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RESEARCH ARTICLE

Functional Increase of Brain Histaminergic Signaling in Huntington's Disease

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Keywords
histamine, histamine receptors, Huntington's disease, hypothalamus, *in situ* hybridization, post-mortem.

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Abstract

To evaluate whether central histaminergic signaling in Huntington's disease (HD) patients is affected, we assessed mRNA levels of histidine decarboxylase (HDC), volume of and neuron number in the hypothalamic tuberomamillary nucleus (TMN) (HD n = 8, controls n = 8). In addition, we assessed histamine N-methyltransferase (HMT) and histamine receptor (H_1R, H_2R and H_3R) mRNA levels in the inferior frontal gyrus (IFG) (n = 9 and 9) and caudate nucleus (CN) (n = 6 and 6) by real-time polymerase chain reaction. In HD patients, TMN volume and neuronal number was unaltered ($P = 0.72$, $P = 0.25$). The levels of HDC mRNA ($P = 0.046$), IFG HMT ($P < 0.001$), H_1R ($P < 0.001$) and H_3R mRNA levels ($P = 0.011$) were increased, while CN H_2R and H_3R mRNA levels were decreased ($P = 0.041$, $P = 0.009$). In HD patients, we observed a positive correlation between IFG H_3R mRNA levels and CAG repeat length ($P = 0.024$) and negative correlations between age at onset of disease and IFG HMT ($P = 0.015$) and H_1R ($P = 0.021$) mRNA levels. These findings indicate a functional increase in brain histaminergic signaling in HD, and provide a rationale for the use of histamine receptor antagonists.

INTRODUCTION

The histaminergic system of the brain is involved in a large variety of functions, such as modulation of the state of arousal, sleep and wakefulness, food intake, learning and memory (18). Neuronal histamine is exclusively produced in the hypothalamic tuberomamillary nucleus (TMN) via the rate-limiting enzyme histidine decarboxylase (HDC) (36). From the TMN, histamine is released into many brain areas including the hypothalamus, prefrontal cortex and hippocampus, and mainly inactivated by histamine N-methyltransferase (HMT) (36). Histamine exerts its functions mainly through three G-protein-coupled histamine receptors (H_1R, H_2R and H_3R), which are widely distributed throughout the brain (18).

In Huntington's disease (HD), an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in the gene encoding the protein huntingtin (26), alterations in the histaminergic system might be expected as the hypothalamus is involved in the disease process (6, 27). Indeed, among all the hypothalamic nuclei, the TMN contains the highest frequency of both nuclear and cytoplasmatic inclusions of mutant huntingtin, the neuropathological hallmark of HD (5). In addition, reductions of the number of orexin-producing neurons in the lateral hypothalamic area have been observed in both HD patients and the R6/2 and YAC128 transgenic mouse models of the disease (5, 8, 25). As orexin was found to be an important activator of TMN neurons (13), reduced orexin levels may thus affect histaminergic signaling. The histaminergic system in HD patients, however, has hardly been studied and the results have been equivocal. So far, only three studies have assessed the distribution of histamine receptors in HD brains, whereas histamine production has not been investigated (17, 23, 37). Post-mortem binding studies found decreases of both H_2R and H_3R in many brain regions, especially the striatum (17, 23). In contrast, H_1R was increased in cortical areas of HD patients (37).

As alterations of the central histaminergic signaling may contribute to a number of debilitating signs and symptoms of HD including cognitive decline, progressive weight loss and sleep disturbances in the present study, we aimed at assessing the functional integrity of the brain histaminergic system in end-stage HD. First, we investigated histamine production by assessing HDC-mRNA expression by *in situ* hybridization in the hypothalamic TMN. Next, we assessed in two main histamine projection brain regions, the inferior frontal gyrus (IFG) and the caput of the caudate nucleus (CN), mRNA levels of three major histamine receptors (H_1R,

H_2Rand H_3R) by real-time polymerase chain reaction (PCR), as well as of the enzyme involved in histamine breakdown, HMT.

METHODS

Post-mortem material

All brain material for HD patients and control subjects was obtained through the Netherlands Brain Bank (NBB), and consisted of formalin-fixed, paraffin-embedded hypothalamic material (HD n = 8, controls n = 8) and snap-frozen material from the IFG (HD n = 9, controls n = 9) and CN (HD n = 6, controls n = 6). Control subjects were matched to HD patients for sex, age, clock time and month of death, post-mortem delay and fixation time (Table 1). Different control subjects were used for each analysis when hypothalamic and frozen tissue for the other anatomic regions were not available from the same patient, while maintaining adequate matching for possible confounding factors. Written informed consent for brain autopsy, and for the use of brain material and medical records for research purposes, was acquired by the NBB from patients or their next of kin. The study was approved by the NBB's ethical board.

The diagnosis of HD was clinically and neuropathologically confirmed in all patients. Additionally, the diagnosis was genetically confirmed (CAG repeat ≥ 39) in all but one patient (NBB 92–105). The latter patient, however, had a positive family history and the clinical features of HD, and had a confirmed Vonsattel grade II HD neuropathology with neuronal intranuclear and cytoplasmic inclusions (34). Exclusion criteria for control subjects were primary neurological and/or psychiatric disorders and glucocorticoid therapy during the last 2 months prior to death, as glucocorticoids can influence the activity of the HDC enzyme (39). Furthermore, any HD patient or control subject receiving histamine receptor (reverse) agonists was excluded.

In situ hybridization (ISH)

For the assessment of HDC-mRNA in the TMN, a 45-mer oligonucleotide probe (GenBank #MGC163399), complementary to bases 599–643 of the human HDC-mRNA sequence was used as described elsewhere (4, 22). The probe was checked for cross homology with other known sequences using Basic Local Alignment Search Tool (2). Interfering sequences were not found in the databases at the National Center for Biotechnology Information and the National Library of Medicine (USA). In addition, an HDC sense probe was used alongside the antisense probe serving as a negative control. No autoradiographic signal was observed in sections incubated with the HDC sense probe.

The probe was 3′-end labeled using terminal deoxynucleotidyl transferase (Roche, Mannheim, Germany) and [^{35}S] dATP (PerkinElmer Watham, MA, USA, Cat. # NEG612H) and purified by ethanol precipitation, similar to what has been described before (22). Hybridization buffer consisted of 0.5 M NaCl, 1× Denhardt's solution, 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid, 10% dextran sulphate, 0.5 mg/mL yeast tRNA, 50% formamide and 200 mM dithiothreitol.

For analysis, every 100th section (6 μm) along the rostro-caudal axis of the TMN was mounted on 2% amino-alkyl-silane-coated slides and dried at 58°C. Following deparaffinization and rehydration, sections were autoclaved for 20 minutes at 120°C under a pressure of 1 bar in 0.01 M citrate buffer (pH 6.0). After delipidation in phosphate-buffered saline containing 0.1% Triton X-100, sections were hybridized in hybridization buffer with approximately 1×10^6 cpm of labeled HDC oligoprobe per slide, coverslipped and hybridized overnight at 42°C.

After hybridization, sections were washed in sequential series of standard saline citrate and dehydrated in graded mixtures of 300 mM ammonium acetate (pH 5.5) and absolute ethanol. Sections were exposed to autoradiographic film (Eastman Kodak Company, Rochester, NY, USA) for 5 days and subsequently, films were developed for 4 minutes in Kodak D-19 developer (Kodak) and fixed in Kodak Maxfix for 5 minutes.

Grey values of individual autoradiograms of TMN sections were related to an existing standard curve. The outcome was multiplied by the area covered by the HDC signal to obtain an estimate for the total amount of HDC-mRNA in the TMN in arbitrary units (AU). This procedure has been extensively described elsewhere (15, 16).

TMN stereology

For an estimation of the total number of TMN neurons, every 100th section of this nucleus was stained by conventional thionin for all subjects. In each section, all TMN neurons with a visible nucleolus, which served as a unique marker for each neuron, were counted using a light microscope at a magnification of 400×. Taking into account the interval distances between individual sections, the total number of TMN neurons was estimated using a method described before. This method is based on well-known stereological methods, including the Cavalieri principle (32). The same method was used to estimate TMN volumes using area measurements on the HDC autoradiograms of the TMN.

Frozen tissue dissection, RNA isolation and real time PCR

Methods for snap-frozen tissue dissection, RNA isolation and cDNA synthesis have been extensively described elsewhere (9). Briefly, cryostat sections of 50 μm in thickness were obtained from snap-frozen IFG or CN. Gray matter areas were separated from white matter using pre-chilled sterile scalpels, and 50 mg of each sample was collected into pre-chilled tubes and immediately put on dry ice. All procedures were conducted at −19°C. Total RNA was isolated from all collected samples by means of a hybrid protocol of Trizol (Invitrogen Life Technologies, Carlsbad, CA, USA) and Qiagen RNeasy Mini Kit™ (Qiagen, Valencia, CA, USA) RNA isolation methods. RNA yields and purity were determined by a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and the RNA integrity Number (RIN) was measured with the use of an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). RNA quality of all samples was sufficient (≥5.00) (11, 14) for quantitative real time PCR analysis (RIN ≥ 5.70; Table 1). For each sample, 500 ng of total RNA was used for synthesis of cDNA, identical to what has been described by others (7).

Primer design

Primer sequences for H_1R, H_2R, H_3R and HMT and GenBank accession numbers are indicated in Table 2. Primer sequences for

Table 1. Clinicopathological data of Huntington's disease patients and control subjects. Abbreviations: yr = years; d = days; h = hours; g = grams; CN = caudate nucleus (snap frozen tissue); CTD = clock time at death; MD = month of death; Fix = fixation time; HD = Huntington's disease; IFG = inferior frontal gyrus (snap-frozen tissue); NBB = Netherlands Brain Bank; ND = not determined.; PMD = post mortem delay; RIN = RNA integrity number; SEM = standard error of mean; TMN = tuberomamillary nucleus (paraffin embedded tissue.

	NBB number		Sex	Age at death (yr)	Age at onset (yrs)	CTD (h)	MD	PMD (h)	Fix (d)	CAG repeat length	Brain weight (gr)	Used for	RIN value (IFG)	RIN value (CN)	Cause of death
Patients	99–108	HD-1	M	49	40	9:15	8	5:45	—	51	1122	IFG	6,20	—	Cachexia secondary to pneumonia
	03–047	HD-2	F	50	35	18:25	6	5:40	55	47	1292	TMN, IFG, CN	5,80	5,70	Pneumonia
	92–105	HD-3	M	54	41	9:55	12	3:50	80	ND	1212	TMN	—	—	Sudden death
	95–060	HD-4	M	57	42	3:30	6	7:30	53	46	1162	TMN, IFG, CN	7,90	7,20	Cachexia
	08–044	HD-5	M	59	50	18:10	5	5:05	52	44	1446	TMN, IFG, CN	7,60	8,30	Legal euthanasia
	01–128	HD-6	M	61	39	10:55	11	10:25	48	43	1380	TMN, IFG	6,80	—	Pneumonia
	09–063	HD-7	F	64	53	21:50	8	5:00	—	46	1075	IFG, CN	7,50	5,70	Pneumonia
	98–047	HD-8	F	67	56	10:10	4	6:05	41	45	1289	TMN, IFG, CN	7,40	7,10	Legal euthanasia
	99–120	HD-9	M	79	54	19:00	10	6:15	34	44	1001	TMN, IFG	6,10	—	Pneumonia and septic shock
	00–109	HD-10	F	80	58	22:30	10	7:15	49	41	906	TMN, IFG, CN	5,70	5,80	Pneumonia
Mean (TMN)				63	48	14:56	8	6:20	52	45	1196		—	—	
Mean (IFG)				63	47	14:51	8	6:33	—	45	1186		6,78	—	
Mean (CN)				63	49	15:45	7	6:05	—	45	1195		—	6,63	
SEM (TMN)				3	3	2:10	1	0:38	5	1	59		—	—	
SEM (IFG)				4	3	2:11	1	0:33	3	1	60		0,28	—	
SEM (CN)				4	4	3:02	1	0:26	2	1	78		—	0,44	

Table 1. *Continued*

	NBB number		Sex	Age at death (yr)	Age at onset (yrs)	CTD (h)	MD	PMD (h)	Fix (d)	CAG repeat length	Brain weight (gr)	Used for	RIN value (IFG)	RIN value (CN)	Cause of death
Controls	97–127	C-1	F	49	—	3:30	4	13:30	165	—	1437	TMN	—	—	Metastasized cervical carcinoma
	98–027	C-2	M	54	—	9:00	12	8:00	59	—	1350	TMN	—	—	Hepatocellular carcinoma
	97–130	C-3	M	58	—	0:00	6	17:00	96	—	1408	TMN	—	—	Aorta dissection
	98–127	C-4	M	56	—	15:45	8	5:25	35	—	1522	TMN	—	—	Cardiac infarction
	92–042	C-5	M	61	—	21:00	4	13:50	52	—	2220	TMN	—	—	Oesophagus carcinoma
	01–069	C-6	F	68	—	12:15	5	5:45	32	—	1153	TMN	—	—	Legal euthanasia
	93–060	C-7	M	79	—	14:00	2	3:00	53	—	1435	TMN	—	—	Hemorrhage from leaking aorta
	00–142	C-8	F	82	—	15:10	12	5:30	36	—	1280	TMN	—	—	Myocardial infarction
	95–007	C-9	M	54	—	9:15	1	9:10	—	—	1305	IFG	7,80	—	Carotid bleeding
	08–073	C-10	F	50	—	20:30	8	4:10	—	—	1332	IFG	6,20	—	Metastasized bronchocarcinoma
	05–034	C-11	M	56	—	0:01	5	14:00	—	—	1313	IFG	7,00	—	Congestive heart failure
	94–114	C-12	M	63	—	16:30	11	26:45	—	—	1154	IFG	5,80	—	Pneumonia
	95–084	C-13	M	72	—	10:00	8	7:25	—	—	1370	IFG	7,70	—	Lungemphesyma
	97–042	C-14	F	65	—	2:00	4	12:50	—	—	1030	IFG	7,10	—	Cardiac arrest
	90–013	C-15	F	68	—	10:00	1	7:35	—	—	1140	IFG	7,10	—	Lung embolia combined with heartfailure
	03–084	C-16	M	82	—	21:15	10	10:00	—	—	1488	IFG	7,10	—	Pleuritis carcinomatosa
	01–104	C-17	F	77	—	20:15	9	5:30	—	—	1343	IFG	8,10	—	Pulmonary metastases / breast carcinoma
	08–073	C-18	F	50	—	20:30	8	4:10	—	—	1332	CN	—	7,90	Metastasized bronchocarcinoma
	95–084	C-19	M	72	—	10:00	8	7:25	—	—	1370	CN	—	7,50	Lungemphesyma
	06–037	C-20	M	66	—	17:45	5	7:45	—	—	1560	CN	—	8,50	Pneumonia
	97–042	C-21	F	65	—	2:00	4	12:50	—	—	1030	CN	—	8,00	Cardiac arrest
	96–032	C-22	F	60	—	11:00	3	8:25	—	—	1275	CN	—	8,30	Metastasized Bronchocarcinoma
	06–008	C-23	F	85	—	23:30	1	4:40	—	—	1075	CN	—	8,20	Coronary Shock
Mean (TMN)				63	—	11:20	7	9:00	66	—	1476		—	—	
Mean (IFG)				65	—	12:11	6	8:50	—	—	1275		7,10	—	
Mean (CN)				66	—	14:07	5	7:30	—	—	1274		—	8,07	
SEM (TMN)				4	—	2:25	1	1:47	16	—	114		—	—	
SEM (IFG)				4	—	2:38	1	1:12	—	—	47		0,25	—	
SEM (CN)				5	—	3:14	1	1:33	—	—	80		—	0,14	
Level of significance (TMN)				0.888*	—	0.689†	0.144†	0.481*	0.798*	—	0.083*		—	—	
Level of significance (IFG)				0.605*	—	0.573†	0.751†	0.094*	—	—	0.258*		—	—	
Level of significance (CN)				0.485*	—	0.737†	0.426†	0.485*	—	—	0.589*		—	—	

*Mann–Whitney-U-Test.
†Mardia–Watson-Test.

Table 2. Primer sequences and GenBank accession numbers. Abbreviations: HMT = Histamine N-Methyl Transferase; H1R = Histamine 1 Receptor; H2R = Histamine 2 Receptor; H3R = Histamine 3 Receptor.

Gene	Forward primer sequence	Reverse primer sequence	Accession Number
HMT	AATGGAGACCTGCTTTGGG	ATCAGGTGGTGCTGTGGC	NM_006895
H1R	CTGGGAGGTTCTGAAAAGG	GCTGAAGACAACTGGGGATT	NM_001098213
H2R	GGAACAGCAGGAACGAGAC	AGTAGCGGGAGGTAGAAGGT	NM_022304
H3R	GAAGATGGTGTCCCAGAGC	CCAGCAGAGCCCAAAGAT	NM_007232

actin-β (ACTβ), complement 3 (C3), glial fibrillary astrocyte protein (GFAP), hydroxymethylbilane synthase (HMBS), hypoxanthine phosphoribosyltransferase 1 (HPRT1), tubulin-α (TUBα) and tubulin-β4 (TUBβ4) were used as reference genes as described before (35). Because of the lack of specific antibodies to H_1R, H_2R and H_3R, protein analysis could not be performed to confirm possible changes in gene expression.

Quantitative PCR

Quantitative PCR (qPCR) was performed as described before (9), in a final volume of 20 µL, using the SYBR Green PCR kit (Applied Biosystems, Carlsbad, CA, USA) and a mixture of sense and antisense primers (2 pmol/µL). qPCR was performed in a GeneAmp 7300 thermocycler PCR program, and data were acquired and processed automatically by sequence Detection Software (Applied Biosystems) and an applied Biosystems Model ABI 5700 Prism Sequence Detection System.

The specificity of the amplification was checked by means of melting curve analysis and electrophoresis of products on an 8% polyacrylamide gel. Sterile water and RNA samples without the addition of reverse transcriptase during cDNA synthesis served as controls. The linearity of each qPCR assay was tested by preparing a series of dilutions of the same stock cDNA in multiple plates.

Normalization strategy

In order to correct for sampling-related differences such as RNA quality and RNA quantity, the normalization strategy from Wang *et al* was used (35). The normalization factor consisted of the geometric mean of ACTβ, HMBS, HPRT1, TUBα and TUBβ for IFG analysis and of C3, GFAP and HMBS for CN analysis. The latter three reference genes were identified based on previous reports (20). The relative absolute amount of target genes was calculated with the use of the following formula: $10^{10} \times E^{-ct}$ [$E = 10^{-(1/slope)}$].

Statistical analysis

All data are presented as mean ± SEM unless otherwise specified. Differences between groups were statistically evaluated by the non-parametric Mann–Whitney–*U* test (two-tailed). Intergroup differences in clock time and month of death were evaluated using the Mardia–Watson test. Spearman's ρ was used for all correlations. $P < 0.05$ was considered to be significant. All statistical analyses were carried out using SPSS Statistics 17.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Control subjects did not differ from HD patients for any of the analyses (TMN, IFG and CN) with respect to sex ($P = 1.00$), age ($P \geq 0.485$), clock time and month of death ($P \geq 0.573$ and ≥ 0.144, respectively), post-mortem delay ($P \geq 0.094$) and fixation time ($P = 0.798$; Table 1).

TMN

Representative autoradiographs are provided in Figure 1. HDC-mRNA expression of HD patients (86.10 ± 5.15) was significantly higher than in matched controls (52.62 ± 1.22; $P = 0.046$; Figure 2). No significant correlation was found between CAG repeat length or age at onset and the amount of HDC-mRNA in HD patients ($\rho = -0.450$, $P = 0.310$ and $\rho = 0.548$, $P = 0.160$, respectively).

HD patients showed a TMN volume of 135 978 ± 19 822 µm^3 and control subjects of 118 163 ± 17 222 µm^3 ($P = 0.72$). Moreover, the estimated number of TMN neurons in HD patients was 35 906 ± 2881, while control subjects had an average of 42 152 ± 2744 neurons ($P = 0.25$; Figure 3). The total TMN neuron numbers for control subjects are quite comparable to those that have been reported in previous studies (1).

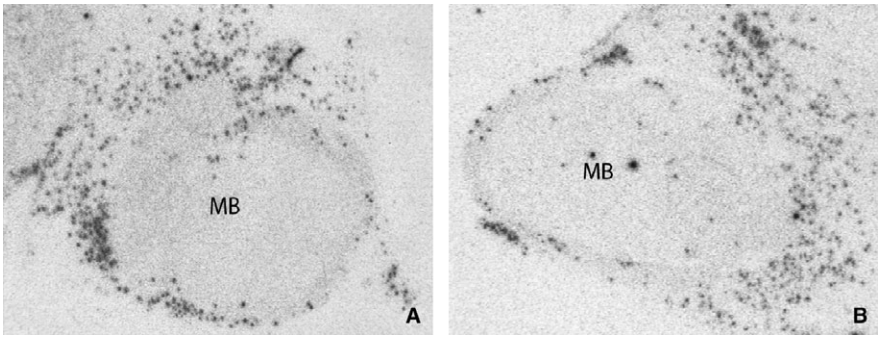

Figure 1. Representative autoradiographic images of L-histidine decarboxylase mRNA expression at the level of the mamillary body (MB) of a Huntington's disease patient (**A**) and a control subject (**B**).

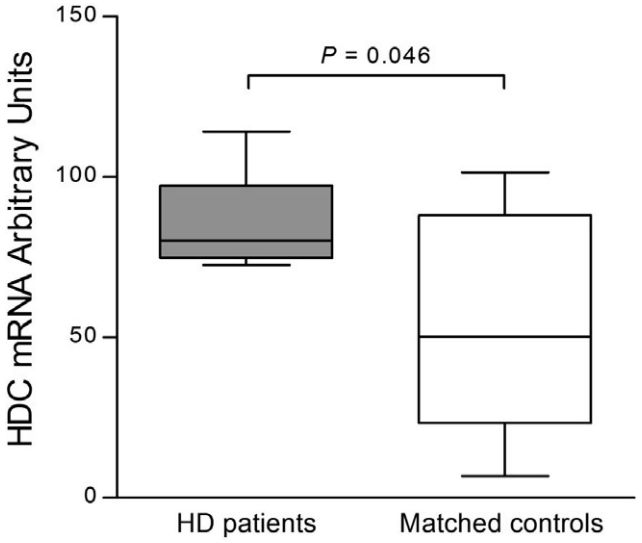

Figure 2. Expression of L-histidine decarboxylase (HDC) in the tuberomamillary nucleus (TMN) using quantitative radioactive *in situ* hybridization. In Huntington's disease (HD) patients (n = 8), HDC expression in arbitrary units was increased compared with matched control subjects (n = 8; $P = 0.046$). Error bars represent minimum and maximum values.

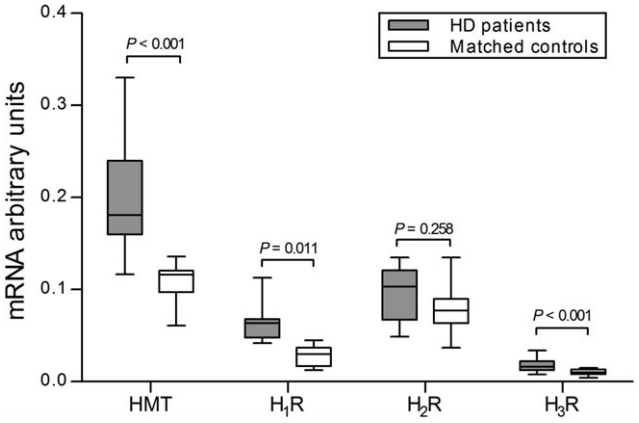

Figure 4. mRNA levels of histamine N-methyl transferase (HMT), histamine 1 (H_1R), histamine 2 (H_2R) and histamine 3 receptor (H_3R) in the inferior frontal gyrus. In Huntington's disease (HD) patients (n = 9), the expression of HMT, H_1R and H_3R in the inferior frontal gyrus was significantly increased compared with matched control subjects (n = 9; $P < 0.001$, $P < 0.001$ and $P = 0.011$, respectively). H_2R mRNA expression was unchanged ($P = 0.258$). Error bars represent minimum and maximum values.

IFG

Compared with controls, HMT-mRNA expression was significantly higher in HD patients ($P < 0.001$). H_1R-mRNA and H_3R-mRNA levels were also increased in HD patients ($P < 0.001$ and $P = 0.011$, respectively). H_2R-mRNA expression was, however, unchanged ($P = 0.258$) (Figure 4).

In HD patients, CAG repeat length showed a significant positive correlation with H_3R-mRNA levels ($\rho = 0.736$, $P = 0.024$), but not with H_1R-mRNA, H2R-mRNA or HMT-mRNA levels (all $P \geq 0.458$). In addition, significant negative correlations were observed in HD patients between the age at onset of disease and HMT-mRNA ($\rho = -0.810$, $P = 0.015$) and H_1R-mRNA ($\rho = -0.786$, $P = 0.021$) levels.

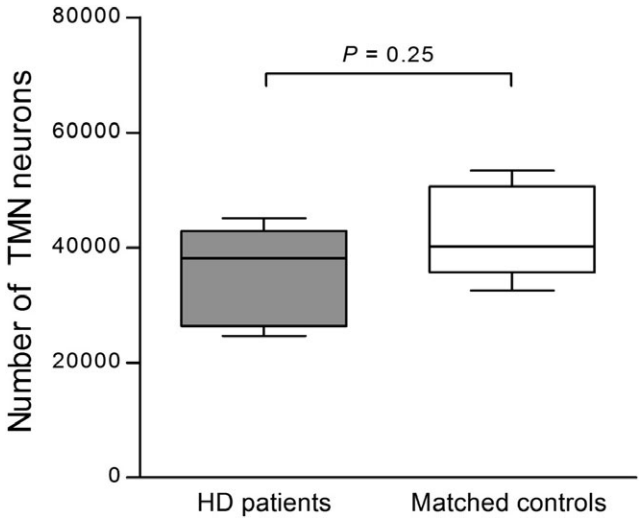

Figure 3. Estimated total number of tuberomamillary nucleus (TMN) neurons. In Huntington's disease (HD) patients (n = 8), the number of TMN neurons was equal to matched control subjects (n = 8) ($P = 0.25$). Error bars represent minimum and maximum values.

CN

In HD patients, H_2R-mRNA and H_3R-mRNA levels were significantly decreased ($P = 0.041$ and $P = 0.009$, respectively; Figure 4). HMT-mRNA and H_1R-mRNA levels, on the other hand, were unchanged in HD patients ($P = 0.937$ and $P = 0.699$, respectively; Figure 5).

No significant correlations were observed among the CAG repeat length, disease duration, Vonsattel grades, mRNA levels of any of the histamine receptors or HMT expression ($P \geq 0.172$).

DISCUSSION

To our knowledge, the present study is the first to demonstrate region-specific changes of the neuronal histaminergic system in HD. HDC-mRNA expression in the TMN, a marker for histamine production, was found to be significantly increased in HD patients. Moreover, increased histamine H_1, H_3 receptor-mRNA levels, as well as HMT-mRNA expression were observed in the IFG. In contrast, a significant decrease of histamine H_2 and H_3 receptor-mRNA expression was found in the CN. A significant positive correlation was observed between CAG repeat length and IFG H_3R-mRNA levels, while obvious negative correlations were found between IFG HMT-mRNA and H_1R-mRNA levels and age at onset of HD. These findings indicate a relationship between the histaminergic system changes and the HD disease process.

Several clues hint at potential functional consequences of histamine over-expression in HD patients. Firstly, because histamine is a

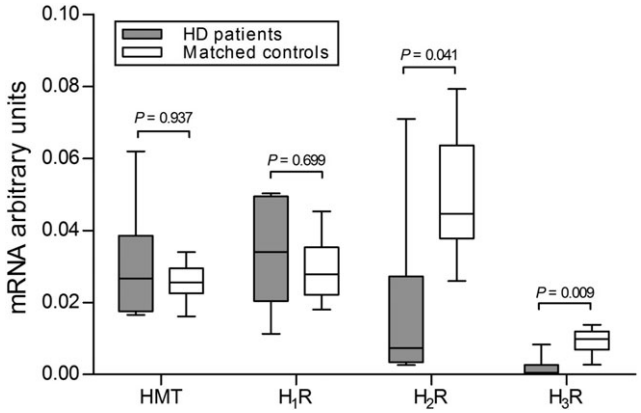

Figure 5. mRNA levels of histamine N-methyl transferase (HMT), histamine 1 (H_1R), histamine 2 (H_2R) and histamine 3 receptor (H_3R) in the caudate nucleus. In Huntington's disease (HD) patients (n = 6), the expression of H_2R and H_3R mRNA in the caudate nucleus was significantly decreased compared with matched control subjects (n = 6; $P = 0.041$ and $P = 0.009$). HMT and H_1R were unchanged ($P = 0.937$ and $P = 0.699$). Error bars represent minimum and maximum values.

wake-promoting neurotransmitter whose levels drop during sleep (10), an increase in histamine production might be responsible, at least partly, for a disruption of the sleep–wake cycle in HD patients (6). The observation that histamine can shift the phase of the biological clock that is generated by the hypothalamic suprachiasmatic nucleus (18) further supports this idea, which implies that an altered histamine production in HD may confer the circadian rhythm disturbances in this disorder (24). Secondly, histamine is a major anorexigenic neurotransmitter (18); hence, changes in histamine levels might underlie the unintended weight loss in HD patients (6). Thirdly, brain histamine is involved in many aspects of cognition, for example, attention (18). An increase in brain histaminergic signaling in HD patients may therefore contribute to cognitive decline, one of the key features of HD.

One can only speculate about the mechanism of the histaminergic system activation in the IFG in HD. A recent report demonstrated the co-aggregation and sequestration of two important transcription factors (Brn-2 and Arnt2) in the hypothalamic supraoptic and paraventricular nuclei of HD transgenic mice (38). As we recently showed that among all the hypothalamic nuclei the TMN contained the highest frequency of both nuclear and cytoplasmic inclusions of mutant huntingtin (5), a potential explanation for our findings may be the interference of mutant huntingtin with the transcriptional regulation of genes involved in the brain histaminergic pathway. However, such interference would decrease rather than increase HDC expression in the TMN. The number of lateral hypothalamic neurons producing orexin is modestly decreased in HD patients (5, 25). Because orexin is known to be a stimulator of histamine production (13), the activation of the histaminergic system in HD cannot be explained this way. The same holds for cortisol that is assumed to inhibit HDC expression (18, 21), while it is increased in early-stage HD patients (7). Therefore, these neuroendocrine alterations are unlikely to account for the activation of the brain histaminergic system. On the other hand, there are no good clinical data available on cortisol levels in end-stage HD patients.

The differential increase in IFG histamine receptor subtype function is likely to have distinct clinical consequences in HD patients. The signal transduction of H_1R, H_2R and H_3R is conducted through different G protein isoforms, by which a variety of downstream proteins are triggered. H_3R, for instance, is negatively coupled to Gi/o proteins and to adenylyl cyclase, while H_2R is coupled to Gsα proteins to stimulate adenylyl cyclase (18). H_1 receptors are involved in learning and memory performance and sleep–wake cycle control. Moreover, stimulation of this receptor seems to increase neuronal activity at a cellular level. H_3 receptors have two main roles. In its role of autoreceptor, the H_3 receptor can regulate the release of histamine in the TMN, while in its role as heteroreceptor, it can directly influence the release of several neurotransmitters such as dopamine and serotonin. Involvement of all three histamine receptors in cognition, emotion, learning and memory have been reported (12, 18). The increase in IFG H_1 and H_3 receptors may be associated with disturbances of sleep–wake cycle and memory in HD patients (6). Moreover, the overexpression of H_3R in the IFG could have a role in disturbed regulation of several neurotransmitters, including dopamine, which is altered to a various degree in both the striatum and cortex of HD patients (3).

The observed decrease in CN H_2R-mRNA and H_3R-mRNA levels is most likely caused by an overall neuronal degeneration and decrease in mRNA expression in this brain area (19, 20). HMT-mRNA and H_1R-mRNA, on the other hand, were unchanged, possibly because of their glial localization, whereas H_2R and H_3R are mostly located in neurons (18). Glial cells appeared to be relatively intact in the CN of our cohort of HD patients as GAFP was unchanged compared with control subjects, in line with previous findings (20). The disequilibrium in CN histamine receptors may play a role in striatal neuronal death (33). Rodent experiments have shown that histamine injections into the substantia nigra (SN) causes inflammation and activation of microglia, which in turn can lead to degeneration of dopaminergic neurons in the SN (29, 33). Other *in vivo* studies have shown that inhibition of endogenous histamine production in rodent models of early-stage Parkinson's disease rescues tyrosine hydroxylase neurons in the SN (21). These data support the neurotoxic role of histamine in pathological changes, suggesting a role for an increase in histaminergic signaling in neuronal cell death in HD patients. Based upon the same observations in Parkinson's disease models, one may presume that H_1R-mRNA, which is upregulated in the IFG of our cohort of HD patients, may be involved in neuronal degeneration (21). The upregulation of HMT-mRNA in the IFG may thus, be interpreted as a protective mechanism to prevent neuronal death in the IFG. The latter would also fit with previous studies in which it was shown that neuronal loss in the frontal cortex is only modest (21% to 29%) compared with the CN (57%), and that the number of glial cells in the cerebral cortex is largely unaffected in HD (11, 30).

In line with our findings of a functional increase in brain histaminergic signaling in HD, are observations by Whitehouse *et al* who demonstrated an increase in the level of H_1 receptors in the cerebral neocortex in HD patients compared with age- and sex-matched controls (37). Levels of histamine metabolites in cerebrospinal fluid have also been shown to be increased in HD patients compared with age-matched controls (28), indicating that the enhanced activity of the histaminergic system in HD is not limited to the mRNA level. We found increases in H_2 and H_3 receptor

mRNA levels in the IFG, but others have reported decreases in binding of both receptors in many brain regions using autoradiography (17, 23). Several confounding factors may account for this discrepancy. In both previous reports, the HD and control subjects were not matched for age and sex, even though the importance of matching was recognized for the rate of histamine production (31). Furthermore, in the report of Martínez-Mir *et al* (23) post-mortem delay in HD patients (range 17.5–42.8 h) was considerably longer than in their control subjects (range 2.0–22.5 h). Hence, in previous reports, these confounding factors together may have caused an underestimation of histamine signaling in HD patients.

Potential limitations of our study include the use of brain material from end-stage HD patients and the use of qPCR on samples from the CN, which is severely affected in HD patients (20). Based on previous reports, however, we identified three genes with stable expression in the CN of HD patients which served as reference genes and were therefore taken to justify the use of qPCR on CN samples (20). Two of the HD patients used in our analysis were legally euthanized, which might imply a less advanced stage of disease compared with the other HD patients. The HDC results of these two patients, however, were 74 and 114 AU, respectively, that is around the mean for HD patients (86 AU). In addition, disease duration was 9 and 11 years, respectively, while the average disease duration in all HD patients was 14 years (range 7–25 years). Thus, the findings in these two HD patients are unlikely to have influenced our findings.

In conclusion, we found an increase in HDC mRNA levels in the TMN, an increase in HMT, H_1R and H_3R mRNA levels in the IFG, and a decrease in H_2R and H_3R mRNA levels, with unchanged mRNA levels of HMT and H_1R in the CN of HD patients. These findings suggest a functional increase of brain histaminergic signaling which may contribute to sleep disturbances, weight loss and neuronal loss in HD patients. Moreover, considering the pivotal role of the histaminergic signaling in cognition, an increase in brain histaminergic signaling in HD may contribute to cognitive decline in this disease. Our findings provide a rationale for the use of histamine receptor inverse agonists in HD patients.

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