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Human brain cytosolic histamine-N-methyltransferase is decreased in Down syndrome and increased in Pick's disease

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Abstract

Histamine-N-methyltransferase (HMT) inactivates the neurotransmitter histamine. Central histaminergic deficits may contribute to the cognitive impairment of neurodegenerative disorders including Alzheimer's disease (AD) and Down syndrome (DS). However, there is no evidence for histaminergic deficits in Pick's disease (PiD). HMT levels were measured in the frontal cortex and cerebellum of brains of patients with AD, DS, and PiD, and normal aged subjects using proteomics techniques. In frontal cortex, HMT was significantly decreased in DS, but significantly increased in PiD compared with controls. HMT levels were comparable in cerebellum of all groups. Elevated HMT in PiD could lead to increased histamine degradation that in turn would be in agreement with impaired cognitive functions of PiD. Decreased HMT in DS would be compatible with findings of decreased histamine synthesis, thus reflecting a compensation mechanism to antagonize reduced synthesis by decreased degradation.

Keywords: Histamine-N-methyltransferase; Cognitive function; Alzheimer's disease; Down syndrome; Pick's disease; Proteomics

Histamine, widely distributed in the brain, acts as a neurotransmitter and is synthesized by L-histidine decarboxylase (HDC) [13]. The catabolism of histamine involves two pathways, methylation by histamine-N-methyltransferase (HMT) producing tele-methylhistamine (t-MH) and oxidative deamination by diamine oxidase (DAO) [1]. Tele-MH is further metabolized by monoamine oxidase B (MAO-B). In the vertebrate central nervous system (CNS), DAO is detectable at a low level [21]. Therefore, brain histamine levels depend on the expression/activity of HMT. Recently HMT was observed mainly in neurons of the bovine CNS, suggesting that histamine could be methylated mainly in postsynaptic or extrasynaptic neurons rather than in astrocytes [16].

Brain histamine has been implicated in the regulation of multiple physiological functions including learning and memory [17,18]. Furthermore, the central histaminergic system is affected in neurodegenerative disorders including Alzheimer's disease (AD). The deficit of the central histaminergic system in AD and Down syndrome (DS) brain was observed in studies on histidine, histamine and histamine handling enzymes (HDC, HMT and histamine-releasing factor) [11,22], suggesting that histaminergic deficits may contribute to the cognitive impairment of neurodegenerative diseases. Additionally, the characteristic neurofunctional abnormalities present in children with DS could be the consequence of a combination of structural and neurochemical aberrations and antihistaminergic treatment affects information processing tested by auditory event-related potentials similar to that seen with anticholinergic treatment [23]. Pick's disease (PiD), one type of frontotemporal dementia (FTD), is characterized by frontal lobe signs including disinhibition and language deficits and may be distinguished clinically from the memory and cognitive impairment of AD [5]. Both AD and PiD are taupathies. However, the tau-positive inclusions of AD (neurofibrillary tangles, dystrophic neurites of neuritic plaques, and neuropil threads) are morphologically and biochemically distinct from the inclusions of PiD (Pick bodies), and AD as well as adult DS brains contain beta-amyloid deposits, which are absent or rare in PiD. Until now, little has been known about the neurochemistry of FTD, and there are no reported studies of brain histamine metabolizing enzymes in PiD. In order to determine the involvement of

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Table 1
Study population and protein levels of HMT

	Control	AD	DS	PiD
Frontal cortex				
Age (years)	62.80 ± 8.61	60.80 ± 10.62	56.00 ± 10.49	59.40 ± 12.10
Postmortem interval (h)	44.20 ± 21.82	46.40 ± 34.70	36.60 ± 24.22	31.60 ± 23.44
N (male/female)	5 (2/3)	5 (1/4)	5 (3/2)	5 (1/4)
HMT protein level	1.30 ± 0.63	0.67 ± 0.25	0.36 ± 0.31**[a]	2.26 ± 0.22*
Cerebellum				
Age (years)	68.50 ± 6.25	59.86 ± 6.47	55.43 ± 8.62	52.33 ± 12.68
Postmortem interval (h)	44.00 ± 25.65	37.29 ± 32.67	30.86 ± 22.50	39.50 ± 26.07
N (male/female)	4 (2/2)	7 (5/2)	7 (4/3)	6 (1/5)
HMT protein level	1.01 ± 1.13	0.83 ± 0.45	0.85 ± 0.47	0.75 ± 0.35

[a] $^{**}P < 0.01$; $^{*}P < 0.05$.

HMT in mechanisms of neurodegeneration of different taupathies (AD, DS and PiD) we investigated the central histaminergic system by measuring HMT levels in different brain regions in the individual disorders. We used an improved proteomics technique, which unambiguously identified HMT in cytosolic fraction of brains with AD, DS, and PiD and normal aged controls.

Postmortem brain samples (Table 1) were obtained from the Brain Bank, Department of Neuropathology, Institute of Psychiatry, King's College, London. AD patients were selected prospectively and examined clinically and fulfilled the National Institute of Neurological Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria for probable AD [26]. The neuropathological diagnosis of 'definite AD' was confirmed using the CERAD criteria [15]. All DS patients were karyotyped and possessed trisomy 21. In all DS brains there were abundant and extensive beta-amyloid deposits, neurofibrillary tangles and neuritic plaques. The neuropathological diagnosis of PiD was made if there were abundant tau-positive intraneuronal inclusions (Pick bodies) in the presence of frontotemporal atrophy and a clinical history of dementia [6]. Control brains were obtained from individuals with no history of neurological or psychiatric illness. The major cause of death was bronchopneumonia in AD, DS, and PiD, and heart disease in controls. After the fresh brain was dissected, coronal slices were immediately frozen and stored at −70 °C until processed. Two brain regions, frontal cortex (superior frontal gyrus) and cerebellum (cerebellar hemisphere), were used in this study.

Brain tissue (1.0 g) was suspended in 10 ml sucrose buffer consisting of 20 mM HEPES (pH 7.5), 320 mM sucrose, 1 mM EDTA, 5 mM dithierythritol, 1 mg/ml of a mixture of protease inhibitors (1 mM PMSF and 1 tablet complete (Boehringer Mannheim) per 50 ml of suspension buffer) and phosphatase inhibitors (0.2 mM Na_3VO_3 and 1 mM NaF). The suspension was homogenized with a glass-teflon potter and centrifuged at 800 × *g* for 10 min at 4 °C to sediment nuclei and unsuspended material. The supernatant was further centrifuged at 10,000 × *g* for 15 min at 4 °C and then at 100,000 × *g* for 1 h to separate fraction enriched with mitochondria-, microsomal- and cytosol-fraction. The supernatant of the last step mainly contained the cytosolic proteins and was used for two-dimensional (2-D) gel electrophoresis. The protein content in the supernatant was determined by a Bradford method [3].

2-D gel electrophoresis was performed essential as reported [12]. Briefly, desalted samples were applied on immobilized pH 3–10 non-linear gradient strips (Amersham, Pharmacia Biotechnology, Uppsala, Sweden). Proteins were focused at 200 V which was gradually increased to 5000 V at 2 V/min and continued at 5000 V for 24 h. The 2-D separation was performed on 12% polyacrylamide gels (Serva, Heidelberg, Germany). The gels were run at 40 mA per gel in an ISO-DALT apparatus (Hoefer Scientific Instruments, San Francisco, CA). After fixation for 12 h in 50% methanol containing 5% phosphoric acid, gels were stained with colloidal Coomassie Blue (Novex, San Diego, CA) for 24 h. Molecular masses were determined by running standard protein markers (Gibco, Basel, Switzerland), covering the range of 10–200 kDa. p*I* values were used as given by the supplier of the IPG strips. The gels were destained with H_2O and scanned in an AGFA DUOSCAN densitometer. Electronic images of the gels were recorded using Photoshop (Adobe) and PowerPoint (Microsoft).

MALDI-MS analysis was performed as described [7] with minor modifications. Briefly, excised spots were destained with 100 μl of 30% (v/v) acetonitrile in 0.1 M ammonium bicarbonate, dried in a Speedvac evaporator and rehydrated with 5 μl of 3 mM Tris–HCl (pH 8.8) containing 50 ng trypsin (Promega, Madison, WI). After 16 h, 8 μl of water was added and the samples were left at room temperature for about 15 min. Seven microliters of 75% acetonitrile containing 0.3% trifluoroacetic acid and the standard peptides des-Arg-bradykinin (Sigma, 904.4681 Da) and adrenocorticotropic hormone fragment 18–39 (Sigma, 2465.1989 Da) were added. The application of the samples was performed with a CyBi™-Well sample processor (CyBio, AG) and 1.5 μl of the peptide mixture was simultaneously applied with 1 μl of matrix, consisting of a satu-

rated solution of α-cyano cinnamic acid in 50% acetonitrile, containing 0.1% trifluoroacetic acid. Samples were analyzed in a time-of-flight mass spectrometer (Reflex 3, Bruker Analytics, Bremen, Germany). An accelerating voltage of 20 kV was applied. Peptide matching and protein searches were performed automatically with the use of in-house developed software [2]. The peptide masses were compared to the theoretical peptide masses of all available proteins from all species. Monoisotopic masses were used and a mass tolerance of 0.0025% was allowed. Unmatched peptides or miscleavage sites were not considered.

Using the ImageMaster 2D Elite software (Amersham Pharmacia Biotechnology), the percentage of the volume of the spot representing HMT was quantified as compared to the total proteins present in partial 2-D gel images including HMT. Between-group differences were investigated by non-parametric Mann–Whitney *U*-test and the significance was set at $P < 0.05$. All statistical analyses were performed by GraphPad Instat2 software, version 2.05. Results were represented as means ± standard deviation.

HMT was identified based on the observed molecular weight (33,615 Da), p*I* 5.1 and probability (1.45E-06) as well as seven matching peptides with SWISS-PROT Accession number P50135. As shown in Table 1, HMT was significantly decreased in frontal cortex of DS but significantly increased in frontal cortex of PiD. In frontal cortex of AD, HMT showed a decrease, but did not reach significance ($P = 0.0952$). In cerebellum of all groups HMT showed comparable levels. The partial 2-D gel images including HMT with the significant difference are presented in Fig. 1.

This is the first report showing increased HMT levels in frontal cortex of PiD. Decreased histamine levels resulting from increased histamine degradation may well be leading to the severe cognitive decline in PiD patients. The underlying cause and mechanism for increased HMT remains elusive. Nothing has been shown so far in histamine metabolism of PiD and one may speculate that increased HMT may be due to pronounced glial proliferation in PiD, although HMT is mainly of neuronal origin. We cannot even draw any conclusions from other neurotransmission systems reported as we failed to detect relevant publications. Oxidative stress, demonstrated in PiD brain [8], cannot be incriminated as it also occurs in DS and AD brains that showed decreased HMT. It remains to be shown whether increased HMT transcription or upregulation at the protein stability accounts for the histaminergic deficit in PiD. It must be mentioned, however, that the increase cannot be simply assigned to 'neurodegeneration' per se and furthermore, differences of histamine handling enzymes according to diseases have already been reported: divergent MAO activity between AD and PiD [24]. From a neuropathological view – the presentation of Pick bodies in PiD and amyloid deposits in DS and AD – one would not necessarily expect similar mechanisms leading to histaminergic deficits in PiD and AD or DS on the other hand.

Significantly reduced HMT levels in DS and the trend of a decrease in the AD brain may be reflecting a compensation mechanism of decreased histamine synthesis in both disorders [22]. Steady-state levels could be kept – maybe at a lower level – by reducing histamine catabolism secondary to decreased histamine biosynthesis. In DS with AD neuropathology and AD we may offer some more information about tentative roles of decreased HMT. The binding potential of histamine H1 receptors showed a significant decrease particularly in the frontal and temporal areas of AD brain [10]. Consequently, histamine levels may in turn decrease and the downregulation of HMT would be a necessary step to prevent further reduction of already decreased histamine. Oxidative stress, described by several authors in DS and AD, could result in deteriorated turnover rates of neurotransmitters as well as the aberrant expression/activity of neurotransmitter handling enzymes. Indeed, peroxidation-induced derangement of the neuronal pool of histamine in synaptosomes and its turnover was reported showing impaired histidine uptake, marked decrease of synaptosomal histamine content, and decreased activity of HDC and HMT [20]. In addition, aldehyde dehydrogenase (ALDH) is one of the major aldo-keto oxidoreductases dehydrogenating imidazoleacetaldehyde, a metabolite of histamine. ALDH levels were reduced in brains of patients with DS [14], but interestingly, ALDH activities were significantly increased in temporal cortex of AD [19]. These results suggest that AD and DS could utilize different mechanisms to metabolize histamine catabolites.

In frontal cortex of AD and DS brain, deficits of the histaminergic system were reported together with reduced choline acetyltransferase activity, an important determinant of memory loss and cognitive decline in dementing disorders [22]. Brain histamine modulates the release of and interacts with acetylcholine, a major mediator of cognitive function and cholinergic agents in turn modulate histamine release and its turnover rate. Improved cognitive perfor-

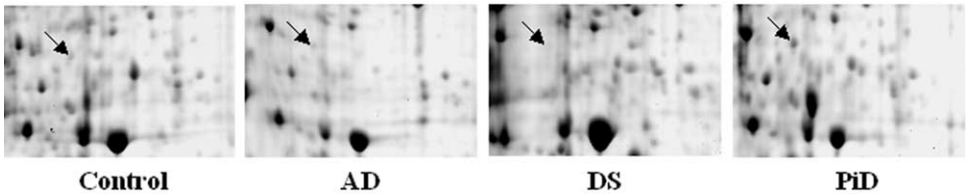

Fig. 1. The partial 2-D gel images of HMT in frontal cortex with significant changes. The arrows indicated the protein spot identified as HMT.

mance has been reported in some mildly demented AD patients undergoing clinical trials with 9-amino-1,2,3,4-tetrahydroacridine (tacrine or THA), an inhibitor of acetylcholinesterase [25]. THA shares structural similarities with inhibitors of HMT [9] and is able to alter histamine metabolism by the inhibition of HMT [4]. Activation of both histaminergic and cholinergic transmission may be a potential experimental treatment for cognitive decline.

We conclude that HMT is differently involved in the pathomechanisms of DS and AD on the one hand and PiD on the other hand. PiD may present with increased HMT and subsequently increased degradation of histamine leading to impaired cognitive function while decreased HMT in DS and maybe AD could be seen as a mechanism to compensate low brain histamine by decreasing the catabolizing principle of HMT.

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