ELSEVIER

Neuroscience Letters 300 (2001) 41–44

Neuroscience Letters

www.elsevier.com/locate/neulet

Decreased brain histamine-releasing factor protein in patients with Down syndrome and Alzheimer's disease

Seong Hwan Kim[a], Nigel Cairns[b], Michael Fountoulakisc[c], Gert Lubec[a,*]

[a]Department of Pediatrics, University of Vienna, Waehringer Guertel 18, A-1090 Vienna, Austria
[b]Brain Bank, Institute of Psychiatry, King's College, London, UK
[c]F. Hoffmann-La Roche, Ltd., Basel, Switzerland

Received 5 December 2000; received in revised form 4 January 2001; accepted 12 January 2001

Abstract

Histamine-releasing factor (HRF) stimulates secretion of histamine that is widely distributed in brain and released as neurotransmitter. Several studies suggested that histaminergic deficits could contribute to the cognitive decline in Alzheimer's disease (AD). Based upon deranged histamine metabolism in brain of patients with AD and Down Syndrome (DS), we aimed to study HRF in brain of AD and DS. We used two-dimensional gel electrophoresis, matrix-assisted laser desorption ionization mass spectroscopy and specific software to quantify HRF. HRF was significantly reduced in temporal cortex, thalamus and caudate nucleus of DS and in temporal cortex of AD as compared to controls. This is the first report to show decreased HRF brain levels in DS and AD suggesting the explanation for the decreased cognitive function in neurodegenerative/dementing disorders.

Keywords: Histamine-releasing factor; Histaminergic deficits; Down syndrome; Alzheimer's disease; Neurodegenerative/dementing disorders

Histamine-releasing factor (HRF) stimulating the secretion of histamine is found in several healthy and tumor cells and this distribution suggests a role for growth and housekeeping [19].

Histamine, widely distributed in human brain [10], is stored within and released from neurons as neurotransmitter but the density of innervation is heterogeneous [7,15]. The cell bodies of histaminergic neurons are localized in the tuberomammillary (TM) nucleus of the posterior hypothalamic region, while their varicose fibers are found in almost all regions of the brain. Histamine released from fiber terminals not only acts on neurons but also on astrocytes and blood vessels [25]. Multiple histamine receptor types were also widely distributed in the brain [6]. Morphological features and widespread distribution of histamine receptors suggest that the histaminergic system could act as a regulatory center for whole-brain activity [25]. Indeed, histamine is involved in the regulation of numerous physiological functions including learning and memory [14,17].

Previous work on histamine and histamine handling enzymes in Alzheimer's disease (AD) and Down syndrome (DS) brain showed impairment of the histaminergic system [21]. Moreover, antihistaminic treatment is leading to DS-like cognitive decline in healthy controls [22]. These findings along with the availability of an analytical technique unambiguously identifying HRF made us examine the histaminergic system at the histamine releasing level quantifying HRF in brain with neurodegenerative/dementing disorders.

Postmortem brain samples were obtained from the Medical Research Council London Brain Bank for Neurodegenerative Diseases, Institute of Psychiatry. 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. 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 [24]. The neuropathological diagnosis of 'definite AD' was confirmed using the CERAD criteria [12]. Normal control brains were obtained from individuals with no history of neurological or psychiatric illness. The major cause of death was bronchopneumonia in DS and AD, and heart disease in controls. After the fresh brain was dissected, coronal slices were immediately frozen and stored at −70°C until required. The frontal

* Corresponding author. Tel.: +43-1-40400-3215; fax: +43-1-40400-3194.
E-mail address: gert.lubec@akh-wien.ac.at (G. Lubec).

0304-3940/01/$ - see front matter
PII: S0304-3940(01)01545-2

(superior frontal gyrus), temporal (superior temporal gyrus), occipital (calcarine sulcus), parietal (superior parietal lobe) cortex, cerebellum (cerebellar hemisphere), thalamus, caudate nucleus of patients with DS ($n = 9$, 55.67 ± 7.48 years old), AD ($n = 13$, 58.54 ± 7.57 years old) and controls ($n = 18$, 50.00 ± 16.94 years old) were used. The postmortem interval of DS, AD and controls was 31.44 ± 19.56 h (between 9 and 72 h), 30.31 ± 26.17 h (between 5 and 96 h) and 38.78 ± 18.84 h (between 14 and 83 h), respectively.

For two-dimensional (2-D) gel electrophoresis, brain sample was suspended in 0.5 ml of sample buffer consisting of 40 mM Tris, 5 M urea (Merck, Darmstadt, Germany), 2 M thiourea (Sigma, St. Louis, MO, USA), 4% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) (Sigma), 10 mM 1,4-dithioerythritol (Merck), 1 mM ethylenediaminetetraacetic acid (EDTA) (Merck), 1 mM phenylmethylsulfonylfluoride (PMSF) (Sigma) and 1 μg/ml of each pepstatin A, chymostatin, leupeptin and antipain. The suspension was sonicated for 30 s and centrifuged at $10\,000 \times g$ for 10 min and the supernatant was centrifuged further at $150\,000 \times g$ for 45 min to sediment undissolved material. The protein concentration of the supernatant was determined by the Coomassie blue method [3]. Samples (1.5 mg) were applied on immobilized pH 3–10 nonlinear gradient strips (IPG, immobilized pH-gradient strips, Pharmacia Biotechnology, Uppsala, Sweden) at both the basic and acidic ends of the strips. The proteins were focused at 300 V for 1 h, after which the voltage was gradually increased to 3500 V within 6 h. Focusing was continued at 3500 V for 12 h and at 5000 V for 48 h. The second-dimensional separation was performed on 9–16% linear gradient polyacrylamide gels. After protein fixation with 40% methanol containing 5% phosphoric acid for 12 h, the gels were stained with colloidal Coomassie blue (Novex, San Diego, CA, USA) for 48 h. The molecular mass was determined by running standard protein markers at the right side of selected gels. The size markers (Gibco, Basel, Switzerland) covered the range 10–200 kDa. The gels were destained with water and scanned in a Personal Densitometer (Molecular Dynamics, Sunnyvale, CA, USA). The images were processed using PhotoShop (Adobe) and PowerPoint (Microsoft) software.

Matrix-assisted laser desorption ionization mass spectroscopy (MALDI-MS) analysis was performed to identify protein spots in 2-D gels. Spots were excised, destained with 50% acetonitrile in 0.1 M ammonium bicarbonate and dried in a speedvac evaporator. The dried gel pieces were reswollen with 3 μl of 3 mM Tris–HCl (pH 8.8) containing 50 ng trypsin (Promega, Madison, WI, USA) and after 15 min, 3 μl of H_2O were added. One microliter was applied onto the dried matrix spot. The matrix consisted of 15 mg nitrocellulose (Bio-Rad) and 20 mg α-cyano-4-hydroxycinnamic acid (Sigma) dissolved in 1 ml acetone: isopropanol (1:1, v/v). A 0.5 μl portion of matrix solution was applied on the sample target. The digest mixtures were analyzed in a time-of-flight PerSeptive Biosystems mass spectrometer (Voyager Elite, Cambridge, MA, USA) equipped with a reflectron. An accelerating voltage of 20 kV was used. Calibration was internal to the samples. The peptide masses were matched with the theoretical peptide masses of all proteins from all species of the SWISS-PROT database. For protein search, monoisotopic masses were used and a mass tolerance of 0.0075% was allowed.

The percentage of the volume of the spot representing HRF was quantified as compared to the total proteins present in partial 2-D gel images including HRF using the ImageMaster 2D Elite software (Amersham Pharmacia Biotechnology).

Between-group differences were investigated by non-parametric Mann–Whitney *U*-test and correlation between either postmortem interval or age and protein level of HRF was evaluated using the Spearman rank coefficient. The significance was set at $P < 0.05$. All statistical analyses were performed by GraphPad Instat2 software, version 2.05.

We showed the presence of HRF protein in human brain using 2-D gel electrophoresis in previous work [9]. The theoretical and the observed M_r and pI values, as well as the matching and total peptides are given in Table 1. We quantified HRF in seven brain regions of DS, AD, and controls in order to detect differences at the protein level. As shown in Table 2, HRF were significantly decreased in temporal cortex of DS and AD. In thalamus and caudate nucleus of DS, HRF were also significantly decreased. Either postmortem intervals or age had no influence on

Table 1
Identification of HRF[a]

M_r (Da)		PI		MALDI-MS	
				Peptides	
Theoretical	Observed	Theoretical	Observed	Matching	Total
19696	22000	4.68	4.70	5	22

[a] Protein separated by 2-D electrophoresis was identified by MALDI-MS, following digestion with trypsin. After the peptide masses were matched with the theoretical peptide masses of all proteins from all species of the SWISS-PROT database, this protein was identified to HRF with SWISS-PROT accession number P13693. The probability of assignment of a wrong identity is 1.00E-04.

Table 2
HRF levels in brain regions of DS, AD and controls[a]

	Control (*n*)	DS (*n*)	AD (*n*)
Cerebellum	2.87 ± 1.35 (10)	1.40 (1)	2.03 ± 0.64 ()
Frontal cortex	1.28 ± 0.88 (8)	1.99 ± 0.72 (6)	1.64 ± 0.81 (7)
Temporal cortex	2.91 ± 1.04 (14)	1.50 ± 0.75** (7)	1.93 ± 0.83* (9)
Occipital cortex	2.52 ± 0.79 (14)	2.05 ± 0.36 (6)	1.96 ± 0.99 (11)
Parietal cortex	2.02 ± 0.60 (7)	2.08 ± 1.10 (6)	1.82 ± 0.97 (6)
Thalamus	2.41 ± 0.42 (9)	1.41 ± 0.39** (6)	1.90 ± 0.99 (7)
Caudate Nucleus	3.35 ± 1.01 (9)	1.17 ± 0.58** (4)	2.43 ± 0.66 (8)

[a] The proteins from seven brain regions of DS, AD and controls were separated on 2-D gels and visualized following stain with colloidal Coomassie blue. In partial gel images, including HRF and neighbouring proteins, the percentage of the volume of spot representing HRF were quantified, compared to the total proteins present. The quantification was performed using the ImageMaster 2D Elite software. In the case that neither the spot representing HRF protein was not separated well with another protein in 2-D gel nor located ambiguously, HRF protein spot were not quantified for correctness. Results were represented as means ± SD (quantified sample number). The statistical analyses were performed by Graphpad Instat2 software and the significance was set at $P < 0.05$. **, $P < 0.01$; *, $P < 0.05$.

the significantly decreased protein levels of HRF (data not shown). The 2-D gel protein spots corresponding to HRF with the significant difference were presented in Fig. 1.

Information on the histaminergic deficit in AD is limited: High densities of neurofibrillary tangles in AD have been found in the vicinity of cortically-projecting histaminergic neurons [20,23] and accumulation of neurofibrillary tangles were reported in the tuberomammillary area in hypothalamus of AD brains [1]. Decreased histamine levels were found in AD brain [11] including reduction in the neuronal pool of hippocampus, hypothalamus and temporal cortex of AD patients [16] but in contradicting reports increased histamine levels in cerebrospinal fluid and brain tissue were demonstrated [4,18]. Histamine measurements per se may be, however, unreliable due to many confounding factors as, e.g. histamine is increasing with post-mortem time [16] but may be valuable when accompanied by determination of histamine-related enzymes.

HRF has not been studied in the human brain yet and we here describe the significant decrease of this protein in

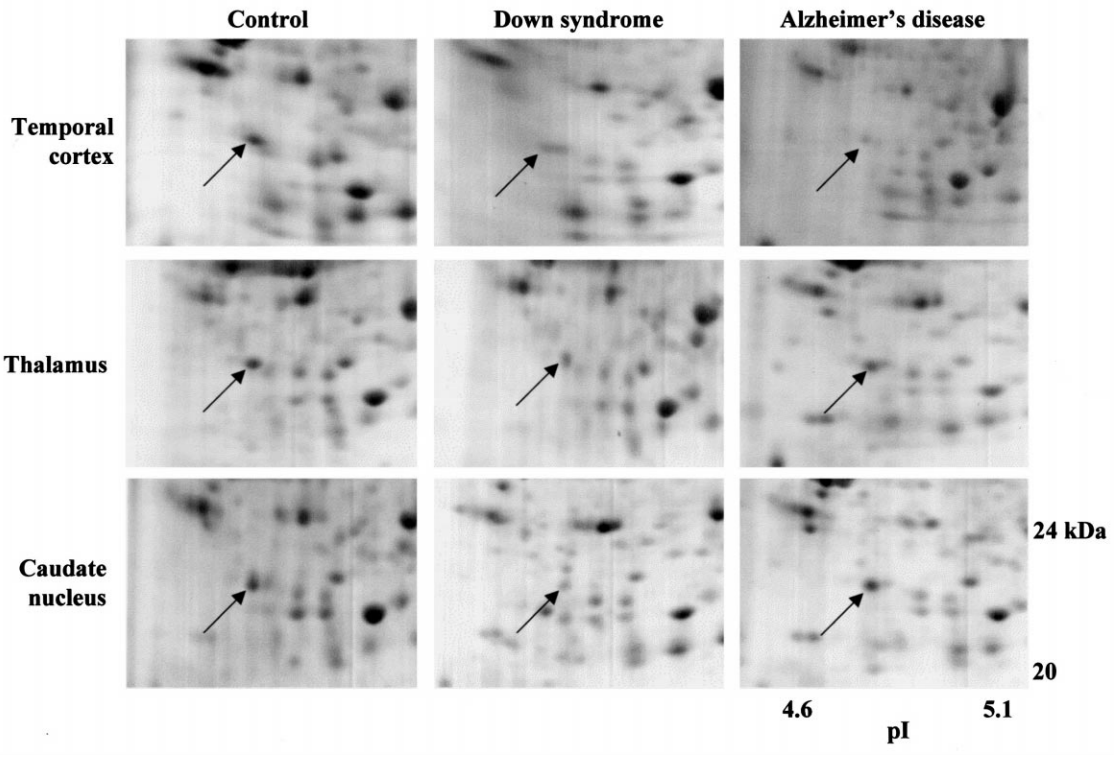

Fig. 1. The partial 2-D gel images of HRF in brain regions with significant changes. The human brain proteins were extracted and separated on an immobilized pH 3–10 nonlinear gradient strip, followed by a 9–16% linear gradient polyacrylamide gel, as stated under experimental procedures. In the gels stained with Coomassie blue, an arrow indicates the protein spot corresponding HRF.

temporal cortex of DS and AD involved in cognitive functions and where decreased histamine was already reported [16]. Decreased brain HRF may well be responsible for decreased brain histamine levels in DS and AD. Furthermore, interestingly HRF were significantly decreased in thalamus and caudate nucleus of DS. Similar decreases of HRF were found in AD but statistically significant changes were not observed. These results suggest that HRF showing the regional specificity could be more vulnerable in DS than AD.

The biological meaning of decreased HRF that could not be affected by postmortem intervals in DS and AD may be decreased cognitive functions, known to be modulated by the histaminergic system by several authors [8,14,17]. Since histamine release as well as its synthesis and turn-over could be regulated by cholinergic agonists that control the release of acetylcholine playing a key role for cognitive functions [5,13], it suggests the relationship between histaminergic system and cholinergic system in the pathogenesis of cognitive defects in DS and AD. The link to the excitatory amino acid neurotransmission system could also be important to be addressed in this context: histamine enhances NMDA-mediated synaptic transmission in hippocampus by modulating long term potentiation and thus probably cognitive functions [2].

We conclude that the decrease of HRF in DS and AD brain regions may lead to a histaminergic deficit found in DS and AD and may help to explain decreased cognitive functions in both neurodegenerative/dementing disorders.

[1] Airaksinen, M.S., Paetau, A., Paljarvi, L., Reinikainen, K., Riekkinen, P., Suomalainen, R. and Panula, P., Histamine neurons in human hypothalamus: anatomy in normal and Alzheimer diseased brains, Neuroscience, 44 (1991) 465–481.

[2] Beckers, J.M., Enhancement by histamine of NMDA-mediated synaptic transmission in the hippocampus, Science, 261 (1993) 104–106.

[3] Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 72 (1976) 248–254.

[4] Cacabelos, R., Yamatodani, A., Niigawa, H., Hariguchi, S., Tada, K., Nishimura, T., Wada, H., Brandeis, L. and Pearson, J., Brain histamine in Alzheimer's disease, Methods Find. Exp. Clin. Pharmacol., 11 (1989) 353–360.

[5] Gulat-Marnay, C., Lafitte, A., Arrang, J.M. and Schwartz, J.C., Regulation of histamine release and synthesis in the brain by muscarinic receptors, J. Neruochem., 52 (1989) 248–254.

[6] Hough, B., Histamine, In G.J. Siegel, B.W. Agranoff, R.W. Albers, S.K. Fisher and M.D. Uhler (Eds.), Basic Neurochemistry: Molecular, Cellular and Medical Aspects, Lippincott Williams and Wilkins, Philadelphia PA, 1999, pp. 293–314.

[7] Inagaki, N., Yamatodani, A., Ando-Yamamoto, M., Tohyama, M., Watanabe, T. and Wada, H., Organization of histaminergic fibers in the rat brain, J. Comp. Neurol., 273 (1988) 283–300.

[8] Kamei, C., Okumura, Y. and Tasaka, K., Influence of histamine depletion on learning and memory recollection in rats, Psychopharmacology (Berl.), 111 (1993) 376–382.

[9] Langen, H., Berndt, P., Roeder, D., Cairns, N., Lubec, G. and Fountoulakis, M., Two-dimensional map of human brain proteins, Electrophoresis, 20 (1999) 907–916.

[10] Lipinski, J.F., Schaumburg, H.H. and Baldessarini, R.J., Regional distribution of histamine in human brain, Brain Res., 52 (1973) 403–408.

[11] Mazurkiewicz-Kwilecki, I.M. and Nsonwah, S., Changes in the regional brain histamine and histidine levels in postmortem brains of Alzheimer patients, Can. J. Physiol. Pharmacol., 67 (1989) 75–78.

[12] Mirra, S.S., Heyman, A., McKeel, D., Sumi, S.M., Crain, B.J., Brownlee, L.M., Vogel, F.S., Hughes, J.P., van Belle, G. and Berg, L., The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease, Neurology, 41 (1991) 479–486.

[13] Oishi, R., Acachi, N., Okada, K., Muroi, N. and Saeki, K., Regulation of histamine turnover via muscarinicand nicotinic receptors in the brain, J. Neurochem., 55 (1990) 899–904.

[14] Onodera, K., Yamatodani, A., Watanabe, T. and Wada, H., Neuropharmocology of the histaminergic neuron system in the brain and its relationship with behavioral disorders, Prog. Neurobiol., 42 (1994) 685–702.

[15] Panula, P., Pirvola, U., Auvinen, S. and Airaksinen, M.S., Histamine-immunoreactive nerve fibers in the rat brain, Neuroscience, 28 (1989) 585–610.

[16] Panula, P., Rinne, J., Kuokkanen, K., Eriksson, K.S., Sallmen, T., Kalimo, H. and Relja, M., Neuronal histamine deficit in Alzheimer's disease, Neuroscience, 82 (1998) 993–997.

[17] Passani, M.B., Bacciottini, L., Mannaioni, P.F. and Blandina, P., Central histaminergic system and cognition, Neurosci. Biobehav. Rev., 24 (2000) 107–113.

[18] Perez, A., Albarran, M.A. and Cacabelos, R., Biochemical studies of body fluids in senile dementia of the Alzheimer type (SDAT) and Multi-infart dementia (MID). Correlation analysis between histamine and vasopressin in CSF and plasma, Acta. Neurol. Scand. (Supplement), 77 (1988) 129.

[19] Sanchez, J.C., Schaller, D., Ravier, F., Golaz, O., Jaccoud, S., Belet, M., Wilkins, M.R., James, R., Deshusses, J. and Hochstrasser, D., Translationally controlled tumor protein: a protein identified in several nontumoral cells including erythrocytes, Electrophoresis, 18 (1997) 150–155.

[20] Saper, C.B. and German, D.C., Hyphothalamic pathology in Alzheimer's diease, Neurosci. Lett., 74 (1987) 364–370.

[21] Schneider, C., Risser, D., Kirchner, L., Kitzmuller, E., Cairns, N., Prast, H., Singewald, N. and Lubec, G., Similar deficits of central histaminergic system in patients with Down syndrome and Alzheimer disease, Neurosci. Lett., 222 (1997) 183–186.

[22] Seidl, R., Hauser, E., Bernert, G., Marx, X., Freilinger, M. and Lubec, G., Auditory evoked potentials in young patients with Down syndrome. Event-related potentials (P3) and histaminergic system, Brain Res. Cog. Brain Res., 5 (1997) 301–309.

[23] Simpson, J., Yates, C.M., Watts, A.G. and Fink, G., Congo red birefringent structures in the hypothalamus in senile dementia of the Alzheimer type, Neuropath. App. Neurobiol., 14 (1988) 381–393.

[24] Tierney, M.C., Fisher, R.H., Lewis, A.J., Zorzitto, M.L., Snow, W.G., Reid, D.W. and Nieuwstraten, P., The NINCDS-ADRDA Work Group criteria for the clinical diagnosis of probable Alzheimer's disease: a clinicopathologic study of 57 cases, Neurology, 38 (1988) 359–364.

[25] Wada, H., Inagaki, N., Yamatodani, A. and Watanabe, T., Is the histaminergic neuron system a regulatory center for whole-brain activity? Trends Neurosci., 14 (1991) 415–418.