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Ligand autoradiographical quantification of histamine H_3 receptor in human dementia with Lewy bodies

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ABSTRACT

Dementia with Lewy bodies (DLB) is a serious age-dependent human neurodegenerative disease, with multiple debilitating symptoms, including dementia, psychosis and significant motor deficits, but with little or no effective treatments. This comparative ligand autoradiographical study has quantified histamine H_3 receptors (H_3R) in a series of major cortical and basal ganglia structures in human DLB and Alzheimer's (AD) post-mortem cases using the highly selective radioligand, [^{3}H] GSK189254.

In the main, the levels of H_3 receptor were largely preserved in DLB cases when compared with aged-matched controls. However, we provide new evidence showing variable levels in the globus pallidus, and, moreover, raised levels of Pallidum H_3 correlated with positive psychotic symptoms, in particular delusions and visual hallucinations, but not symptoms associated with depression. Furthermore, no correlation was detected for H_3 receptor levels to MMSE or IUPRS symptom severity.

This study suggests that H_3R antagonists have scope for treating the psychotic symptomologies in DLB and other human brain disorders.

1. Introduction

Dementia with Lewy bodies (DLB) is the second most prevalent human dementia. This is a seriously debilitating human disease, with multiple prevalent symptoms, including dementia, psychosis (hallucinations and fluctuating consciousness) and significant motor deficits, but with little or no effective treatments [1]. The histaminergic system plays an important role in central nervous system regulation and behaviour through its role as an autoreceptor, regulating the synthesis and release of histamine and as a heteroreceptor, negatively regulating the release of a variety of other key neurotransmitters including acetylcholine, dopamine, glutamate and gamma-aminobutyric acid [2–4]; reviewed in [5]. Given its widespread distribution and influence upon multiple neurotransmitter systems, H_3 antagonists are promising clinical candidates for the treatment of age-related dementias, such as DLB [6–8].

There are indications that histamine deficits are present in dementias, such as Alzheimer's Disease (AD), however it is unknown whether these are specific to certain brain regions, changes in histamine receptor numbers, or are specific for AD amongst other neurodegenerative disorders. The importance of the histaminergic system in AD is difficult to assess due to a number of conflicting reports. For example, histamine levels in AD brains have been reported to be increased in temporal and frontal cortex, basal ganglia and hippocampus [9]. However, other studies have shown decreases in histamine content in the hypothalamus, hippocampus and temporal cortex [10,11]. Histaminergic cell bodies are also located in the TMN, where neurofibrillary tangles (NFTs) are also found. NFTs are particularly concentrated in the region containing histaminergic perikarya compared with surrounding areas [12,13] and together with cholinergic basal forebrain nuclei, the TMN has been described as an early affected subcortical nucleus for the presence of NFT [14]. The number of histaminergic cell bodies in the TMN was shown to be similar to that of normal brains [12]. In contrast, another group showed a significant reduction in large-sized histamine containing neurons in the TMN where numerous NFTs were found, indicative of a central histaminergic dysfunction [13]. Histamine decarboxylase (HDC) activity, also a common marker of

Abbreviations: DLB, Dementia with Lewy Bodies; AD, Alzheimer's Disease; ADHD, attention deficit hyperactivity disorder; NFTs, neurofibrillary tangles; PM, post mortem delay; RαMHA, R-α-methylhistamine; MMSE, Mini Mental State Examination; UPDRS, Unified Parkinson Disease Rating Scale; MCID, Microcomputer Imaging Device.

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the histaminergic system, has been shown to be decreased in AD compared with elderly controls [15]. Whilst there are conflicting data about the histamine content in the brain of AD patients, one recent study using a highly selective H_3R ligand had shown the level of H_3R expression to be unaltered in the late stages of human AD compared to age matched controls, as well as in TASTPM mice (a mouse model of AD) compared with wild type mice [6,16].

Understanding the molecular structure of the H_3R has increased considerably and a number of H_3R antagonists have been identified and a few (pitolisant and GSK189254) have entered advanced clinical development focusing on narcolepsy, cognitive and psychotic disorders [8,18,19]. The histaminergic system innervates several structures that are known to be involved in cognition such as the basal forebrain, cerebral cortex, cingulate cortex, amygdala and thalamus [20]. High levels of H_3R have been shown to be expressed in the cerebral cortex [21], which is densely innervated by cholinergic neurons. In neuropsychiatric disorders such as AD, attention deficit hyperactivity disorder (ADHD) and schizophrenia, cognitive deficits play a major role in the disease [22]. Increased brain histamine is also positively correlated with age and may play a role in decreasing acetylcholine uptake [23]. It is thought that H_3R antagonists may be able to prevent the reduction in acetylcholine through its heteroreceptor characteristic [24–26]. H_3Rs are also highly expressed in the basal ganglia in both rodent and human brains [27–29].

Ligand autoradiography is a very useful technique to define the topology and quantify receptors in post-mortem brain slices. GSK189254 is derived from a novel benzazepine series of H_3R antagonists [6] that are structurally distinct from other recently described non-imidazole H_3R antagonists. GSK18925 has been shown to significantly improve performance of rats in diverse cognition paradigms, including passive avoidance, water maze, object recognition and attentional set shift [4,5]. The data thus far for H_3R antagonists point to a possible therapeutic potential for diseases where cognitive deficits are already present such as AD and other dementias, including DLB. These complex brain diseases also display multiple symptoms in addition to dementia which may be targeted through the histaminergic system. In this present study, [^{3}H] GSK189254 was utilised to quantify levels of cortical and basal ganglia H_3Rs in normal human aged post-mortem brains, and in a series of DLB and AD cases (the latter for comparative purposes) with detailed connected clinical information.

2. Materials & methods

2.1. Determining the working concentration of [^{3}H] GSK189254 for autoradiography

Saturation binding assays using [^{3}H] GSK189254 were performed essentially as described previously [6], in 50 mM Tris-HCl, pH 7.7 containing 5 mM EDTA and a concentration range of 0.01–8 nM for radioligand. Non-specific binding was determined using 1 μM R-α-methylhistamine (RαMeH). The assay was terminated by rapid filtration through a Whatman GF/B filters pre-soaked in 10 mM sodium phosphate dibasic pH 7.4, which were washed (3 × 3 ml) using iced cold 10 mM sodium phosphate dibasic pH 7.4, using a Brandell 24-place cell harvester.

[^{3}H] GSK189254 bound selectively to the hH_3R vs hH_4R, and the two major hH_3R isoforms, namely hH_3 445 and hH_3 365, transiently expressed in HEK293 cells [6], displayed very similar K_D values of 0.16 ± 0.04 and 0.24 ± 0.07 nM, respectively (Supplementary Fig. 1). The concentration of radioligand used was, therefore, selected as approximately 2× mean K_D to ensure that each autoradiography run detected at least 65% of available receptor binding sites.

2.2. Human case details and diagnostic criteria

All human brain tissue were obtained from Newcastle Brain Tissue Resource Bank LREC (Newcastle and Tyneside) with full ethical approval (2002/295). Frozen tissue was collected at autopsy and 1 cm coronal slices from the left hemisphere were snap frozen in liquid Arcton (ICI) and stored at −70 °C. The sections were then stored at −80 °C. Prior to sectioning, tissue slices were warmed to 15 °C and blocks containing the striatum were sub-dissected and mounted onto cryostat chucks with 8% carboxymethylcellulose. Coronal sections were cryostat sectioned at a thickness of 20 μm using a Brights OTF cryostat onto Vectabond-coated glass slides, air dried for 1–2 h and stored at −80 °C prior to receptor autoradiography. The right hemisphere was used for histopathological examination, following formalin fixation and paraffin embedding. Cortical and hippocampal neurofibrillary tangles were demonstrated using a modification of Palmgren's silver technique [30] and the von Braunmühl silver impregnation technique [31] was used to identify senile plaques in 25 μm thick frozen sections cut from tissue blocks adjacent to those taken for paraffin processing. Counts of NFTs and neuritic plaque number were made from fields across the entire cortical ribbon, as described in [32]. Lewy-bodies in the substantia nigra were visualised by the use of haematoxylin and eosin staining, cortical Lewy-bodies and dystrophic neuritis were detected using ubiquitin immunohistochemistry on 5 μm thick paraffin embedded sections. Neurones in the substantia nigra were quantified following cresyl fast violet staining of 20 μm thick paraffin sections.

Control cases had no history of psychiatric or neurological disorder and had no neuropathological indications of Lewy-body disease (DLB) or any other neurological disorder. DLB cases were clinically diagnosed by the presence of a progressive cognitive impairment seen in conjunction with at least two of the following symptoms: recurrent visual hallucinations; fluctuating cognition with pronounced variations in attention and alertness; spontaneous motor features of parkinsonism [33]. DLB cases were distinguished from AD by the presence of brain stem and cortical Lewy-bodies, Lewy neurites in the CA2/3 and endplate segments of the hippocampus [33], and by lower or moderate Alzheimer-type pathology with fewer NFT than found in AD.

2.3. Human cases used

The 43 cases selected for this study were cut at the level of the striatum (caudate nucleus and putamen) corresponding to coronal brain levels 9–15 using the Coronal Map of Brodmann Areas in the human Brain [34]. Of the 43 cases, 12 were control cases, 16 DLB cases and 15 AD cases (Table 1). For each case 5 replicates were used to measure 3 total and 2 non-specific radioligand binding.

Summary of the 43 human cases chosen for the study. PM delay = post mortem delay, that is, time between death and freezing of the tissue to allow for post-mortem examination.

No significant differences were seen with age or PM delay in these cases ($p > 0.05$). No gross significant differences were seen between the male and female cases in respective groups ($p > 0.05$) (not shown).

2.4. In vitro autoradiography of human brain tissue using [^{3}H] GSK189254

The autoradiography method used was essentially as described previously [6]. In brief, human brain sections were left to equilibrate to room temperature for 1 h before the protocol commenced. Human sections were incubated in (50 mM Tris, 5 mM EDTA pH 7.7) containing 2 × K_D (approximately 0.5 nM) [^{3}H] GSK189254 (specific activity = 81 Ci/mmol, stored at −20 °C, gift from Dr Medhurst, GSK, Harlow, UK) for 1 h at RT, until equilibrium is reached. Non-