included the Mini-Mental State Examination (MMSE),¹⁶ the Cambridge Cognitive Examination (CAMCOG)¹⁷ and the Neuropsychiatric Inventory (NPI).¹⁸ Each patient was diagnosed as having the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorder Association "probable" AD by consensus between two experienced clinicians.¹⁹ Controls had no signs or symptoms of cognitive disturbance and did not meet criteria for mild cognitive impairment. Moreover, all controls scored within the normal range of cognitive tests; all scored ≥ 27 on the MMSE and >90 on the CAMCOG, >2 SDs above the cut-off scores denoting cognitive impairment.

Preparation of ^{123}I -5IA-85380

5- ^{123}I -A85380 was produced from the corresponding stan-nyl precursor, 5-SnBu₃-A85380, by electrophilic iododestannylation. The radiolabelling was performed according to a previous report,¹⁰ with slight modifications. Reagents were added to carrier-free Na ^{123}I (1110–2500 MBq), in approximately 100–200 μl of 0.05 M NaOH, in the following order: 20 μl of 2 M HCl, 300 μg of 5-SnBu₃-A85380 (in 100 μl ethanol), 50 μg (50 μl) Chloramine-T. The mixture was stirred for 30 min at room temperature. Then, 100 μg (10 μl) of NaHSO₄ and 100 μl of 2 M HCl were added and the mixture was stirred for 15 min at 65°C. The mixture was then purified by reverse-phase high performance liquid chromatography and the solvent was removed by rotary evaporation. The 5- ^{123}I -A85380 was formulated as 185 MBq in 5 ml (0.9%) of saline for intravenous injection containing up to 1.5 mg ascorbic acid and filtered through a 0.22 μm filter. The 5- ^{123}I -A85380 was produced with an isolated mean (SD) radiochemical yield of 60% (4.6%; $n=21$) and had a radiochemical purity of $>98\%$.

Acquisition

^{123}I -5IA-85380 Scanning

Subjects were imaged using a triple-detector rotating γ camera (Picker 3000XP; Picker, Twinsburg, Ohio, USA) fitted with a low-energy high-resolution fan-beam collimator. At 2 h after a bolus intravenous injection of 185 MBq of ^{123}I -5IA-85380, one hundred and twenty 15 s views over a 360° orbit were acquired from each detector on a 128×128 matrix with a pixel size and slice thickness of 3.5 mm. Scanning at 2 h after injection was

based on the results from time-activity curve analysis and practical issues of scanning the patient group. Imaging time was 30 min. Image reconstruction was performed using ramp-filtered back-projection with a Butterworth filter (order 13, cut-off 0.2 cycle/cm) to produce the transverse sections. The data were resampled to generate images of dimension 64×64 with cubic voxels of 4 mm. The reconstructed images were corrected for γ ray attenuation using the Chang method,²⁰ with an attenuation coefficient of 0.11/cm.

$^{99\text{m}}\text{Tc}$ -HMPAO scanning

Using the same triple-detector rotating γ camera, and within 3 months of ^{123}I -5IA-85380 scanning, all subjects underwent a $^{99\text{m}}\text{Tc}$ -HMPAO SPECT scan (a marker of rCBF) with the exception of one patient with AD who died before this could be undertaken. Approximately 10 min after injection of 500 MBq $^{99\text{m}}\text{Tc}$ -HMPAO (Ceretec, GE Healthcare, Berkshire, UK), one hundred and twenty 15 s views over a 360° orbit were acquired from each detector on a 128×128 matrix with a pixel size and slice thickness of 3.5 mm. Imaging time was 30 min. Image processing and reconstruction were performed using identical parameters for the processing and reconstruction of ^{123}I -5IA-85380 scans. The reconstructed images were similarly corrected for γ -ray attenuation using Chang's method (attenuation coefficient 0.11 cm^{-1}). Subsequently, both ^{123}I -5IA-85380 and $^{99\text{m}}\text{Tc}$ -HMPAO scans were transferred to a personal computer for further analysis.

Data analysis

Semiautomated ROI analysis was performed on all imaging data using the biomedical imaging program ANALYZE (V.4.0, Mayo Clinic, Rochester, Minnesota, USA). Using an affine transform (12 parameters), each image volume was registered to match a rCBF $^{99\text{m}}\text{Tc}$ -HMPAO SPECT template in standard MNI space (Montreal Neurological Institute, <http://www.bic.mni.mcgill.ca>) using statistical parametric mapping (SPM99, Wellcome Department of Cognitive Neurology, London, UK). The voxel size of images before and after spatial normalisation was preserved. The resulting output volumes were contained within the dimensions held by the template bounding box (x, y, z ; ie, $-90:91$ mm, $-126:91$ mm and $-72:109$ mm, respectively), which describes the extent of the output volume relative to the anterior commissure. As the required registration accuracy was of a similar order to the spatial resolution, image registration using purely linear transformations was considered adequate.²¹ The template image was generated from 39 elderly controls (16 of whom participated in this nicotinic SPECT study) by spatial normalisation of each scan to the generic SPECT template in SPM99; this was followed by creation of a mean image from the registered scans that was smoothed with an 8 mm FWHM Gaussian filter.

Next, a ROI map (fig 1A), which has been previously described,^{22,23} containing a set of 19 predefined regions and modified to conform to standard MNI space, was applied to each registered nicotinic SPECT scan (fig 1B). The mean count per voxel was calculated for each region in both hemispheres. Subsequently, in order to allow for variability in global tracer uptake between subjects and avoid masking any regional changes, the ratio of mean count per voxel to mean cerebellum count per voxel was calculated for each region and each subject, producing intensity-normalised data. The procedure was then repeated for $^{99\text{m}}\text{Tc}$ -HMPAO SPECT imaging data.

Statistical analysis

Data were analysed using the SPSS V.11. Continuous variables for each group were tested for normality of

Figure 1 Region of interest map overlaid on to (A) ^{99m}Tc -hexamethylenepropyleneamine oxime single-photon-emission CT (SPECT) template in standard Montreal Neurological Institute (MNI) space used for image registration and (B) a mean nicotinic ^{123}I -5-iodo-3-[2(S)-2-azetidylmethoxy] pyridine SPECT image in standard MNI space of 16 controls. Notice the increased uptake in the thalamus in (B), corresponding to a rich density of nicotinic receptors compared to the cerebral cortex. B, basal; L, left; R, right.

distribution using the Shapiro–Wilk test and visual inspection of variable histograms, whereas homogeneity of variance was examined using Levene's test. Subsequently, differences in normalised regional activity ratios between groups for both nicotinic and rCBF imaging data were investigated by analysis of covariance (controlling for effects of age) using either F (equal variance) or W (non-equal variance) statistics. Correlations between nicotinic imaging data and clinical measures were investigated by Pearson's r or Spearman's r where appropriate.

RESULTS

Subjects

Table 1 shows the demographic characteristics of subjects. Groups were similar with respect to gender but there was a significant, though numerically small, difference in age (patients with AD were older than controls; mean (SD) difference 5.4 (8) years; $p = 0.01$). Age was therefore included as a covariate in all analyses. As expected, MMSE and

Table 1 Demographic and clinical characteristics of subjects studied with ^{123}I -5-iodo-3-[2(S)-2-azetidylmethoxy] pyridine single-photon-emission CT

	Control	AD	Significance
n	16	16	
Sex (M:F)	10:6	7:9	NS
Age	75.4 (4.5)	80.8 (6.6)	0.01*
MMSE (maximum 30)	28.8 (1.1)	17.2 (5.7)	<0.001†
CAMCOG (maximum 107)	97.9 (3.8)	59.7 (18.3)	<0.001†
NPI	NA	14.4 (12.5)	
Medications			
Cholinesterase inhibitors			
Donepezil	0	5	
Galantamine	0	2	
Rivastigmine	0	0	

AD, Alzheimer's disease; CAMCOG, Cambridge Cognitive Examination; F, female; M, male; MMSE, Mini-Mental State Examination; NA, not applicable; NPI, Neuropsychiatric Inventory; NS, not significant.

Values expressed as mean (SD).

F test, *control > AD.

Welch (W) test, †control > AD.

CAMCOG scores in patients with AD were lower than in controls ($p < 0.001$). Seven patients with AD were receiving cholinesterase inhibitors (for >3 months) at the time of the study (donepezil, $n = 5$; galantamine, $n = 2$).

Group differences in ^{123}I -5IA-85380 and ^{99m}Tc -HMPAO binding

Table 2 shows the mean ratios (adjusted for age) of ^{123}I -5IA-85380 activity within each ROI for controls and patients with AD. Compared with controls, significant reductions in uptake were identified bilaterally in frontal ($F_{1,29} \geq 9.9$, $p \leq 0.004$) and striatal ($F_{1,29} \geq 9.9$, $p \leq 0.004$) regions, in right medial temporal ($F_{1,29} = 4.8$, $p \leq 0.04$) and pons ($F_{1,29} = 12.7$, $p < 0.001$) regions in patients with AD. Figure 2 shows an example of scans. Because of the possibility that treatment with cholinesterase may have affected the results, additional post hoc analyses were carried out. Significantly reduced uptake was still found bilaterally in frontal ($F_{1,22} \leq 5.5$, $p \leq 0.03$) and striatal ($F_{1,22} \leq 4.8$, $p \leq 0.04$) regions and pons ($F_{1,22} = 16.2$, $p < 0.001$) in the nine untreated patients with AD compared to the controls. Moreover, there was no difference in uptake between the nine subjects with AD who were medication-free and the seven on cholinergic treatment (greatest deviation, $F_{1,13} = 2.3$, $p > 0.15$).

Table 2 presents mean intensity-normalised ^{99m}Tc -HMPAO activity ratios (adjusted for age) within each ROI for controls and patients with AD. Compared to controls, significant reductions in rCBF in patients with AD were observed bilaterally in frontal ($F_{1,29} \geq 5.9$, $p \leq 0.02$), central (sensory-motor) ($F_{1,29} \geq 4.5$, $p = 0.04$), parietal ($F_{1,29} \geq 9.3$, $p \leq 0.005$), striatal ($F_{1,29} \geq 8.4$, $p \leq 0.007$), temporal and medial temporal ($F_{1,29} \geq 5.5$, $p \leq 0.03$) regions. In order to illustrate the sense of overlap between controls and patients with AD in ^{123}I -5IA-85380 and ^{99m}Tc -HMPAO, individual mean uptake values for each type of SPECT scan were plotted for each group in selected brain regions (fig 3, frontal; fig 4, striatum).

Correlations of ^{123}I -5IA-85380 uptake with clinical and cognitive measures

In the group with AD, there were no significant correlations between ^{123}I -5IA-85380 uptake in any region and age ($r \leq 0.35$, $p \geq 0.18$), MMSE ($r \leq 0.31$, $p \geq 0.25$), CAMCOG ($r \leq 0.29$, $p \geq 0.28$) or NPI total score ($p \leq 0.46$, $p \geq 0.06$). Age effects were also investigated in the control group, which revealed no significant relationship between 5IA binding and age ($p \geq 0.07$).

Differences in patterns of uptake between ^{123}I -5IA-85380 and ^{99m}Tc -HMPAO SPECT

The pattern of nicotinic binding relative to rCBF was also investigated. In order to establish which regions differed significantly in uptake of ^{123}I -5IA-85380 relative to ^{99m}Tc -HMPAO, the ratio of normalised ^{123}I -5IA-85380 and ^{99m}Tc -HMPAO uptake values in each ROI was calculated for each subject and the mean was calculated for each group. One-sample t tests were carried out for each region within groups, where significant deviations from unity represent changes in nicotinic binding relative to blood flow. In both controls and patients with AD, nicotinic binding significantly deviated bilaterally from rCBF in the occipital region ($t \geq 2.8$, $p \leq 0.014$), thalamus ($t \geq 13.1$, $p < 0.001$), striatum ($t \geq 3.3$, $p \leq 0.006$) and pons ($t \geq 5.7$, $p < 0.001$). In controls, nicotinic uptake differed from rCBF bilaterally in medial temporal ($t \geq 3.6$, $p \leq 0.002$), and in left central ($t = 3.9$, $p = 0.001$) and left parietal ($t = 3.1$, $p < 0.008$) regions.

Table 2 Summary of age-adjusted intensity-normalised ^{123}I -5-iodo-3-[2(S)-2-azetidylmethoxy] pyridine and $^{99\text{m}}\text{Tc}$ -hexamethylenepropyleneamine oxime uptake values obtained from the region of interest in controls and patients with Alzheimer's disease

ROI (voxels)	^{123}I -5IA-85380		$^{99\text{m}}\text{Tc}$ -HMPAO	
	Control	AD	Control	AD
L frontal (1614)	0.84 (0.03)	0.78 (0.06)	0.85 (0.04)	0.80 (0.06)
R frontal (1614)	0.82 (0.04)	0.76 (0.05)	0.82 (0.04)	0.78 (0.04)
L central (290)	0.83 (0.05)	0.81 (0.09)	0.88 (0.04)	0.83 (0.07)
R central (290)	0.80 (0.07)	0.75 (0.08)	0.82 (0.04)	0.78 (0.08)
L parietal (503)	0.90 (0.06)	0.87 (0.09)	0.92 (0.05)	0.85 (0.06)
R parietal (503)	0.84 (0.06)	0.81 (0.08)	0.88 (0.05)	0.79 (0.08)
L occipital (578)	0.82 (0.07)	0.81 (0.09)	0.91 (0.06)	0.86 (0.08)
R occipital (578)	0.79 (0.05)	0.77 (0.10)	0.87 (0.05)	0.83 (0.08)
L temporal (720)	0.86 (0.06)	0.81 (0.09)	0.88 (0.06)	0.80 (0.06)
R temporal (720)	0.79 (0.05)	0.75 (0.08)	0.81 (0.05)	0.75 (0.08)
L medial temporal (239)	0.77 (0.06)	0.72 (0.07)	0.84 (0.07)	0.75 (0.07)
R medial temporal (239)	0.83 (0.08)	0.76 (0.09)	0.88 (0.07)	0.79 (0.10)
L thalamus (185)	1.28 (0.06)	1.22 (0.11)	1.01 (0.07)	0.98 (0.08)
R thalamus (185)	1.26 (0.06)	1.22 (0.09)	1.01 (0.07)	0.97 (0.08)
L striatum (399)	0.93 (0.07)	0.85 (0.06)	0.98 (0.06)	0.92 (0.06)
R striatum (399)	0.96 (0.07)	0.88 (0.07)	1.02 (0.07)	0.94 (0.08)
L cerebellum (1024)	1.02 (0.02)	1.03 (0.03)	1.02 (0.01)	1.04 (0.03)
R cerebellum (1024)	0.98 (0.02)	0.97 (0.03)	0.98 (0.01)	0.97 (0.03)
Pons (144)	0.99 (0.07)	0.89 (0.07)	0.82 (0.05)	0.78 (0.07)

AD, Alzheimer's disease; HMPAO, hexamethylenepropyleneamine oxime; L, left; R, right; ROI, region of interest; 5IA-85380, 5-iodo-3-[2(S)-2-azetidylmethoxy] pyridine. Values expressed as mean (SD). Values in bold denote significant differences.

Figure 2 Transverse sections of ^{123}I -5-iodo-3-[2(S)-2-azetidylmethoxy] pyridine single-photon-emission CT scans in standard Montreal Neurological Institute space of a healthy control and a patient with Alzheimer's disease (AD). Note the decreased uptake in both pons and striatum in patients with AD relative to controls. Images are intensity scaled to corresponding mean count per voxel in cerebellum.

The role of ^{123}I -5IA-85380 and $^{99\text{m}}\text{Tc}$ -HMPAO in discriminating controls and patients with AD

To investigate the ability of nicotinic and rCBF scans to discriminate 15 patients with AD from 16 controls, linear discriminant analysis (linear discriminant analysis-forced entry) was performed separately using nicotinic and rCBF measures as independent variables. For each analysis, the most discriminating regions were chosen (ie, left and right frontal, left and right striatum and pons for nicotinic; bilateral frontal, parietal and medial temporal for HMPAO). For nicotinic SPECT, the function accounted for approximately 44% of the variance (Wilks λ 0.56; $\chi^2 = 15.5$, $df = 5$, $p = 0.008$) and resulted in 73% (sensitivity) of patients with AD and 88% (specificity) of controls being correctly classified. For HMPAO

Figure 3 Scatter plots of individual ^{123}I -5-iodo-3-[2(S)-2-azetidylmethoxy] pyridine and $^{99\text{m}}\text{Tc}$ -hexamethylenepropyleneamine oxime (HMPAO) mean uptake values in controls and patients with Alzheimer's disease (AD) in the frontal cortex.

Figure 4 Scatter plots of individual ^{123}I -5-iodo-3-[2(S)-2-azetidinylmethoxy] pyridine and $^{99\text{m}}\text{Tc}$ -hexamethylenepropyleneamine oxime (HMPAO) mean uptake values in controls and patients with Alzheimer's disease (AD) in the striatum.

SPECT, the discriminant function accounted for 36% of variance between groups (Wilks λ 0.64; $\chi^2 = 12.6$, $df = 6$, $p = 0.05$), resulting in 80% (sensitivity) of patients with AD and 81% (specificity) of controls being correctly classified. Misclassification of controls as patients with AD occurred in two cases using nicotinic SPECT and in three cases using HMPAO, none of which were jointly miscategorised. Misclassification of patients with AD as controls occurred in four cases with nicotinic SPECT and in three cases with HMPAO, with only one patient with AD being jointly miscategorised.

DISCUSSION

This study has shown decreased ^{123}I -5IA-85380 uptake, presumably reflecting $\alpha 4\beta 2$ receptor loss, in patients with AD compared to controls bilaterally in frontal and striatal regions as well as in the right medial temporal and pons. Reductions in the frontal cortex seem to be consistent with previous in vitro postmortem and in vivo PET imaging studies in patients with AD,^{5,12} where 52% of nicotine and 55% of acetylcholine-binding sites were found in patients with AD. Reduced ^{123}I -5IA-85380 uptake in striatum in patients with AD is in agreement with most, though not all,²⁴ autopsy studies showing significant reductions in nicotinic binding sites in caudate,^{2,10} putamen²⁵ and whole striatum,²⁶ and may reflect loss of corticostriatal projections. Interestingly, marked amyloid deposits, particularly in frontal and striatal regions, were observed in patients with mild AD relative to controls using an amyloid-binding PET tracer termed Pittsburgh Compound-B.²⁷ Temporal lobe deficits in nAChR, especially in the hippocampus and entorhinal cortex, have been identified in AD in numerous postmortem studies^{3,7,8,10,28,29} and with ^{11}C -Nicotine PET imaging.¹² We found only modest reductions on the right side. It is possible that the reduction in nAChR in the temporal cortex may indicate either end-stage or severe AD and the relative preservation in this region observed may reflect the inclusion of earlier, milder cases. A previous study reported no significant differences between controls and subjects with mild AD in insular cortex, suggesting nicotinic receptor density in temporal areas may be relatively preserved early in the disease process, perhaps because of receptor upregulation.¹⁰ Alternatively, previous demonstrations of nicotinic receptor loss in the temporal cortex may reflect reductions in another subtype, with relative preservation of the $\alpha 4\beta 2$ receptor.

SPECT measures of rCBF and receptor availability are both influenced by atrophy and partial volume effects, and so both nicotinic and rCBF scans should be equally affected by neural degeneration. Thus, where there is overlap between the results of nicotinic $\alpha 4\beta 2$ receptor and rCBF loss—that is, in frontal, striatal and temporal regions—this could be a result of atrophy. Overall, our results suggest that there is some selective loss of nicotinic receptors in patients with AD that was greater than the general functional deficits shown on rCBF scans.

Demographical and neuropsychological measures (age, MMSE, CAMCOG and $\text{NPI}_{\text{total}}$) did not correlate with 5IA uptake in patients with AD. Several possible explanations could include small sample size, cognitive and clinical or SPECT measures lacking sensitivity, or that $\alpha 4\beta 2$ receptor loss occurred early in the disease process, even before symptoms appeared. Another reason could lie in that most of our patients with AD were in the mild-moderate stages of illness; inclusion of a larger heterogeneous group ranging from very early to more severe disease may improve sensitivity in detecting a significant correlation between imaging and cognitive scores. However, results appear to support previous studies showing that treatment of AD with nicotine and nicotinic agonists have limited effects on cognitive functioning—that is, only in attention, but not in memory³⁰⁻³²—while an autopsy report revealed that the decline in cognitive function does not correlate with loss of nicotine acetylcholine receptors in either AD or DLB.³³ Also, patients tended to lack behavioural and psychiatric disturbances—for example, NPI scores were low and insufficient numbers had psychotic features for correlates to be examined. Autopsy studies have revealed possible links between delusions and the muscarinic receptor, although the $\alpha 7$ nicotinic receptor has been associated with visual hallucinations in DLB,³⁴ and the $\alpha 4\beta 2$ subtype has also been shown to be linked with fluctuations in this group.^{35,36} However, fluctuations are prominent in DLB rather than in AD, and it will be important for future studies to examine associations with ^{123}I -5IA-85380 uptake in vivo in DLB.

The group with AD was slightly older than the control group ($p = 0.01$), therefore statistical analyses were used in an attempt to adjust for such age differences despite no significant relationship between nicotinic uptake and age in either group for any brain region. Seven patients with AD were being treated with cholinesterase inhibitors at the time of their ^{123}I -5IA-85380 scan. Those patients not on medication showed the same differences from controls as the whole group. However, although the use of cholinergic medication could not have accounted for our results, it is still possible that cholinergic treatment produces small changes in the $\alpha 4\beta 2$ receptor, as has been demonstrated after long-term use of tacrine.³⁷ Serial studies before and after treatment is required to address this issue.

A difference in pattern of uptake was demonstrated between ^{123}I -5IA-85380 and $^{99\text{m}}\text{Tc}$ -HMPAO scans in both study groups. Nicotinic-binding patterns in pons, occipital, thalamic and striatal regions significantly differed from rCBF patterns in controls and patients with AD (fig 1). The pons and thalamus are cholinergic-rich brain regions with high concentrations of $\alpha 4\beta 2$ -nAChR. Uptake was consistent with the known distribution of nAChR in humans, providing validation of this ligand as being specific for the cholinergic system and not simply a distribution reflecting perfusion. The uptake pattern observed in controls also resembled the pattern of uptake reported from previous SPECT studies using ^{123}I -5IA-85380 in healthy volunteers.^{14,15} The difference in uptake between ^{123}I -5IA-85380 and $^{99\text{m}}\text{Tc}$ -HMPAO were generally in regions with a greater density of nicotinic binding sites, but other reasons that

explain the distribution of these radiotracers in the current dataset may exist.

Both ^{123}I -5IA-85380 and $^{99\text{m}}\text{Tc}$ -HMPAO SPECT imaging demonstrated comparable ability in this dataset to correctly classify controls and patients with AD. The discriminant functions accounted for 44% of the variance between groups using nicotinic SPECT and 36% using HMPAO SPECT. The unexplained variance was probably due to the other ROI variables not entered into the models as well as random experimental and physiological variations between subjects. Sensitivity in identifying patients with AD was 73% for nicotinic SPECT and 80% for HMPAO SPECT whereas specificity was 88% and 81%, respectively. These values are in keeping with previous $^{99\text{m}}\text{Tc}$ -HMPAO SPECT imaging studies on AD.^{38,39} Eleven controls (11/16; 69%) were correctly and mutually classified with nicotinic or HMPAO SPECT and this was lower for patients with AD (8/15; 53%), but clearly much larger studies are needed to investigate diagnostic utility.

In summary, we have demonstrated significant reductions using 5IA SPECT in patients with AD in cortical and striatal regions, with a pattern distinct from that obtained using perfusion (rCBF) SPECT. Together with previous studies in healthy volunteers and autopsy studies in patients with AD, this validates ^{123}I -5IA-85380 SPECT as an in vivo marker of the $\alpha 4\beta 2$ receptor subtype. Further studies, including those in very early disease (mild cognitive impairment), other dementias and serial imaging are required to further assess its role in diagnosis, progression of disease and response to treatment.

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Authors' affiliations

J T O'Brien, S J Colloby, S Pakrasi, E K Perry, I G McKeith, Institute for Ageing and Health, Newcastle University, Wolfson Research Centre, Newcastle General Hospital, Newcastle upon Tyne, UK

S L Pimlott, West of Scotland Radionuclide Dispensary, North Glasgow University Hospitals NHS Trust, Western Infirmary, Glasgow, UK

D J Wyper, Department of Clinical Physics, South Glasgow University Hospitals NHS Trust, Southern General Hospital, Glasgow, UK

E D Williams, Regional Medical Physics Department, Sunderland Royal Hospital, Sunderland, UK

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NEUROLOGICAL PICTURE

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Involvement of the recurrent artery of Heubner with contralateral middle cerebral artery infarction

An 86-year-old Chinese woman presented with left-sided weakness for 6 h. Her medical history included hypertension, ischaemic heart disease, atrial fibrillation and hypercholesterolaemia. She was conscious and communicative, with blood pressure 166/102 mm Hg and irregular pulse (102 beats/min). Examination showed right-gaze deviation

and left-hemiparesis (Medical Research Council power grade 0 and 2 in the upper and the lower extremity, respectively, National Institute of Health Stroke Scale 11 points).

Computed tomography of the brain showed hyper-dense right middle cerebral artery (MCA) and early ischaemic changes in its territory (fig 1A,B).

Transcranial Doppler sonography showed right MCA occlusion with flow diversion to the ipsilateral anterior cerebral artery (ACA). Low flow velocity in the contralateral A1-ACA suggested its hypoplastic nature.

After 2 days, she developed increased weakness in the left-lower extremity (Medical Research Council power grade 0), transient choreiform movements of the right upper extremity, followed by right hemiparesis (power grade 3, National Institute of Health Stroke Scale 19 points) and new speech difficulties (non-fluent aphasia, syntax errors, repetition impairment and word-finding difficulty). Repeated brain computed tomography showed an established right MCA infarction, brain swelling with mild midline shift and subfalcine herniation (fig 1C). New infarcts were noted in the right ACA territory, left caudate head and internal capsule.

In subfalcine herniation, the expanding hemisphere forces the ipsilateral cingulate gyrus under the falx-cerebri, compressing the ipsilateral ACA. Recurrent artery of Heubner is an inconstant branch of ACA, usually arising just beyond the anterior communicating artery. It supplies blood to the anteromedial part of the head of the caudate nucleus and the anteroinferior internal capsule. In patients with hypoplastic A1-ACA, both A2 segments and their branches are perfused by one patent ACA. In our patient, subfalcine herniation caused compression of the right ACA and compromised the left recurrent artery of Heubner, with resultant infarctions in the corresponding territories.

Figure 1 Temporal evolution in right middle cerebral artery (MCA) infarct. Non-enhanced computed tomography scan of the brain performed 6 h after symptom onset, showing a dense right MCA (A) together with early ischaemic changes in its territory (B). Brain computed tomography scan repeated after 3 days (C) shows well-defined hypodensity in the right MCA. A mass-effect and subfalcine herniation (curved arrow) are seen with resultant infarctions in the territories of the ipsilateral anterior cerebral artery (ACA) and contralateral recurrent artery of Heubner. A schematic diagram (D) shows the absent left A1-ACA; both recurrent arteries of Heubner receive perfusion from the right A1-ACA.

Vijay K Sharma, Bernard P L Chan

Division of Neurology, National University Hospital, Singapore

Correspondence to: Dr V Sharma, Department of Medicine, National University Hospital, 119074, Singapore; drvijay@singnet.com.sg

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