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RESEARCH PAPER

Characterization of histamine H_3 receptors in Alzheimer's Disease brain and amyloid over-expressing TASTPM mice

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Background and purpose: Histamine H_3 receptor antagonists are currently being evaluated for their potential use in a number of central nervous system disorders including Alzheimer's Disease (AD). To date, little is known about the state of H_3 receptors in AD.
Experimental approach: In the present study we used the radiolabelled H_3 receptor antagonist [^{3}H]GSK189254 to investigate H_3 receptor binding in the amyloid over-expressing double mutant APPswe × PSI.MI46V (TASTPM) transgenic mouse model of AD and in post-mortem human AD brain samples.
Key results: No significant differences in specific H_3 receptor binding were observed between wild type and TASTPM mice in the cortex, hippocampus or hypothalamus. Specific [^{3}H]GSK189254 binding was detected in sections of human medial frontal cortex from AD brains of varying disease severity (Braak stages I–VI). With more quantitative analysis in a larger cohort, we observed that H_3 receptor densities were not significantly different between AD and age-matched control brains in both frontal and temporal cortical regions. However, within the AD group, [^{3}H]GSK189254 binding density in frontal cortex was higher in individuals with more severe dementia prior to death.
Conclusions and implications: The maintenance of H_3 receptor integrity observed in the various stages of AD in this study is important, given the potential use of H_3 antagonists as a novel therapeutic approach for the symptomatic treatment of AD.
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Keywords: H_3 receptor; Alzheimer's Disease; [^{3}H]GSK189254; TASTPM mouse; neocortex

Abbreviations: Aβ, β-amyloid; AD, Alzheimer's Disease; MMSE, Mini-Mental State Examination; PEI, polyethyleneimine; TMN, tuberomamillary nucleus; WT, wild type

Introduction

Alzheimer's Disease (AD) is characterized by deficits in a number of neurotransmitter systems which are believed to result in cognitive dysfunction as well as neuropsychiatric behaviour. Loss of cholinergic neurons in the basal forebrain is one of the most prominent and consistent events occurring in AD (Whitehouse *et al.*, 1982), and provided the rationale for the development of cholinergic replacement therapies such as acetylcholinesterase inhibitors (Bartus *et al.*, 1982). Prominent cell loss also occurs in glutamatergic pyramidal neurons of the cortex and hippocampus (Greenamyre *et al.*, 1988), while deficits in 5-hydroxytryptaminergic, GABAergic, noradrenergic and dopaminergic pathways have also been described, although the degree to which these correlate with cognitive and/or behavioural changes in AD can vary (Ramirez *et al.*, 2005). The density of certain receptors linked to these neurotransmitter pathways are modulated in AD brain, including 5-HT_{2A} receptors which are decreased in temporal cortex compared with age-matched controls (Lai *et al.*, 2005).

The histaminergic system has also been implicated in AD, although its importance is difficult to assess due to a number of conflicting reports (Fernández-Novoa and Cacabelos, 2001). For example, histamine levels in AD brains have been reported to be increased in areas such as temporal and frontal cortex, basal ganglia and hippocampus (Cacabelos *et al.*, 1989). However, other studies have shown decreases in histamine content in the hypothalamus, hippocampus and temporal cortex of AD brains (Mazurkiewicz-Kwilecki and

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Nsonwah, 1989; Panula *et al.*, 1998). Histaminergic cell bodies are solely located in the tuberomamillary nucleus (TMN) of the posterior hypothalamus (Brown *et al.*, 2001). Despite some reports showing the occurrence of neurofibrillary tangles in the TMN of AD patients, the distribution and number of histaminergic cell bodies was very similar to that of normal brains (Airaksinen *et al.*, 1991). In contrast, another group showed a significant reduction in large-sized histamine containing neurons in the TMN where numerous neurofibrillary tangles were found, indicative of a central histaminergic dysfunction (Nakamura *et al.*, 1993). High levels of histamine have also been reported in cerebrospinal fluid and serum of AD patients, although this may originate from mast cells as well as the central nervous system (CNS) (Fernández-Novoa and Cacabelos, 2001).

The physiological effects of histamine are mediated through four G-protein-coupled 7-transmembrane receptor subtypes, namely H_1, H_2, H_3 and H_4 (Brown *et al.*, 2001; nomenclature follows Alexander *et al.*, 2008). Few studies have investigated histamine receptor subtypes in AD brain. A positron emission tomography study has demonstrated a decrease in frontal and temporal H_1 receptors in AD patients (Higuchi *et al.*, 2000), while the number of H_2 receptors in temporal cortex and striatum has been reported to be normal in AD post-mortem brains (Perry *et al.*, 1998). We recently showed preliminary evidence for qualitatively normal H_3 receptor binding in AD medial temporal cortex (Medhurst *et al.*, 2007), while there are no reports to date describing H_4 receptors in AD brain.

Among the histamine receptor subtypes, H_3 receptors play an important regulatory role in the CNS. Activation of H_3 autoreceptors can inhibit histamine synthesis and release from histaminergic neurons (Arrang *et al.*, 1983), while activation of H_3 heteroreceptors can inhibit release of other neurotransmitters such acetylcholine, noradrenaline, dopamine and 5-HT from non-histaminergic neurons (Schlicker *et al.*, 1994; Blandina *et al.*, 1996; Brown *et al.*, 2001). Conversely, blockade of H_3 receptors with selective antagonists can increase the release of neurotransmitters involved in cognitive processes (Fox *et al.*, 2005; Medhurst *et al.*, 2007). Selective H_3 receptor antagonists have been shown to improve performance in a diverse range of rodent cognition paradigms (Hancock and Fox, 2004; Witkin and Nelson, 2004; Medhurst *et al.*, 2007), and can also increase wakefulness (Brown *et al.*, 2001; Barbier *et al.*, 2004). This has led to the development of H_3 receptor antagonists for the potential treatment of several CNS disorders including cognitive dysfunction in AD (Passani *et al.*, 2004; Esbenshade *et al.*, 2008).

GSK189254 is a novel, highly selective H_3 receptor antagonist which shows efficacy in a number of cognition paradigms in rats (Medhurst *et al.*, 2007). Given the limited information on H_3 receptors in AD, we investigated H_3 receptor binding using [^{3}H]GSK189254 in the double mutant APPswe × PSI.MI46V (TASTPM) transgenic mouse model of AD. In these mice, over-expression of both human amyloid precursor protein (hAPP695swe) and presenilin-1 (PS1.M146V) transgenes results in β-amyloid (Aβ) deposition from 3 months and cognitive deficits from 6 to 8 months of age (Howlett *et al.*, 2004). In addition, we carried out a detailed analysis of H_3 receptor binding with [^{3}H]GSK189254 in human post-mortem AD neocortex samples using both autoradiography and saturation binding studies. These studies demonstrate maintenance of H_3 receptor integrity even in severe AD, an important finding given that H_3 receptor antagonists are being pursued as a novel symptomatic treatment for AD.

Methods

TASTPM transgenic mice

All experimental procedures were conducted in compliance with the Home Office Guidance on the operation of the Animals (Scientific Procedures) Act 1986 under the authority granted in personal and project licenses, and procedures were reviewed and approved by the GlaxoSmithKline Procedures Review panel. Adequate measures were taken throughout to minimize pain or discomfort. TASTPM transgenic mice over-expressing human amyloid precursor protein and presenilin-1 cDNAs harbouring the swedish and M146V mutations respectively were generated as previously described (Richardson *et al.*, 2003; Howlett *et al.*, 2004). Thirteen or 16-month-old wild type (WT) and TASTPM mice were used for saturation binding ($n = 5$ per group) and autoradiography ($n = 6–8$ per group) studies respectively, ages by which significant cognitive deficit and Aβ load would have been present for over 6 months (Howlett *et al.*, 2004).

Human brain tissues

For autoradiography studies, human medial frontal gyrus tissues of varying disease severity [AD Braak stages I, II, IV, V and VI; Braak and Braak (1991), male or female, ages 72–90 years, non-neurological cause of death] were obtained from the Netherlands Brain Bank following informed patient consent, approval of local ethics and GlaxoSmithKline human tissue committees and compliance with the Human Tissue Act (2006). Twenty μm frozen sections were prepared as previously described (Roberts *et al.*, 2004). AD plaque pathology was confirmed in adjacent sections using monoclonal 1E8 antibody (1 : 1000 dilution) raised against the 13–27 fragment of Aβ as previously described (Howlett *et al.*, 2004).

For saturation binding studies, tissues from a well-characterized cohort of community-based, longitudinally follow-up AD patients (Hope *et al.*, 1997; 1999) were used. Cognitive functioning was assessed 4-monthly from study recruitment to death (mean follow-up 3.5 years) with the Mini-Mental State Examination (MMSE, scores of 0–30, Folstein *et al.*, 1975). At death, informed consent was obtained from next of kin prior to the removal of brain, and tissues from the frontal (orbitofrontal gyrus, Brodmann area 11) and temporal (mid-temporal gyrus, Brodmann area 21) cortices of a maximum of 27 AD patients as well as 12 non-neurological controls were dissected, homogenized and stored at −75°C as previously described (Lai *et al.*, 2003). Tissues from both regions were not available for all subjects, and n values for each assay are specified in Table 1. All samples were from severe AD (Braak V–VI) in this cohort.

H_3 receptor autoradiography

Autoradiography studies were carried out based on previous methods (Roberts *et al.*, 2004; Medhurst *et al.*, 2007). Twenty

Table 1 Demographic and [^{3}H]GSK189254 saturation binding variables of controls and AD patients

	Controls maximum n = 12	*AD maximum n = 27*
Age (years)	75 ± 3	81 ± 2
Sex (M/F)	6/6	11/16
Post-mortem interval (h)	43 ± 8	38 ± 5
[^{3}H]GSK189254		
Frontal cortex		
K_D	39.6 ± 4 (9)	73.8 ± 20 (19)
B_{max}	14.7 ± 1.0 (9)	12.3 ± 1.4 (19)
Temporal cortex		
K_D	39.6 ± 6 (6)	46.4 ± 4 (22)
B_{max}	10.0 ± 2.0 (6)	11.1 ± 0.9 (22)

Data are mean ± SEM. Numbers in parentheses represent the *n* available for each region.
AD, Alzheimer's Disease; B_{max}, binding density (in fmol mg^{-1} protein); F, female; K_D, binding affinity (in pM); M, male.

μm frozen sections of human medial frontal gyrus, TASTPM or WT mouse brains were thaw-mounted on to gelatin-coated slides and stored at −80°C until time of assay. Sections were incubated in assay buffer [50 mmol·L^{-1} Tris-HCl, pH 7.7 and 5 mmol·L^{-1} ethylenediaminetetraacetic acid (EDTA)] containing 1 nmol·L^{-1} [^{3}H]GSK189254 for 60 min at room temperature (22°C). On anatomically adjacent sections, non-specific binding was determined in the presence of 10 μmol·L^{-1} imetit. Following incubation, all sections were rinsed five times for 3 min at 4°C in Tris-HCl buffer with the addition of 5 mmol·L^{-1} $MgCl_2$. The sections were then quickly dipped in distilled water at 4°C to remove buffer salts and dried in a stream of cool air. Once dried, the sections were analysed by digital autoradiography using a Beta-Imager 2000 Instrument (Biospace, Paris, France). Adjacent sections were also stained with cresyl fast violet to allow for anatomical orientation. Amyloid plaque pathology was confirmed in adjacent sections using monoclonal 1E8 (1:1000) antibody raised against the 13–27 fragment of Aβ as previously described (Howlett *et al.*, 2004).

In vitro H_3 receptor saturation binding

H_3 receptor binding in WT and TASTPM mouse brains was determined based on methods previously described using [^{3}H]GSK189254 (Medhurst *et al.*, 2007). For preparation of membranes, tissue from mouse whole brain (~13-month-old TASTPM and age-matched WT control) was resuspended (1 g of tissue to 10 mL) in 50 mmol·L^{-1} Tris-HCl, 140 mmol·L^{-1} NaCl, 1 mmol·L^{-1} EDTA buffer (pH 7.4 at 4°C) and homogenized using a polytron P10 (2 × 10 s bursts at full speed). The homogenate was centrifuged at approximately 48 000× *g* in a Sorval Evolution RC centrifuge at 4°C for 20 min (SS34 rotor). The pellet was rinsed with water, resuspended in assay buffer (50 mmol·L^{-1} Tris-HCl, pH 7.4) and centrifuged as before. The final cell pellet was resuspended in assay buffer and frozen at −80°C until required.

Membranes (corresponding to approximately 20 μg of protein per well) and [^{3}H]GSK189254 (20 nmol·L^{-1}–0.02 nmol·L^{-1}) were incubated in polypropylene tubes in a final volume of 200 μL of 50 mmol·L^{-1} Tris-HCl, pH 7.7, at 25°C containing 5 mmol·L^{-1} EDTA. Non-specific binding was determined in the presence of 10 μmol·L^{-1} Imetit. Reactions were conducted at 30°C for 45 min. The experiments were terminated by rapid filtration through Whatman GF/B filters (Whatman, Maidstone, UK) [presoaked in 0.3% (v/v) polyethyleneimine (PEI)]. The filters were washed with 4 × 2 mL aliquots of ice-cold buffer containing 50 mmol·L^{-1} Tris-HCl, pH 7.7, at 25°C and 5 mmol·L^{-1} $MgCl_2$. Filters were dried, and added to vials each containing 4 ml of Ultima Gold MV scintillation fluid (Hewlett Packard, Palo Alto, CA, USA). Radioactivity was determined by liquid scintillation spectrometry using a Packard Tri-Carb 2500TR liquid scintillation counter (PerkinElmer Life and Analytical sciences, Boston, MA, USA). Protein concentrations were determined using the Bradford assay method (Bio-Rad protein assay Kit; Bio-Rad, York, UK) with bovine serum albumin as a standard.

For human AD studies, aliquots of frozen brain homogenates were thawed, diluted in assay buffer (50 mmol·L^{-1} Tris-HCl, pH 7.4) and added to six concentrations (0.05–5 nmol·L^{-1}) of [^{3}H]GSK189254 in triplicate for 2 h at 25°C. A concurrent series of assay tubes were set up with the addition of 10 μmol·L^{-1} unlabelled thioperamide maleate to define non-specific binding, which constituted <10% of total binding. An aliquot of the diluted homogenate was used for protein determination using the Coomasie blue method (Pierce Biotech Inc., Rockford, IL, USA). Incubation was terminated by rapid filtration in a cell harvester (Molecular Devices Ltd., Sunnyvale, CA, USA) with ice-cold sodium phosphate buffer through 0.1% PEI-treated GF/B glassfibre filters (Whatman BDS, Maidstone, UK). Filters were then dried and membrane-bound radioactivity was measured by liquid scintillation spectrometry with a Wallac Beta counter.

Data analysis

For autoradiography studies in TASTPM and WT mice, [^{3}H]GSK189254 binding in cortex, hippocampus and hypothalamus was measured from five sections per animal and six measurements per region. This was determined previously to be a statistically validated number of sections. The levels of specific bound radioactivity were determined via the Beta-imager by counting the number of beta particles from delineated areas and the results are expressed as mean specific binding counts per minute per square millimeter (cpm·mm^{-2}; *n* = 6–8 animals per group), and the data were analysed using a repeated measures ANOVA approach (using Statistica v6.0 StatSoft Inc. software). For human brain, the levels of specific bound radioactivity in the brain areas were determined via the Beta-imager by counting the number of beta particles from delineated whole section areas (delineated area in the range of 86–252 mm^2 across all sections measured with a mean delineated section area of 155.60 mm^2) and the radioligand binding signal was expressed as counts per minute per section (cpm per section) for each Braak stage.

For saturation binding studies in TASTPM and WT mice, specific binding was analysed to estimate binding parameters B_{max} (total number of binding sites) and K_D (binding affinity) using GraphPad Prism 3.0 by GraphPad Software Inc. (San Diego, CA, USA). For human AD brain saturation binding studies with [^{3}H]GSK189254, Scatchard transformation of data was performed using EBDA and LIGAND software

(McPherson, 1985) to calculate K_D and B_{max}. Dementia severity (mean of the last five MMSE scores before death, i.e. MMSE5) was correlated with B_{max} using Pearson's product moment as previously described (Lai *et al*., 2003; 2005). In all cases, binding isotherms were best fitted to single sites with Hill coefficients (N_H) around 1.

Chemicals
[^{3}H]GSK189254 (6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin - 7 - yl)oxy] - *N* - methyl - 3 - pyridinecarboxamide hydrochloride; specific activity 81 Ci·mmol^{-1}) was prepared through a contract with GE Healthcare, UK. Imetit and thioperamide maleate were obtained from Tocris Cookson Inc. (Bristol, UK). All other chemicals were obtained from Invitrogen (Paisley, UK) or Sigma-Aldrich Co. (St Louis, MO) and were of reagent grade.

Results

TASTPM mice
Autoradiographic analysis of coronal brain sections (Figure 1A) from 16-month-old WT (i) and TASTPM (ii) mice revealed extensive specific [^{3}H]GSK189254 binding (>80%) within the cerebral cortex, hippocampus and hypothalamus, while binding levels were negligible following co-incubation with 10 μmol·L^{-1} imetit to define non-specific binding (iii). The presence of amyloid plaques observed in TASTPM mice (v) confirmed significant amyloid load in comparison with the lack of plaques seen in WT mice (iv). Quantification of H_3 receptor binding revealed no significant differences between WT and TASTPM mice (n = 6–8 per group) in cortex, hippocampus and hypothalamus (Figure 1B).

Saturation binding was carried out in whole brain membranes from 13-month-old WT and TASTPM mice using [^{3}H]GSK189254 (Figure 1C). Specific binding represented >90% total binding. Saturation analysis with [^{3}H]GSK189254 in WT and TASTPM mice yielded B_{max} values of 428 ± 64 and 455 ± 104 fmol·mg^{-1} protein respectively and K_D values of 0.4 ± 0.02 and 0.33 ± 0.04 nmol·L^{-1} respectively, with no significant differences being observed between the groups (P > 0.05, Student's *t*-test).

Human AD brain H_3 receptor autoradiography
Specific [^{3}H]GSK189254 binding (>75%) was observed in sections of medial frontal AD cortex from Braak stages 0–1, II, IV, V and VI (Figure 2 lower panels). Plaque pathology was also evident in adjacent sections stained with the 1E8 antibody to total Aβ (Figure 2 upper panels). While quantitative comparisons of H_3 receptor binding in AD versus control brains was restricted in this cohort due to limited number of brains

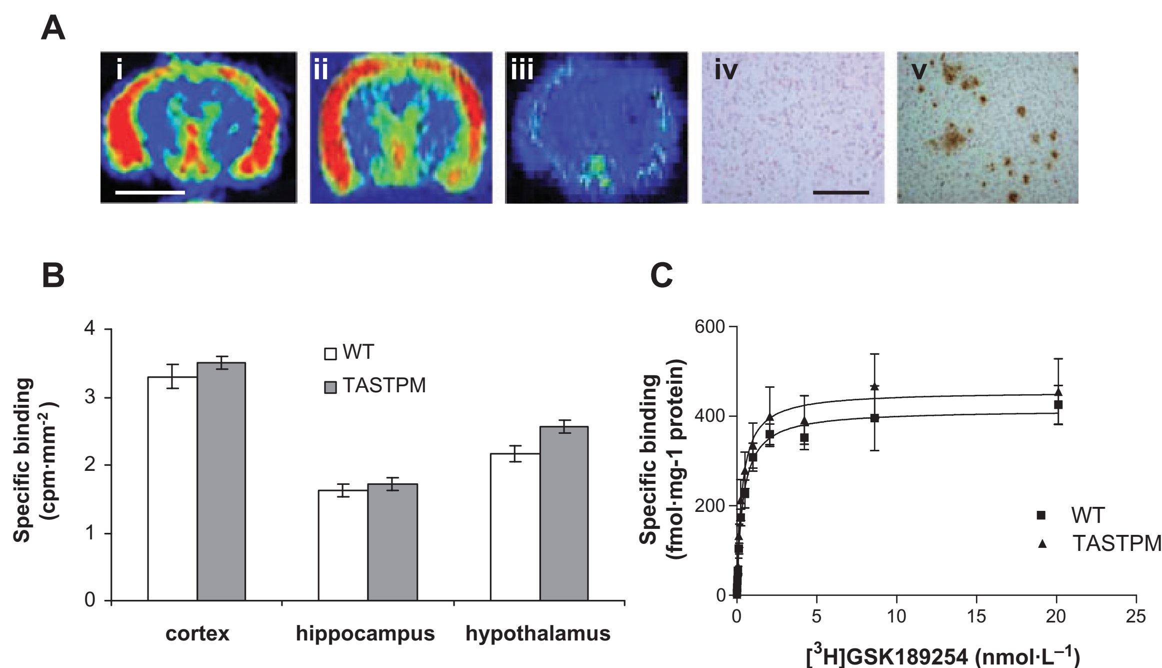

Figure 1 H_3 receptor binding in TASTPM and WT mice measured using [^{3}H]GSK189254 and real time autoradiography. (A) Representative pseudo-coloured images of coronal brain sections showing specific [^{3}H]GSK189254 binding in cortex and hypothalamus of WT (i) and TASTPM mouse (ii), but negligible binding was observed in the presence of 10 μmol·L^{-1} imetit used to determine non-specific binding (iii). Scale bar = 2 mm. Lack of 1E8 staining shown in WT mouse (iv) but significant 1E8 staining of amyloid plaques in cortex of TASTPM mouse (v). Scale bar = 100 μm. (B) Quantitative bar graphs showing specific [^{3}H]GSK189254 binding in cortex, hippocampus and hypothalamus of WT and TASTPM mice (mean ± SEM; n = 4–6 per group). No significant differences were observed between WT and TASTPM mice in any of these brain regions. Non-specific binding was determined in the presence of 10 μmol·L^{-1} imetit. (C) Saturation binding analysis for [^{3}H]GSK189254 to H_3 receptors in whole brain of WT and TASTPM mice (n = 5 pooled brains per group). Mean saturation binding curves (±SEM) are shown. No significant differences were observed in B_{max} or K_D calculated from individual saturation binding curves. TASTPM, double mutant APPswe × PSI.MI.MI46V transgenic mouse; WT, wild type.

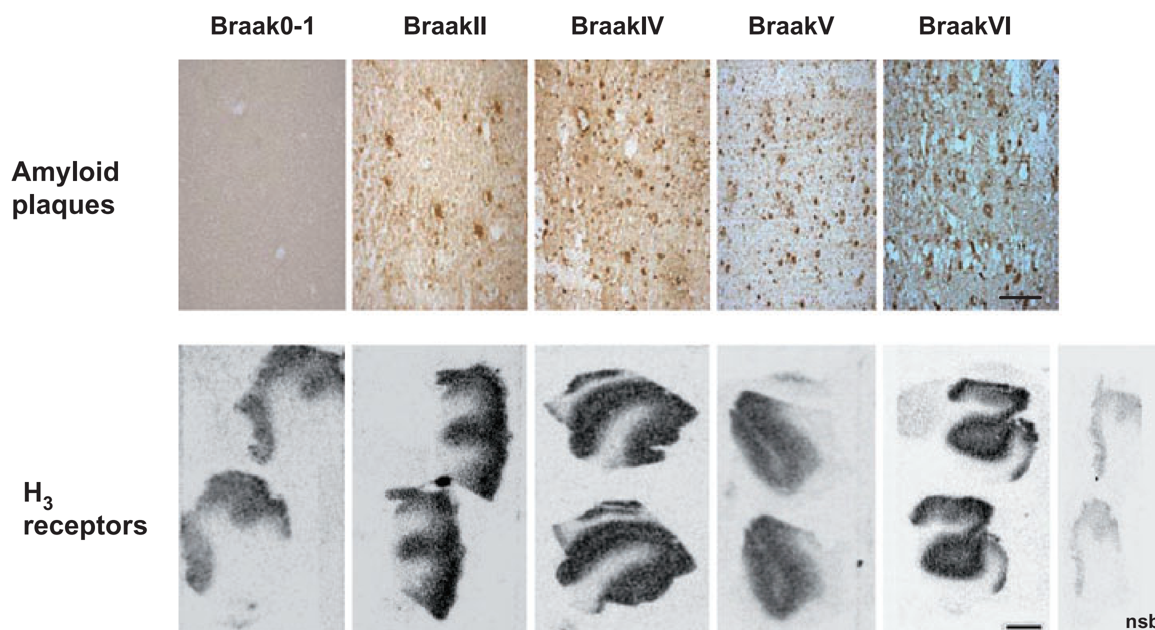

Figure 2 In vitro autoradiography of [^{3}H]GSK189254 binding to human brain H_3 receptors in medial frontal cortex from different Braak stages of AD. Upper panels show increasing Aβ plaque pathology across AD Braak stages 0-I, II, IV, V and VI detected using 1E8 antibody to total Aβ (scale bar 25 μm). Lower panels show specific H_3 receptor binding maintained across all Braak stages even where severe plaque pathology is present (scale bar 1 mm). Non-specific binding was determined in the presence of 10 $\mu mol \cdot L^{-1}$ imetit. Aβ, β-amyloid; AD, Alzheimer's Disease.

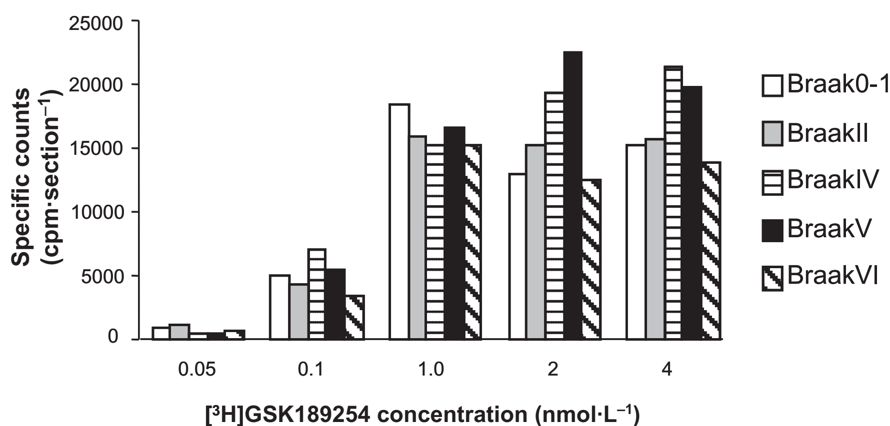

Figure 3 Semi-quantitative analysis of [^{3}H]GSK189254 specific binding to human brain H_3 receptors in medial frontal cortex from different Braak stages of AD determined using autoradiography. Comparable binding levels were observed in each Braak stage across the radioligand concentrations tested and binding appeared saturable. Data shown are from each individual expressed as specific binding in cpm per section. Non-specific binding was determined in the presence of 10 $\mu mol \cdot L^{-1}$ imetit. AD, Alzheimer's Disease.

available, specific H_3 receptor binding was clearly saturable at all disease stages (Figure 3).

Human AD brain H_3 receptor saturation binding

We also performed quantitative analysis of H_3 receptor binding by [^{3}H]GSK189254 saturation assays in a separate, larger cohort of AD and control brains (see Methods). A representative plot of binding data obtained from AD frontal cortex is shown in Figure 4A. Full saturation of binding was achieved, and [^{3}H]GSK189254 binding was of high specificity (>90% total) at radioligand concentrations around K_D. The mean frontal and temporal cortex H_3 receptor binding parameters and demographic details from control and AD patients are shown in Table 1. The age and post-mortem interval of controls and AD subjects were matched, and there were no differences in [^{3}H]GSK189254 binding parameters between the two groups (Student's *t*-test, $P > 0.05$). Interestingly, within the AD group, frontal cortex [^{3}H]GSK189254 B_{max} correlated negatively with the mean of the last five MMSE scores before death (Figure 4B; MMSE5). MMSE5, rather than pre-death MMSE, was used as an indicator of dementia severity to avoid floor effects associated with prolonged terminal states typically seen in AD (Lai *et al.*, 2003). Therefore, in the AD group, [^{3}H]GSK189254 binding density was higher in individuals that had more severe dementia (lower MMSE5) prior to death. However, this association was not observed in the temporal cortex (Figure 4C).

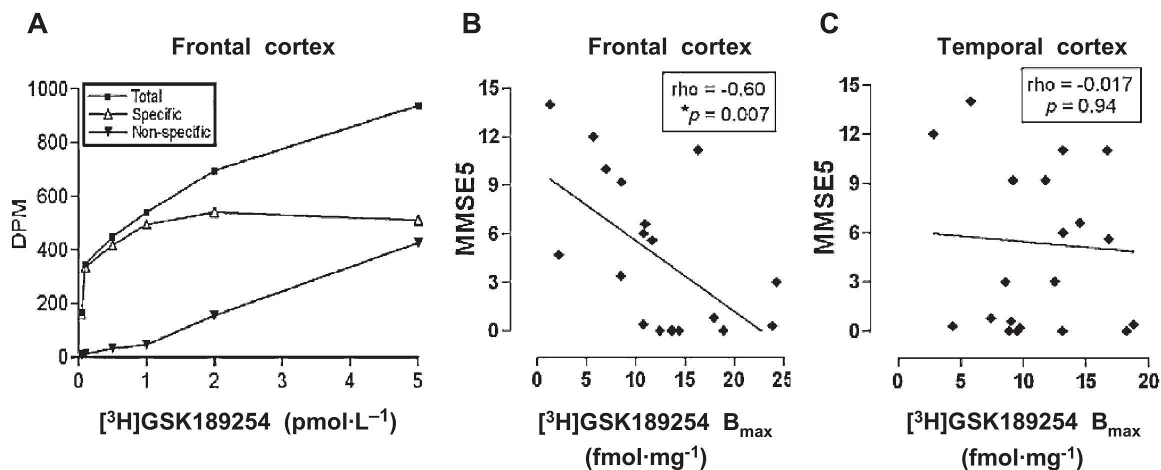

Figure 4 (A) Representative saturation binding plot of [^{3}H]GSK189254 in post-mortem frontal cortex of an AD patient, with K_D = 68 pM and B_{max} = 14.1 fmol·mg^{-1} protein. DPM represents disintegrations per minute. (B, C) Correlation between MMSE5 (mean of last five Mini-Mental Examination scores before death) and B_{max} (in fmol·mg^{-1} protein) for frontal (n = 19) and temporal (n = 22) cortex respectively. *Significant Spearman correlation (P < 0.05). AD, Alzheimer's Disease; DPM, disintegrations per min; MMSE, Mini-Mental State Examination.

Discussion

In the present study, we have shown that H_3 receptor binding does not significantly alter in amyloid over-expressing TASTPM transgenic mice compared with WT mice, or in human AD brain compared with control brain. These results showing preservation of H_3 receptors even in late stage AD are important, given that H_3 receptor antagonists are currently being considered as a novel approach for the symptomatic treatment of AD.

Progressive cognitive decline is a key characteristic of AD and related dementias, and enhancing cognitive performance in these diseases represents a complex challenge, given the involvement of numerous neurotransmitter systems and brain regions (Corey-Bloom, 2002). Current therapies such as cholinesterase inhibitors provide only minimal benefit to a subset of patients and for a limited period of time, so a number of alternative symptomatic strategies are being pursued, including the development of selective histamine H_3 receptor antagonists (Johnson *et al.*, 2004).

It is well established that H_3 receptors can modulate the release of multiple neurotransmitters involved in cognitive processes through a pre-synaptic inhibitory mechanism (Blandina *et al.*, 1996; Fox *et al.*, 2005). By blocking this feedback loop, structurally diverse non-imidazole H_3 antagonists including GSK189254, ABT-239 and BF2.649 have been shown to increase the release of a number of neurotransmitters including acetylcholine and dopamine in the cortex, consistent with blockade of H_3 heteroreceptors (Fox *et al.*, 2005; Ligneau *et al.*, 2007; Medhurst *et al.*, 2007; Esbenshade *et al.*, 2008). In addition, efficacious effects of multiple H_3 receptor antagonists have been described in a huge array of rodent cognition paradigms involving different aspects of learning and memory, and the involvement of different neural substrates. These have been previously reviewed in detail and the majority of studies support pro-cognitive effects of H_3 receptor antagonists (Witkin and Nelson, 2004; Esbenshade *et al.*, 2008). For example, GSK189254 shows efficacy in a diverse battery of cognition models in rats (passive avoidance, water maze, attentional set shift and novel object recognition paradigms) when dosed acutely or repeatedly (Medhurst *et al.*, 2007). These effects on multiple neurotransmitters coupled with their pro-cognitive benefit makes H_3 receptor antagonists an attractive therapeutic approach for a number of CNS disorders including AD, other dementias and cognitive dysfunction in schizophrenia (Passani *et al.*, 2004).

Given the interest in the potential development of H_3 receptor antagonists for AD, we were interested in determining the integrity of H_3 receptors in a transgenic mouse model of AD, as well as in human post-mortem AD brains. We have previously shown that [^{3}H]GSK189254 is a suitable radioligand for studying H_3 receptor binding in rat as well as human brain, although its affinity for the rat H_3 receptor is ~10-fold lower for the human receptor (Medhurst *et al.*, 2007). Dense specific binding was observed in rat striatal, cortical, thalamic, hippocampal and hypothalamic areas as well as substantia nigra, with minimal binding in white matter areas, consistent with previous studies using other H_3 receptor radioligands (Pollard *et al.*, 1993; Barbier *et al.*, 2004). In the current study, we observed a similar binding affinity and distribution pattern in mouse brain compared with the rat, with H_3 receptor binding being particularly prominent in cerebral cortex, hippocampus and hypothalamus. Using both autoradiography and saturation binding analysis in homogenates, no differences were observed in H_3 receptor density between TASTPM and WT mice, despite a significant amyloid load being present in cortical areas of TASTPM mice at 13–16 months of age. Given that it was previously not possible to distinguish degrees of cognitive deficit in these mice (Howlett *et al.*, 2004), we did not investigate any possible correlation between H_3 receptor expression and severity of cognitive deficit as we did with the MMSE5 in human AD brains. However, while significant amyloidosis and cognitive deficits (determined using the object recognition paradigm) are observed in this model, neurodegeneration is not a major

pathological component (Howlett *et al.*, 2004; 2008), so this may account for the lack of changes in neuronal H_3 receptors observed in the current study. We therefore also investigated H_3 receptor binding in human AD brains where neurodegeneration would have clearly occurred.

[^{3}H]GSK189254 has previously been shown to label specific H_3 receptor binding sites in human control and AD medial temporal cortex, with localization appearing consistent with a neuronal localization (Medhurst *et al.*, 2007) in agreement with other studies (Martinez-Mir *et al.*, 1990; Anichtchik *et al.*, 2001). In the present study, we extended these preliminary observations to the medial frontal cortex of the same individuals, and into another very well-characterized cohort of AD and control medial and temporal cortex samples that have been described previously (Hope *et al.*, 1997; 1999). Using autoradiography, specific H_3 receptor binding was observed in medial frontal cortex samples of individuals with AD pathology of Braak stage I, II, IV, V and VI. These data were similar to our previous observations showing H_3 receptor binding in medial temporal cortex of the same individuals, suggesting that H_3 receptor expression is prevalent throughout the disease process and even in severe disease within two cortical areas predominantly affected by AD pathology. Unfortunately, a robust quantitative comparison in this cohort of AD brains compared with controls was not possible due to the limited sample number available, although saturable binding in each individual subject appeared comparable across the different Braak stages.

However, we were able to quantify [^{3}H]GSK189254 binding using cortical homogenates from a larger cohort of AD (Braak V–VI) and control brains with more detailed clinical information regarding disease severity in terms of cognitive function. The K_D for [^{3}H]GSK189254 in human brain was ~10-fold lower than that observed in TASTPM and WT mice, consistent with previous species differences in pharmacology observed between human and rat H_3 receptors (Medhurst *et al.*, 2007). Both control and AD neocortex exhibited low levels of [^{3}H]GSK189254 binding (B_{max} ~ 10–15 fmol·mg^{-1}) which were not significantly different between the two groups, consistent with the autoradiography studies in the smaller cohort described earlier. This suggests that H_3 receptors may not be directly involved in AD, but may become significant as a negative modulator of neurotransmitter systems especially those that are damaged in severe disease, serving to exacerbate the deficits. The majority of cortical H_3 receptors are believed to be heteroreceptors on intrinsic neurons mediating cholinergic and monoaminergic function rather than autoreceptors on histaminergic neurons, although these may also be present (Cumming *et al.*, 1991; Pollard *et al.*, 1993; Blandina *et al.*, 1996; Fox *et al.*, 2005). Therefore, it would follow that an H_3 receptor antagonist could potentially ameliorate neurotransmitter deficit and improve cognition insofar as these cognitive processes are mediated by cholinergic and monoaminergic neurotransmitter systems.

Interestingly, although there were no differences between AD and control brains, frontal cortex [^{3}H]GSK189254 binding density within the AD group appeared higher in patients with more severe dementia prior to death (based on MMSE5), although this was not the case in temporal cortex. The increase in H_3 receptor binding in frontal cortex is difficult to comprehend. Perhaps it is a compensation mechanism to counteract changes elsewhere in the histaminergic system in severe AD such as a decrease in frontal cortex H_1 receptors, although a decrease in these receptors is also observed in the temporal cortex (Higuchi *et al.*, 2000). In addition, the functional consequence of increased H_3 receptor density could be a further decrease in cognitive neurotransmitters and hence further exacerbation of cognitive deficits, and so would not be a positive compensatory effect. Alternatively, the increase in H_3 receptor binding in brains of individuals with more severe dementia could be simply related to loss of cholinergic neurons. The reason for the regional difference observed between frontal and temporal cortex is also not clear, although it may reflect differences in AD pathology in different cortical regions. One could speculate that it reflects a region-specific difference in function of the H_3 receptor, or that the functioning of systems being modulated by H_3 receptors (e.g. acetylcholine and monoamines) has reached a nadir and are incapable of being activated due to the early selective degeneration of the temporal lobe compared with the frontal cortex (Wilcock and Esiri, 1987; Scahill *et al.*, 2002). However, given the relatively small sample size, further studies are required to explore this potential difference in a larger cohort of AD and control brains.

To our knowledge, this is the first quantitative study assessing H_3 receptor binding density in AD brains. In studies with brains from other neurodegenerative conditions such as Parkinson's Disease, H_3 receptor binding was either increased or unchanged (Anichtchik *et al.*, 2001), highlighting the importance of studying different cohorts. The fact that we consistently observed H_3 binding in severe dementia in both our cohorts strengthens the conclusion that H_3 receptors appear relatively spared in AD, and are available for potential therapeutic blockade by H_3 antagonists.

While we have demonstrated little difference in H_3 receptor binding in AD versus control brain, this does not provide information on whether receptor coupling or other downstream events are affected. For example, given that H_3 receptor antagonists can increase the release of various neurotransmitters (Schlicker *et al.*, 1994; Blandina *et al.*, 1996), the effects of this potential therapeutic intervention in AD could be modulated if there were changes in density of other receptors mediating the responses to the increased neurotransmitters released following H_3 receptor blockade. Further detailed studies are required to understand whether mechanisms downstream from the H_3 receptor are affected in AD, and could result in different responses to H_3 receptor antagonists than in non-diseased individuals.

In conclusion, we have shown in the TASTPM mouse model of AD where significant Aβ plaque load is present, that H_3 receptor binding was no different from WT mice. In addition, we have demonstrated both qualitatively and quantitatively that H_3 receptor binding is preserved throughout different Braak stages of AD, and does not appear significantly different in AD brains compared with age-matched controls. This maintenance of H_3 receptor integrity is important, given the potential use of H_3 antagonists as a novel therapeutic approach for the symptomatic treatment of AD.

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Conflict of interest

AD Medhurst, JC Roberts, SH Brown and S Roman are employees of GlaxoSmithKline.

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