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Preservation of cortical histamine H_3 receptors in ischemic vascular and mixed dementias

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ABSTRACT

Aim: Histamine H_3 receptor antagonists have been proposed as a novel therapeutic approach for the symptomatic treatment of Alzheimer's disease (AD). However, it is unclear whether there is a neurochemical basis for extending their potential use in vascular and mixed dementias. In this study, we measured cortical H_3 receptors in patients with subcortical ischemic vascular dementia (SIVD) and mixed SIVD/AD (MIX).
Materials and methods: Radioligand binding assays using [^{3}H]GSK189254 were used to measure H_3 receptors in the postmortem frontal cortex, anterior cingulate gyrus and hippocampus of a cohort of longitudinally assessed SIVD, MIX and age-matched controls.
Results: H_3 receptor levels were unchanged in SIVD and MIX in all areas studied. Furthermore, frontal H_3 receptor densities negatively correlated with predeath assessment of cognition using Mini-Mental State Examination (MMSE) scores.
Conclusion: Our data suggest that H_3 receptors are preserved in SIVD and MIX, thus supporting further assessments of H_3 antagonists as potential therapeutics in these dementias.

1. Introduction

Vascular dementia (VAD) is the second most common cause of dementia after Alzheimer's disease (AD) in both developed and developing countries [1,2]. VAD is closely linked to cerebrovascular disease, and is a direct consequence of cerebral hemorrhages, infarcts and white-matter lesions [3]. Several subtypes of VAD have been described based on the size, number and location of infarcts as well as the presence of white matter or other vascular lesions [4]. Of these, subcortical ischemic vascular dementia (SIVD) has a relatively homogenous clinical presentation characterized by frontal deficits (e.g., executive dysfunction) and relatively mild dementia [5], both of which are thought to arise from the disruption of frontal-subcortical loops or long association fibers by lacunar infarcts or deep white matter disease [6]. Furthermore, concomitant VAD and AD (mixed dementia) is now considered to be more prevalent than previously recognized, and will become increasingly common in elderly patients [6,7]. However, unlike AD, where findings of cortical cholinergic deficits and glutamatergic dysfunction have led to development of cholinesterase inhibitors (ChEIs) and memantine as the mainstay of pharmacologic treatment at different stages of disease [8], there is at present no widely approved pharmacotherapy for VAD. Indeed, the healthcare focus has been on primary prevention by identifying and treating risk factors such as hypertension [9]. There is some evidence that cholinergic deficits occur in hereditary forms of subcortical VAD in the absence of AD [10], and clinical trials using ChEIs on VAD have reported modest improvements in cognitive assessment scores with unclear clinical significance [11]. Similarly, studies on the glutamatergic system in vascular and mixed dementias have only just begun [12,13], and the limited efficacy of memantine indicates that further investigations are needed before its widespread use in VAD can be supported [11,14].

Therefore, there is a clear need for the identification of novel targets for rational therapeutic strategies based on a good understanding of the neurochemical status of VAD. For example, there is increasing evidence of critical roles in learning and cognition for neuromodulator systems utilizing histamine [15]. Histaminergic neurons are localized to the tuberomamillary nucleus and project throughout the cortex, where their diverse functions are mediated by four currently known receptor subtypes (H_1–H_4, see [16]). Of these, the H_3 receptors have important regulatory roles in the brain, both as inhibitory autoreceptors [17] and

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as heteroreceptors which modulate neurotransmitter release in multiple systems [18–20]. Indeed, selective H_3 receptor antagonists have been shown to improve performance in rodent models of cognition and AD [21–23], as well as increase cortical acetylcholine, noradrenaline and dopamine release, leading to their development as potential therapeutics for cognitive disorders [24,25]. We have previously shown that whilst H_3 receptors are preserved in AD neocortex, higher H_3 densities also correlated with more severe dementia [21,26], further supporting a potential role for H_3 receptor antagonists in AD treatment. However, the status of H_3 receptors in vascular and mixed dementias remains unclear at present. In this study, we measured H_3 receptors using a specific radioligand [21] in the postmortem brains of a well characterized, longitudinally assessed cohort of SIVD and mixed SIVD/AD.

2. Materials and methods

2.1. Patients and clinical assessments

Institutional Review Board (IRB) approval from Oxford and Singapore had been obtained for this study, which consisted of a maximum of 12 non-demented elderly controls and 21 SIVD patients, of which six also had significant AD pathology. The subjects were recruited in a longitudinal study of aging and dementia (Oxford Project to Investigate Memory and Ageing, OPTIMA, see http://www.medsci.ox.ac.uk/optima), whose postmortem tissues are now part of the Thomas Willis Oxford Brain Collection. The majority of OPTIMA subjects had been assessed annually from study recruitment to death with a battery of cognitive assessments, including the CAMCOG, a self-contained, cognitive part of the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX) [27]. CAMCOG (maximum score = 107) incorporates all the items in the Mini-Mental State Examination (MMSE [28]) and covers additional domains such as orientation, attention and perception. CAMCOG has previously been found to be useful for the detection of dementia after stroke [29]. At autopsy, informed consent was obtained from next-of-kin before removal of brain, after which tissue blocks from one hemisphere were dissected and fresh frozen, while the contralateral hemisphere was formalin fixed and processed for pathological assessments. Vascular dementia was clinically diagnosed using NINDS-AIREN operationalized criteria [3], while neuropathological determination of SIVD was predicated on findings of microinfarcts, lacunae, white matter and small vessel disease in subcortical structures [30]. The six subjects who were considered to have mixed SIVD/AD (MIX) satisfied “probable” or “definite” AD diagnosis based on the Consortium to Establish a Registry for Alzheimer's disease (CERAD) criteria [31] in addition to SIVD criteria. Braak staging of neurofibrillary tangle involvement [32] was also performed. All control subjects died from non-neurological causes and had no history of dementia or psychiatric conditions. Controls also did not meet neuropathological diagnostic criteria for neurodegenerative diseases. Depending on tissue availability, not all assays were performed for all subjects.

2.2. Radioligand and chemicals

Tritiated GSK189254 (6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)oxy]-N-methyl-3-pyridinecarboxamide, specific activity 81.0 Ci/mmol) was a kind gift of GlaxoSmithKline. Thioperamide maleate was purchased from Tocris Bioscience, UK. Unless otherwise stated, all other chemicals were from Sigma-Aldrich Co. (USA) and of reagent grade.

2.3. Tissue processing

Frozen tissues from various cortical regions (frontal cortex, anterior cingulate gyrus, hippocampus) were thawed on ice, dissected free of meninges and white matter, then homogenized with an Ultra Turrax homogenizer (10 s maximum setting) and washed in ice-cold Tris–HCl buffer to obtain brain homogenates for radioligand binding assays before storage at −80 °C. Frontal cortex consisted of tissues dissected from Brodmann areas 9 and 46 (dorsolateral prefrontal cortex), while hippocampal tissues consisted of sections incorporating CA1–CA3 as well as the dentate gyrus [33].

2.4. Radioligand binding assays

Saturation binding assays of histamine H_3 receptors with [^{3}H] GSK189254 were performed as previously described [26] with slight modifications. Briefly, frozen brain homogenates were thawed, diluted 1:4 vol./vol. in assay buffer (50 mM Tris–HCl, pH 7.4) and added to 6–7 concentrations (0.05–5 nM) of radioligand in triplicates for 1 h at room temperature. A parallel series of assays were set up with the addition of 10 μM unlabelled thioperamide maleate to define non-specific binding. An aliquot of the diluted homogenate was used for protein determination (Pierce Coomasie Plus, Thermo Fisher Scientific, USA). Incubation was terminated by rapid filtration in a cell harvester (Molecular Devices, USA) with ice-cold sodium phosphate buffer through 0.1% polyethylenemine-treated Whatman GF/B glassfibre filters (GE Healthcare, USA), then air-dried and punched into scintillation vials for the measurement of membrane-bound radioactivity using liquid scintillation spectrometry with a Wallac Beta counter (PerkinElmer, USA).

2.5. Data analyses

Scatchard transformation of radioligand binding data was performed using EBDA and LIGAND software [34] to calculate K_D (binding affinity, in nM) and B_{max} (binding density, in fmol/mg protein). In all cases, binding isotherms were best fitted to single sites with Hill coefficients (N_H) around 1. Statistical analyses were performed using SPSS 13.0 for Windows software (SPSS Inc, USA), with normality of data tested by Komogorov–Smirnov tests. Since the sample sizes of the groups were unequal, comparisons of normally distributed variables between control, SIVD and MIX were performed by one-way analyses of variance (ANOVA) followed by *post-hoc* Tamhane's T2 tests. Pearson's product moment was used in correlations of neurochemical variables with dementia severity (defined as mean CAMCOG and MMSE scores up to one year predeath). Appropriate non-parametric tests were used for ordinal or non-normally distributed variables. Results were considered statistically significant if $p<0.05$.

3. Results

3.1. Demographic and disease variables in controls and dementia patients

Table 1 shows that age, length of follow-up and postmortem interval did not differ significantly among the groups (ANOVA with *post-hoc* Tamhane, $p>0.05$). There are more males (67%) than females in both controls and SIVD. However, neurochemical variables were not significantly different between males and females in any group (Student's *t* tests, $p>0.05$, data not shown). As expected, the majority of SIVD patients (11 of 15) had little or no neurofibrillary tangle burden, as indicated by Braak stage 0–II, while none was higher than Braak stage III/IV, similar to controls (Table 1). In contrast, all MIX patients had extensive, Braak stage V/VI tangle involvement. In agreement with previous findings [5], cognitive impairment in SIVD was relatively mild. Therefore, while both predeath MMSE and CAMCOG scores of SIVD were significantly lower than controls, they were still higher than the scores for MIX patients, whose cognitive impairments were comparable to AD [35,36].