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Similar deficits of central histaminergic system in patients with Down syndrome and Alzheimer disease

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Abstract

In order to study whether Alzheimer-like neuropathological changes involve the central histaminergic system we measured the concentration of histamine, its precursor histidine as well as the activity of histidine decarboxylase (HDC) and histamine-*N*-methyltransferase (HMT) in frontal cortex of aging Down syndrome (DS) patients, Alzheimer patients and control individuals. The study populations were also investigated for choline acetyltransferase (ChAT) activity, since reduced ChAT activity is an established biochemical hallmark in DS and Alzheimer disease (AD). HDC and ChAT activity were reduced in brains of both DS and Alzheimer patients versus control patients. Additionally, we observed a significant decrease of histamine levels in the DS group. Histamine levels in AD brains tended to be decreased. Histidine concentrations and HMT activities were comparable between the three groups. Thus, our results for the first time show histaminergic deficits in brains of patients with DS resembling the neurochemical pattern in AD. Neuropathological changes may be responsible for similar neurochemical alterations of the histaminergic system in both dementing disorders. © 1997 Elsevier Science Ireland Ltd.

Keywords: Alzheimer disease; Central histaminergic system; Down syndrome; Histamine; Histamine-*N*-methyltransferase; Histidine decarboxylase; Postmortem brain analysis

It is well established that an intimate relationship exists between Down syndrome (Trisomy 21, DS) and Alzheimer disease (AD). All brains of DS patients beyond age 30 show AD-like neuropathological changes, i.e. deposition of βA4 amyloid protein, neurofibrillary tangles and senile plaques [34]. There is even a genetic link between these two conditions since several groups could localize the gene for βA4 amyloid precursor protein on the long arm of chromosome 21 [15,29,31]. Neurochemical investigations revealed that many central neurotransmitter systems are similarly affected in aging DS and in Alzheimer patients, respectively [11]. A major biochemical consistent finding in both dementing disorders is a decrease in central choline acetyltransferase (ChAT) activity [35] and damage to the cholinergic system is thought to be an important determinant of memory loss and other functional deficits in AD [10].

Data from several studies showed that central histaminergic system is also affected by AD. Airaksinen et al. found numerous neurofibrillary tangles in the tuberomammillary area of the hypothalamus, where cell bodies of histaminergic neurons are located [1]. Postmortem evaluation of histamine concentration in several brain regions of Alzheimer patients led to contradictory results. While Cacabelos et al. reported an increase of central histamine levels [6], Mazurkiewicz-Kwilecki et al. found deficits of the endogenous diamine [22].

In order to study whether AD-like neuropathological changes involve the central histaminergic system we measured the concentration of histamine, its precursor histidine as well as the activity of histidine decarboxylase (HDC) and histamine-*N*-methyltransferase (HMT) in frontal cortex of aging DS, Alzheimer patients and control individuals. Since reduced ChAT activity represents a biochemical hallmark for AD and DS [11,14,28], we al-so examined the activity of the acetylcholine synthesizing enzyme.

Brain samples (frontal cortex, gyrus frontalis superior,

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grey matter) of DS patients ($n = 8$), Alzheimer patients ($n = 9$) and control adults ($n = 9$) were obtained from the MRC London Brain Bank for Neurodegenerative Diseases, Department of Neuropathology, Institute of Psychiatry, London, UK. All patients with AD or DS were clinically assessed by psychiatrists during life. AD patients fulfilled the NINCDS/ADRDA criteria for probable AD [33] and all DS patients expressed the phenotype characteristics of DS. Neuropathological investigations revealed abundant β-amyloid plaques and neurofibrillary tangles in all diseased brains. The brains of the age-matched control group were chosen as an unselected consecutive series from individuals with no neurological or psychiatric history (Table 1).

Frontal cortex was dissected while the brain was kept frozen. The freezing chain was never interrupted by any handling and samples were transported to the Viennese laboratory on dry ice. Chemicals were of the highest commercially available purity. ^{14}C-labeled aectyl coenzyme A was obtained from Sigma (St. Louis, USA). All other radiochemicals were obtained from NEN (Vienna, Austria). For measurement of histamine and histidine brain tissue was homogenized in 19 volumes of 0.1 M HCl and centrifuged at 15 000 *g* for 20 min. Until determination the samples were stored at −80°C. Histamine in the supernatants was quantified by a high performance liquid chromatography (HPLC) method with fluorescence detection [25]. For measurement of histidine, carboxymethyl-l-cysteine was added to the supernatant fraction as internal standard. Concentration of histidine was determined by HPLC with fluorimetric detection after derivatization with *o*-phthaldialdehyde as previously described by Singewald et al. [30]. A coupled radioenzymatic assay was employed to determine the activity of HDC [20]. This method depends on the formation of radio-labeled histamine in the assay which is methylated in situ by HMT to radio-labeled *N*-tele-methylhistamine. Frontal cortices were homogenized with eight volumes of 0.1 M sodium phosphate (pH 7.2). The incubation mixture consisted of 40 μl of homogenate and 10 μl of a reaction cocktail containing [ring 2,5–^{3}H]histidine (40–60 Ci/mmol; 1 μCi) pyridoxal-5-phosphate (0.5 nmol); *S*-adenosylmethionine (5 nmol) and 2 μl HMT preparation (NEN, Austria, Vienna). The reaction was linear at 37°C for 180 min. As for the assay, the reaction was stopped with 40 μl of 5 M NaOH after incubation of the samples for 120 min. The radio-labeled product was then extracted into 1 ml of $CHCl_3$ and the organic layer washed with 200 μl of 5 M NaOH. After back extraction of [^{3}H]*N*-tele-methylhistamine into 200 μl of 1 M HCl the acid layer was washed with 5 ml Tris–HCl buffer (pH 8.0), which contained 5 M NaOH to give a final pH of 7.5–8.5. This solution was loaded on an Amberlite GC 50 ion-exchange column (100–200 mesh; Fluka, Basel, Switzerland); the column was washed with 5 ml of Tris–HCl buffer (pH 8.0). Finally bound [^{3}H]*N*-tele-methylhistamine was eluted with 1.5 ml 1 M HCl into a scintillation vial that contained 3.5 ml 0.3 M sodium acetate buffer (pH 4.5) and sufficient 5 M NaOH to bring the pH between 4.5 and 5.0. Scintillation liquid (10 ml; Ultima Gold, Packard, Austria) and 0.1 M diethylhexylphosphoric acid was added. After shaking the vials radioactivity was measured by liquid scintillation counting.

In order to measure HMT activity brain tissue was disperged with a glass-Teflon homogenizer in 10 volumes of 0.1 M sodium phosphate buffer (pH 7.2). After centrifugation (24 500 *g* for 20 min) the supernatant was used for the radioenzymatic assay. According to the assay by Prast et al. [26] the reaction mixture consisted of 30 μl supernatant or of 30 μl 0.1 M sodium phosphate buffer (pH 7.2; blanks), 70 μl 0.1 M sodium phosphate buffer (pH 7.9) containing 4.5 μmol histamine and 1.65 μCi *S*-adenosyl-l-(methyl-^{3}H)-methionine (15 Ci/mmol). Under these conditions the assay was linear within 1 h. Triplicate samples were incubated at 37°C for 30 min. The reaction was terminated by addition of 75 μl of 2.5 M potassium borate (pH 11.0) and 1.25 ml of toluene/isopentyl alcohol (3:2). This and all following steps of extraction were done using the method of Bowsher et al. [4]. After vortexing and centrifugation at 1500 *g* for 5 min, a 1 ml aliquot of the organic phase was transferred to another culture tube containing 250 μl of 0.5 M HCl. The tubes were vortexed, centrifuged and the organic phases were removed by aspiration. A 200 μl aliquot of the aqueous phase was

Table 1

Study population and neurochemical findings in postmortem frontal cortex of the study population

	Control group	Down syndrome	Alzheimer disease
Age (years)	58.50 ± 7.58	55.88 ± 2.82	71.44 ± 5.14
Postmortem interval (h)	42.38 ± 6.85	32.38 ± 7.32	29.00 ± 5.23
Sex (male/female)	6:2	6:2	3:6
n	8	8	9
Histidine (μg/g tissue)	67.03 ± 10.23	71.27 ± 12.73	44.17 ± 8.39*
Histamine (ng/g tissue)	80.04 ± 7.64	55.66 ± 4.30*	61.03 ± 6.05**
HDC (pmol/mg protein/h)	37.72 ± 6.07	15.18 ± 1.80*	18.60 ± 2.39*
HMT (nmol/mg protein/h)	1.96 ± 0.25	2.01 ± 0.18	1.52 ± 0.20*
ChAT (nmol/mg protein/h)	3.20 ± 0.38	1.33 ± 0.20*	1.17 ± 0.24*

Values represent means ± SEM; $*P < 0.05$, $**P = 0.083$.

then transferred to scintillation vial containing 1 ml of 1 M potassium phosphate (pH 7.1) and 10 ml scintillation liquid. The vials were shaken and quantified as a two phase system by liquid scintillation counting.

ChAT activity was determined by a radiochemical procedure of Fonnum by calculating the rate of the formation of [^{14}C]acetylcholine from choline and ^{14}C-labeled coenzyme A [12]. A sample of frontal cortex from each subject was homogenized by a potter in an ice-bath in 150 volumes of 10 mM EDTA, 0.5% Triton X-100 (pH 7.4). This homogenate (40 μl) was incubated with 30 mM NaCl, 20 mM EGTA, 8 mM choline chloride, 0.2 nM [^{14}C]acetylcoenzyme A (0.8 mCi/mmol) and 10 mM sodium phosphate buffer (pH 7.4), at 37°C for 30 min. The reaction was stopped by adding 5 ml of ice-cold 10 mM sodium phosphate buffer (pH 7.4) and placing the tubes on ice. Labeled acetylcholine formed during this reaction was extracted with 1 ml heptanon-3 containing 1% tetraphenylborate. After vigorous shaking the tubes were centrifuged (at 5000 rpm for 7 min) and 500 μl aliquots of the organic phase were transferred to counting vials and processed for liquid scintillation counting.

Protein was measured according to the method of Bradford using a commercially available kit (Bio-Rad, Vienna, Austria) and bovine serum albumin as a standard [5].

All data were given as means ± SEM. Comparison of groups was calculated using the Mann–Whitney-*U*-test. Statistical significance was considered at the $P < 0.05$ level. Correlations between age, postmortem time, ChAT, histamine, histidine, HDC and HMT were evaluated using linear regression analysis.

The characteristics of the corresponding groups regarding age, sex, postmortem delay and results of neurochemical investigations are depicted in Table 1.

We observed a reduction of histamine levels in frontal cortex of DS (–28%). Alzheimer brains also showed a minor, but not a statistically significant loss of the endogenous diamine concentration (–24%). HDC activity in both groups of diseased brains was significantly reduced versus HDC activity in brains of the control panel (–60% for DS and –51% for AD, respectively). In contrast to HDC activity, HMT activity as well as histidine levels in control and DS groups were comparable. ChAT activity was significantly decreased in patients with DS and AD (–58 and –63%). No correlations were found between the investigated parameters except a significant correlation between ChAT activity and HDC activity in the DS panel ($P = 0.02$, $r = 0.79$).

It was found that brains of DS patients had significantly reduced histamine levels. At unchanged levels of histamine precursor histidine and unchanged activity of the degrading enzyme HMT, this reduction may be explained by the concomitant decrease of its synthesizing enzyme HDC. Additionally, we determined reduced ChAT activity, a biochemical hallmark for the cholinergic deficit in AD and DS [7,15,21,27], significantly and strongly correlating with HDC activity in our DS patients. The reduction of both enzyme systems may be related to the concomitant loss of cholinergic and histaminergic neurons.

Comparable HMT activities in brains of DS patients and control individuals could be explained as a major part of HMT is not localized in neurons containing HDC [3]. Moreover, there is evidence for extraneuronal distribution of HMT (e.g. in glial cells, [13]).

Similar decreases of HDC and ChAT activities were found in patients with AD. Although we did not observe statistically significant changes of histamine levels in AD, they were in the range of levels detected in brains of DS. The decreased HMT activities and histidine levels in AD, however, discriminate the two diseases pointing to different mechanisms involved in pathobiochemistry of histaminergic deficits. The histaminergic deficit in DS has never been reported before but our results on brain histamine in frontal cortex of Alzheimer patients are in accordance with the findings from Mazurkiewicz-Kwilecki and Nsonwah [21] who reported decreased histamine at unchanged histidine levels in AD. Cacabelos et al., on the contrary, described an up to 3-fold increase of histamine in frontal cortex of Alzheimer patients [6]. Discrepancies to our results are obvious, as study groups are simply not comparable. Cacabelos et al. do not provide classification of the disease, nor were clinical, neuropathological and data on histamine metabolism described. Confounding factors in our study are the sex differences between the AD group and the control and DS patients and the long high postmortem time. A long postmortem time, however, was shown not to interfere with parameters of histamine, HMT and HDC as shown in a pilot study (data not shown) and postmortem times of control brains do not correlate with enzyme activities of HMT and HDC or with histamine. The crucial period seems to be defreezing which did not occur as the samples were handled and dissected by a brain bank in the frozen state. Another factor that has to be taken into account is the age mismatch between controls and patients with AD. The impaired histaminergic system may help to explain dementia in both disorders as a series of animal studies has provided evidence for a pivotal role of histamine for cognitive function [8,9,17–19]. Furthermore, brain histamine modulates the release of acetylcholine, a major mediator of cognitive functions [10], in vivo [27] and cholinergic agents in turn modulate histamine release and its turnover rate [16,24]. Miyasaki et al. recently demonstrated that histamine elicited an ameliorating effect on scopolamine-induced learning deficits via histamine H_1 receptors in mice indicating a close relationship between the histaminergic and cholinergic systems controlling learning and memory processes [23]. A link between histamine and an other memory processing system was reported by Bekkers [2] demonstrating the enhancement of *N*-methyl-**d**-aspartate (NMDA) mediated synaptic transmission by histamine in the hippocampus. According to a current concept

the NMDA receptor is considered as a major structure involved in long-term potentiation, a model for memory formation [32].

In conclusion, we demonstrate histaminergic deficits in brains of patients with DS. In AD brains low histamine levels along with decreased HDC were found thus resembling the histaminergic deficit of DS. The histaminergic deficits may contribute to the clinical manifestation of dementia in both disorders.

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