The human histaminergic system in health and neuropsychiatric disorders
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Chapter 9

Increased expression of histamine methyltransferase-mRNA and astrocyte markers in the prefrontal cortex in schizophrenia

Ling Shan, Unga Unmehopa, Ai-Min Bao and Dick F. Swaab
Manuscript in preparation
Abstract

It is known that supplementation of histamine may have favorable therapeutic effects in schizophrenic patients. The present study investigates the possible histaminergic changes in the postmortem superior frontal gyrus (SFG) in schizophrenia. We used quantitative real time polymerase chain reaction (qPCR) to determine the mRNA levels of (i) the four major G protein-coupled histamine receptors HRs (H_{1A-R}), (ii) the enzyme involved in histamine metabolism, i.e. histamine methyltransferase (HMT) and. Because several aspects of the function of the histaminergic system are presumed to be dependent on glia cells, we also determined the mRNA-expression of several glia markers for astrocytes, oligodendrocytes or microglia. We found that the HMT-mRNA levels were significantly (P = 0.025) up-regulated in the SFG of schizophrenic patients, together with a trend (P = 0.065) of approximately 1.7-fold elevation of the histamine releasing factor histamine release factor (HRF)-mRNA expression. In addition, an increased expression of the astrocytemarkers, i.e. glial fibrillary acidic protein (GFAP)-, aldehyde dehydrogenase (ALDH1)- and vimentin (VIM)-mRNA were observed in the SFG of schizophrenic patients. HMT appeared to be expressed in the human SFG exclusively in neurons, and not in astrocytes. Our data imply an increased release and turnover of histamine in the PFC of schizophrenic patients.

Key words : Schizophrenia, Histamine, qPCR, Superior frontal gyrus, Astrocytes

Introduction

A core feature of schizophrenia is impairment of cognitive functions (Lewis and Lieberman, 2000). In 1938 it was found that subcutaneous injections of histamine may result in favorable therapeutic responses in schizophrenic patients (Heleniak and O’Desky, 1999), which led to the supposition that the histaminergic system is involved in the pathophysiology of this disorder (Tandon, 1999).

Neuronal histamine is exclusively produced in the tuberomamillary nucleus (TMN), located in the posterior hypothalamus by key enzyme histidine decarboxylase (HDC). The TMN projects to a large number of brain areas (Haas and Panula, 2003), including the human prefrontal cortex (PFC), which contains well-organized histamine-positive fibers (Panula et al., 1990). The brain histaminergic system is involved in quite a number of functions, such as attention and cognition, which are disturbed in schizophrenic patients (Lewis and Lieberman, 2000). There are a few observations indicating alterations in the histaminergic system in schizophrenia. Both positron emission tomography studies and postmortem brain sample studies show decreased histamine receptor-1 (H_{1R}) binding in the frontal and cingulate cortex of schizophrenic patients (Iwabuchi et al., 2005, Nakai et al., 1991). In addition, in several open-label clinical trials, famotidine, an H_{2R}-antagonist, was shown to have antipsychotic effects and to reduce negative symptoms in schizophrenia (Rosse et al., 1996, Kaminsky et al., 1990, Oyewumi et al., 1994). In post-mortem human brain, increased H_{3R} radioligand-binding was found in the dorsolateral PFC of schizophrenic patients (Jin et al., 2009). Moreover, elevated levels of tele-methylhistamine (t-MH), the major histamine metabolite, were found in cerebrospinal fluid (CSF) in patients with chronic schizophrenia, indicating increased central histaminergic activity (Prell et al., 1996, Prell et al., 1995). The present study aims at providing a more complete picture of the histaminergic terminating changes in the postmortem superior frontal gyrus (SFG) in schizophrenia. We used quantitative real time polymerase chain reaction (qPCR) to deter-
mine the mRNA levels of (i) the four major G protein-coupled HRs (H₁-₄R) (Haas et al., 2008), (ii) the enzyme involved in histamine metabolism, histamine methyltransferase (HMT) and (iii) the newly identified histamine releasing factor (HRF) that induces histamine release from human mast cells and basophilic leucocytes (Langdon et al., 2008). Because several aspects of the functioning of the histaminergic system are thought to be dependent on glia cells, we also determined the mRNA-expression of several glia markers, namely glial fibrillary acidic protein (GFAP), aldehyde dehydrogenase (ALDH1) and vimentin (VIM) as astrocyte markers (Jing et al., 2007, Pekny and Pekna, 2004, Eng et al., 2000), proteolipid protein (PLP) as an oligodendrocyte marker (Duncan et al., 1987, Lees et al., 1984), and cluster of differentiation molecule 11β (CD11b), cluster of differentiation molecule 68 (CD68) and homo sapiens major histocompatibility complex, class II, DR alpha (HLA-DR) as microglia markers (Koning et al., 2007). Furthermore, in order to see whether HMT up regulation is due to the in astrocytes activation we reported, we performed in-situ hybridization of HMT-mRNA in combination with immunocytochemistry of glial fibrillary acidic protein (GFAP), the marker of astrocytes.

Materials and methods

Postmortem brain material

All postmortem tissues were obtained through the Netherlands Brain Bank (NBB), all with prior permission from the patient or next of kin for brain autopsy and for the use of the brain material and clinical data for research purposes. Freshly frozen SFG tissue samples from 7 clinically diagnosed schizophrenia patients were studied with 7 well-matched controls free from neuropsychiatric disorders and neuropathological alterations. The Braak stages indicating the progress of Alzheimer’s Disease (Braak and Braak, 1991) were determined by systematic neuropathological analyses as described before (van de Nes et al., 1998). For clinico-pathological information, see Table 1. A qualified psychiatrist ensured adherence to the criteria for the presence, duration and severity of the symptoms of schizophrenia, as well as warranting the systematic exclusion of other psychiatric and neurological disorders, all according to the DSM-IV.

qPCR study in the SFG tissue

Details of dissection of the grey matter from the snap-frozen postmortem SFG tissue, subsequent RNA isolation, cDNA synthesis and qPCR procedures have been described in our previous studies(Shan et al., 2012). Briefly: RNA extracted from the frozen tissue was of high integrity (RNA integrity number RIN: mean 7.7, range 6.2-8.9), which was well within the quality requirements for qPCR analysis (Fleige and Pfaffl, 2006). qPCR was performed in a reaction volume of 20 µl, using the SYBR Green PCR kit (Applied Biosystems, California, USA) and a mixture of sense and antisense primers (2 pmol/µl). For detailed primer information see Table 2. Reactions were run in a GeneAmp 7300 thermocycler under the following conditions: 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 sec at 95 °C and finally 1 min at 60 °C. Data were acquired and processed automatically by the Applied Biosystems Sequence Detection Software. Specificity of amplification was checked by means of melting curve analysis and electrophoresis of products on an 8% polyacrylamide gel. Sterile water (non-template control) and omission of reverse transcriptase (non-RT control) during cDNA synthesis served as negative controls. Amplification efficiency was determined by running qPCR reactions on a dilution series of pooled cDNA from all the subjects. The resulting cycle threshold (Ct) values were plotted against the inverse log of each dilution and the slope of this curve was then used to calculate the efficiency as follows: efficiency (E) = 10^(-1/slope). The normalization factor was based upon the geometric mean of the following 4 - out of 9 - potential reference genes selected by geNorm analysis (Vandesompele et al., 2002): i.e. KLHDC5 (Homo sapiens kelch domain containing 5), EF1alpha (Homo sapiens eukaryotic translation elongation factor 1 alpha), GAPDH (Glyceraldehyde-3-phosphate dehydrogenase),
<table>
<thead>
<tr>
<th>NBB no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>DD (Years)</th>
<th>pH CSF</th>
<th>PMI (hours)</th>
<th>CTD (hours)</th>
<th>Braak AD</th>
<th>RIN</th>
<th>Subtype of Schizophrenia</th>
<th>Cause of death; Medication</th>
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<td>04-040*</td>
<td>76</td>
<td>F</td>
<td>37</td>
<td>6,5</td>
<td>4:40</td>
<td>3:55</td>
<td>1</td>
<td>7</td>
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<td>Liver failure; Temazepam, Zuclopentixol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pancreas carcinoma; Morphine, Nitrázepam, Oxazepam, Olanzapine</td>
</tr>
<tr>
<td>05-046*</td>
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<td>F</td>
<td>31</td>
<td>7,24</td>
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<td>3:30</td>
<td>0</td>
<td>8,9</td>
<td>Schizophrenia, depression</td>
<td>Schizophrenia with paranoide manic depression episodes, dementia</td>
</tr>
<tr>
<td>93-143*</td>
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<td>10:20</td>
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<td>Pneumonia; Haloperidol, Clonazepam</td>
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<tr>
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<td>2</td>
<td>6,12</td>
<td>5:20</td>
<td>4:00</td>
<td>4</td>
<td>6,7</td>
<td>Schizophrenia with paranoide manic depression episodes</td>
<td>Cachexia; Oxazepam, Morphine</td>
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<tr>
<td>10-021</td>
<td>59</td>
<td>M</td>
<td>5,93</td>
<td>12:30</td>
<td>22:00</td>
<td></td>
<td>6,3</td>
<td></td>
<td>Schizophrenia paranoid type</td>
<td>Schizophrenia with paranoide manic depression episodes</td>
</tr>
<tr>
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<td>68</td>
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<td>10</td>
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<td>Paranoid catatonic schizophrenia</td>
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<td>8,3</td>
<td>Schizophrenia with paranoide manic depression episodes</td>
<td>Lärinxcarcinoma; Morphine, Dormicum, Nozinan, Temazepam</td>
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<tr>
<td>Median</td>
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<td></td>
<td>7</td>
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<td>2:52</td>
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<td>Control</td>
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<td>8,8</td>
<td>6,3</td>
<td>1,3</td>
<td>7,7</td>
<td></td>
<td></td>
<td>Congestive cardial failure; Morphine</td>
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<td>8,8</td>
<td></td>
<td></td>
<td>Cachexia and dehydration; Haloperidol, Fentanyl</td>
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<tr>
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<td>F</td>
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<td>7:20</td>
<td>3</td>
<td>3</td>
<td>6,3</td>
<td></td>
<td></td>
<td>Cardial decompensation and emphysema; Prednisolon, Oxazepam</td>
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<tr>
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<td>9:10</td>
<td>13:50</td>
<td>1</td>
<td>7,7</td>
<td></td>
<td></td>
<td>Acute renal failure; Morphine,</td>
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<tr>
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<td>56</td>
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<td>8,7</td>
<td></td>
<td></td>
<td>Myocardial infarction; Oxazepam</td>
</tr>
<tr>
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<td>71</td>
<td>M</td>
<td>6,54</td>
<td>18:45</td>
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<td>7,9</td>
<td>7,6</td>
<td></td>
<td></td>
<td>Coloncarcinoma with livermetastases; Morphine</td>
</tr>
<tr>
<td>Median</td>
<td>72</td>
<td></td>
<td></td>
<td>6,45</td>
<td>7:20</td>
<td>11:22</td>
<td></td>
<td>7,9</td>
<td></td>
<td>Respiratory insufficiency; Morphine, Lorazepam, Clonidine, Pentobarbital</td>
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<tr>
<td>SEM</td>
<td>4,6</td>
<td></td>
<td></td>
<td>0,07</td>
<td>0,37</td>
<td>2:12</td>
<td></td>
<td>0,32</td>
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</table>

Note: Braak stage for AD: progression of pathological changes in Alzheimer disease was according to (Braak et al., 1993). P value: Differences between two groups were tested using the Mann-Whitney U-test.

BW = Brain weight, CTD = Clock time at death, F= female, FT= fixation time in days, M= male, MON= month of death, NBB = Netherlands Brain Bank number, ND= no data, PMD= post-mortem delay, SEM.= Standard error mean.
ERBP (deoxynucleotidyltransferase, terminal, interacting protein 2). The relative absolute amount of target genes was calculated with the use of the following formula: \(10^{\text{delta} \times \text{E}^{-\text{delta}}}\). The relative mRNA expression of the target gene was obtained by dividing the calculated absolute amount of transcript by the normalization factor.

**HMT-mRNA in situ hybridization followed by immunocytochemical staining of GFAP**

Since both GFAP and HMT-mRNA were up-regulated in schizophrenia (see results), and glial cells were presumed to contribute to histamine inactivation in the brain (Huszti et al., 1990), we performed a combination of HMT-mRNA in situ hybridization and GFAP immunocytochemistry on formalin-fixed paraffin-embedded 6 µm-sections of PFC tissues from the same subjects, provided the relevant tissues were available (6 schizophrenia subjects and 3 control see Table 1), in order to determine the possible location of HMT-mRNA in astrocytes.

**In situ hybridization for HMT-mRNA in the PFC tissue**

For in situ hybridization of HMT-mRNA we used a novel Locked Nucleic Acid (l) and 2’-O-methylRNA (m) modified oligonucleotide, 5’-labeled with fluorescein (FAM5’) (Ribotask, Odense, Denmark) probe that was complementary to base pair 179-197 of the human HMT-mRNA sequence (Genbank NM_001024074.2: FAM5’-lTmUmCl-CmCmAlGmUmUIcMClTmCmUlTmUm-CIT-3’). In addition, we used an HMT-mRNA sense probe as a negative control (FAM5’-lAmG-mAlAmAmGmGmGlGmAmAlCmUmGmGlGmAlA-3’). PFC sections were collected and mounted on Super frost double Plus slides (Merk, Darmstadt, Germany) using a water bath set at 48°C. After mounting they were dried over 2
nights in a stove at 37°C. The sections were deparaffinized in xylene for 10 min, then cleared for another 10 min in fresh xylene. Subsequently, the tissue was rehydrated in a graded ethanol series (2x 5 min in 100% ethanol, 1 min in each 96%, 80% and 60% ethanol) and washed twice for 2 min in PBS. Sections were washed for 5 min in 0.01M citrate buffer pH 6.0 and subsequently microwaved in 0.01M citrate buffer pH 6.0 for 2x 5 min at full power (700W). Sections were allowed to cool down to room temperature for 30 minutes and were subsequently washed in PBS for 2x 5min and deproteinated as follows: the sections were incubated in 0.2 N HCl for 20 min, washed twice in PBS for 5 min and deproteinized for 15 min in a solution containing proteinase K (Invitrogen, Carlsbad, CA, USA) (5 ug/ml) in 1x prot K buffer (2 mM CaCl2, 10 mM Tris/HCl, pH 7.5). To stop the reaction, the sections were incubated for 30 sec in glycin buffer (26.6mM glycin in PBS) and washed 2x 5 min in PBS. Lipids were removed from the tissue by a 10 min incubation in PBS-Triton (PBS containing 0.1% (v/v) Triton X-100 (Sigma)) and a subsequent 2x 5 min rinse in PBS. Sections were prehybridized in 200μl HBF overnight while covered with Nescofilm at room temperature in a humidified chamber. The next day the probe was diluted in HBF to a final concentration of 50nM, denatured at 95°C for 5 min and cooled on ice. Sections were hybridized in hybridization mix at 57°C for 90 min, washed for 5 min in 5xSSC at 57°C, 5 min in 2x SSC at57°C, 5 min 0.2xSSC at 57°C and 5 min in PBS at room temperature, and pre-incubated with TBS-milk (1% non-fat dry milk (Elk, Campina) in TBS, pH 7.6) for 1hr at room temperature, followed by a 3hr incubation with sheep IgG, anti-fluorescein-Fab fragments coupled to alkaline phosphatase 1:3000 (Roche, Mannheim, Germany) in Supermix-milk (SUMI-milk: 0.25% gelatin (Merck, Darmstadt, Germany) (w/v), 0.5% Triton X-100 (v/v) in TBS-milk, pH 7.6). Next, the slides were washed 2x 5 minutes with 0.1M Tris, 150mM NaCl buffer pH 7.5, then, after a prewash in 100mM Tris-HCl pH 9.5, 100mM NaCl, 5mM MgCl₂, the sections were developed in NBT (Sigma, Steinheim, Germany)-BCIP (Roche, Mannheim, Germany) coloring solution (337.5 μg/ml Nitro Blue Tetrazolium Chloride, 175.4μg/ml 5-Bromo-4-Chloro-3-indolyl phosphate, toluidine salt, 240μg/ml Levamisole in 100mM Tris-HCl pH 9.5, 100mM NaCl, 5mM MgCl₂) for approximately 25 minutes at room temperature under dark conditions. Sections were then washed in aqua dest, rinsed in 100% Methanol (Sigma, Steinheim, Germany) for 5 minutes and washed again in aqua dest. Finally, the sections were cover slipped with Mowiol (10% (w/v) Mowiol 4-88 (Calbiochem, Merck, Darmstadt, Germany) in 0.1M Tris-HCl pH 8.5, 25% (v/v) glycerol) aqueous mounting medium and stored in a lighttight box at 4°C.

**Immunocytochemical staining of GFAP**

Sections that were double-labeled for HMT-mRNA and GFAP were first hybridized in situ as described above for HMT-mRNA and subsequently immunocytochemically stained for GFAP. After the in situ hybridization procedure, sections were processed as follows: Following methanol treatment and rinsing in aqua dest, sections were washed in TBS for 2x5 minutes. Subsequently, sections were pre-incubated in TBS-1.25% milk (w/v, Elk, Campina, Woerden, The Netherlands) for 45 minutes at room temperature and then incubated in rabbit IgG against pan-GFAP (DAKO, Glostrup, Denmark) at 1:1000 dilution in Supermix-2.5% (w/v) milk for 1 hour at room temperature, followed by an overnight incubation at 4°C in a moist chamber. The next day, sections were washed in TBS for 20 minutes and then incubated in biotinylated goat anti-rabbit IgG, diluted 1:400 in Supermix for 1 hour at room temperature. After washes in TBS for 20 minutes, sections were incubated in ABC complex (Vector Laboratories, Burlingame, CA) for 1 hour. After 2 washes in TBS, sections were equilibrated for 5 min in acetic acid buffer (29.6 mM acetic acid, 70.4 mM sodium acetate, pH 5.0) and then developed in AEC solution (0.05% (w/v) 3-amino-9-ethylcarbazole in acetic acid buffer, 0.015% H₂O₂) for approximately 15 minutes, washed in TBS and finally cover slipped in Mowiol.
Statistical analysis

Since the distribution of data did not always follow the normal distribution pattern, the differences between the groups were statistically evaluated by the nonparametric Mann-Whitney U-test. Correlations were tested by Spearman’s correlation coefficient. Intergroup differences in clock time and month of death were evaluated using the Mardia-Watson-Wheeler test. To screen for potential confounding factors, Spearman’s rank-order correlations were carried out for age, sex, post-mortem interval (PMI) and clinical data versus all variables (Table 1). Those showing significant correlations were retained as covariates in group comparisons (Multivariate/Univariate ANOVA). Tests were two-tailed and values of P < 0.05 were considered to be significant. Percentage-changes of mRNA levels were calculated using the mean values.

Results

Possible confounders

Control subjects did not differ from schizophrenia patients with regard to sex (P = 1.00), age (P = 0.653), clock time and month of death (P = 0.103 and P = 0.969, respectively), post-mortem interval (P = 0.337), Braak stages of Alzheimer’s disease (P = 0.876) and RIN values (P = 0.481) (Table 1).

Alterations of histaminergic system in the SFG

A significant (P = 0.025) 2.1-fold increase in HMT-mRNA expression was found in schizophrenia patients, together with a trend (1.7-fold, P = 0.064) towards an increase in HRF-mRNA levels (Figure 2). Interestingly, age was observed to be positively correlated with HRF- (rho = 0.893, P = 0.007) and H1R-mRNA levels (rho = 0.857, P = 0.014) in the control subjects, but not in the schizophrenia patients. No significant differences were found between schizophrenic patients and control subjects in H1-R-mRNA levels (P ≥ 0.110). A very low expression of HDC-mRNA signal Ct (cycle of threshold) value (33 ± 0.45 median ± standard error of the mean) was observed in schizophrenic patients as well as in controls (P = 0.749), showing that the local histamine production is negligible.

Increased astrocyte marker gene expression in the SFG

The expression of the astrocyte-associated genes GFAP, ALDH1 and VIM was significantly increased in schizophrenic patients (P = 0.025, 0.048, and 0.048, respectively, Table 3). In the same brain area, oligodendrocyte-associated genes, PLP (P = 0.565), and microglia-associated genes, CD11b (P = 0.277), HLA-DR and CD68 (P = 0.482 and 0.338 respectively) did not show significant changes (Table 3). In addition, significant positive correlations were found between HRF and glia markers tested for mRNA such as GFAP (P = 0.023), with ALDH1 (P = 0.003) and VIM (P = 0.003), in schizophrenic patients (Table 4), while no significant correlations were observed in control subjects (P ≥ 0.253, Table 4).

Table 3 Glia associated gene expression in patients with Schizophrenia (n = 7) and Controls (n = 7)

<table>
<thead>
<tr>
<th></th>
<th>Astrocyte markers</th>
<th>Oligodendrocyte marker</th>
<th>Microglia associated genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GFAP</td>
<td>ALDH1</td>
<td>VIM</td>
</tr>
<tr>
<td>Control</td>
<td>2.87</td>
<td>0.04</td>
<td>0.23</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>10.38</td>
<td>0.18</td>
<td>0.33</td>
</tr>
<tr>
<td>M-W test P-value</td>
<td>0.025*</td>
<td>0.048*</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

M-W, Mann-Whitney U-test; all values are Median, Two-way analysis of variance, P-value, * P-value < 0.05.
ALDH1, aldehyde dehydrogenase; CD11b, cluster of differentiation molecule 11β; CD68, cluster of differentiation molecule 68; GFAP, glial fibrillary acidic protein; PLP, proteolipid protein; HLA-DR, Homo sapiens major histocompatibility complex, class II, DR alpha.
Chapter 9

Specificity of HMT-mRNA in situ hybridization in the SFG

The specificity of HMT-mRNA in situ hybridization was supported by the exclusive localization of HMT-mRNA (blue staining) in neurons and GFAP staining (red) in astrocytes in the SFG (Figure 1A). In addition, it was supported by the failure of the sense probe to show a blue signal while the red GFAP staining was present (Figure 1B). Higher intensity of the HMT-mRNA signals was observed in the deep layers of the SFG. No co-localization of HMT-mRNA and GFAP in the PFC was found to be homogeneously distributed over the PFC but absent in neurons (Figure 3). The HMT-mRNA in situ hybridization signal (blue) labeling was only observed in neurons of the same probe to show a blue signal while the red GFAP staining was present (Figure 1B).

Table 4 Summary of the Correlations between histaminergic genes and glia markers

<table>
<thead>
<tr>
<th></th>
<th>Astrocyte markers</th>
<th>Oligodendrocyte marker</th>
<th>Microglia associated genes</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>GFAP</td>
<td>ALDH1</td>
<td>VIM</td>
</tr>
<tr>
<td>HMT</td>
<td>0.000, 1.000</td>
<td>0.429, 0.337</td>
<td>-0.214, 0.645</td>
</tr>
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<td>HRF</td>
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<td>0.536, 0.215</td>
<td>0.250, 0.589</td>
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<td>H2R</td>
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<td>-0.357, 0.432</td>
<td>0.179, 0.702</td>
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<tr>
<td>H3R</td>
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<td>-0.357, 0.432</td>
<td>0.179, 0.702</td>
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<td>H4R</td>
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<td>0.143, 0.760</td>
<td>-0.643, 0.119</td>
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</table>

Note: Spearman's rank-order correlations; Rho value, P value; * < 0.05, ** < 0.01
ALDH1, aldehyde dehydrogenase; CD11b, cluster of differentiation molecule 11β; CD68, cluster of differentiation molecule 68; GFAP, glial fibrillary acidic protein; H1-4R, histamine 1-4 receptor; HMT, histamine-N-methyltransferase; HRF, histamine release factor; PLP, proteolipid protein; HLA-DR, Homo sapiens major histocompatibility complex, class II, DR alpha
rons, and thus no co-localization of HMT-mRNA and GFAP-immunoreactivity was present in the PFC (Figure 3).

**Figure 1.**
Boxplot showing the median, 25th-75th percentiles and the range of the HMT-mRNA expression (A) and HRF-mRNA expression (B). Note that the expression of HMT-mRNA levels is significantly higher in schizophrenic patients (P = 0.025). The HRF-mRNA expression shows a trend to be higher in schizophrenia (P = 0.064); P value is from the Mann-Whitney U test.

**Discussion**

The present study showed, to our best knowledge for the first time, that the histamine inactivating enzyme HMT-mRNA is significantly up-regulated, together with a clear trend of HRF-mRNA elevation in the SFG of schizophrenic patients. In addition, the expression levels of the astrocyte-associated genes, GFAP, ALDH1 and VIM, were significantly increased in the SFG of schizophrenia. Moreover, HRF-mRNA was positively correlated with the expression of astrocyte-associated genes. Furthermore, we observed that HMT-mRNA was absent from astrocytes, but expressed exclusively in neurons. Therefore, the HMT-mRNA up regulation cannot be explained simply by the observed astrocyte activation in schizophrenic patients. Our current study implies an increased release and turnover of histamine in the PFC of schizophrenic patients.

The SFG not only plays an important role in self-awareness (Goldberg et al., 2006), but also in mediating impulse-control (Goldstein and Volkow, 2002, Rose et al., 2011), the dysfunction of which ability has been implicated in the pathophysiology of schizophrenia (Smee et al., 2011). Consistent with our finding that the expression of HMT-mRNA was significantly increased and that HRF-mRNA showed a trend in the same direction, the

**Figure 2.**
Glial fibrillary acidic protein (GFAP) immnocytochemistry and histamine methyltransferase (HMT)-mRNA in situ hybridization in the human prefrontal cortex (PFC). (A) Specific HMT-mRNA in situ hybridization signal (blue) was observed after hybridization with HMT anti-sense probe in the PFC of the schizophrenic patient NBB #05-046 with GFAP (red) double staining. Inset shows detail of the HMT-mRNA neuronal staining and astroglial GFAP staining. (B) Absence of blue staining after hybridization with sense probe in adjacent section from same subjects. Scale bar = 5 μm.
main histamine metabolite t-MeHA was reported to be 2.6-fold elevated in lumbar puncture CSF of schizophrenic patients (Prell et al., 1995).

We observed a trend towards an increase of HRF-mRNA levels in the SFG of schizophrenic patients. Moreover, a positive correlation between HRF-mRNA and age was observed in controls. HRF was first discovered as the translationally controlled tumor protein in Ehrlich ascites tumor cells and MacDonald characterized it as a histamine-releasing factor (see review in (Telerman and Amson, 2009)). Since downregulation of HRF protein expression was observed in temporal cortex of Alzheimer’s disease and Down’s syndrome, it has been proposed that reduced HRF may be associated with cognitive and memory deficits (Kim et al., 2001). It should be noted, however, that a previous study reported a down-regulation of HRF-mRNA (1.4–1.9 fold) in the hippocampus also of schizophrenic patients (Chung et al., 2003). In addition, although MacDonald showed that HRF induced histamine release from human mast cells and basophilic leucocytes (Langdon et al., 2008), it is at present far from clear whether this protein could also affect neuronal histamine release or is involved by a different mechanism. Recent molecular and functional studies suggest that HRF facilitated the GDP-GTP exchange of Ras homology enriched in brain (Rheb) (Dong et al., 2009, Choi and Hsu, 2007), which is critical for postnatal brain development (Zou et al., 2011).

The observed augmentation of the expression of astrocyte-associated genes, GFAP-, ALDH1-, VIM-mRNA, together with earlier reported increased expression of GFAP protein levels in the same brain area in schizophrenia (Toro et al., 2006) and of the – in astrocytes localized - S100B glutamate transporter in the PFC (Rothermundt et al., 2009, Nanitsos et al., 2005), suggest the presence of gliosis in schizophrenia (Schnieder and Dwork, 2011). It should also be noted that the patients in the present study had been on anti-psychotic medication. According to a postmortem study this may induce up-regulation of astrocyte-associated genes (Toro et al., 2006). However, Fatemi et al. and Konopaske et al. have systematically examined the chronic administration of such medication in rats and rhesus monkeys and found it reduced astrocyte markers such as GFAP or S100B at the protein level (Fatemi et al., 2008, Konopaske et al., 2008). Be that as it may, the up-regulation of astrocyte-associated genes in the present study is unlikely to be the result of administration of antipsychotic medication.

Although the H1-R-mRNA expression levels were not found to be changed in the SFG of schizophrenic patients in the present study, the increased local turnover of histamine might result in changes in HRs-occupancy. This may, at least partly,
explain the previous reports showing a lower H3R binding (Jin et al., 2009) and H1R binding (Iwabuchi et al., 2005, Nakai et al., 1991) in schizophrenia. Since specific and sensitive antibodies for the HRs are unavailable, their localization within the SFG remains at present unresolved. The H1-4R localizations and protein expression are topics that merit further investigation.

In summary, a significant up-regulation of HMT-mRNA and slightly elevated HRF-mRNA, together with a higher expression of astrocyte-associated genes were observed in the PFC of schizophrenic patients, which imply an increased release and turnover of histamine in this brain area. Their contribution to the cognitive impairment deserves further study.

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References
Lees MB, Samiullah M, Laursen RA. Structural analogies between myelin basic protein and proteolipid. Prog Clin Biol Res. 1984;146:257-64.


