The human histaminergic system in health and neuropsychiatric disorders
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Chapter 10

General Discussion

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Manuscript in preparation
Neuronal histamine is exclusively produced in the hypothalamic tuberomamillary nucleus (TMN) via the key enzyme histidine decarboxylase (HDC). These histaminergic TMN neurons project to a large number of brain areas (Haas et al., 2008) where they play important roles in basic physiological functions, such as the sleep-wake cycle, energy and endocrine homeostasis, sensory and motor functions, cognition, attention, and learning and memory (Haas et al., 2008).

For the current thesis, we introduced a number of alterations in an otherwise routine protocol in order to create optimal in situ hybridization conditions for quantification of the mRNA expression of HDC in formalin-fixed, paraffin-embedded archival postmortem human brain tissue. These alterations included (1) omitting glycogen as a carrier for precipitation during purification of the oligo-probes, (2) dissolving the labeled probe contained within the pellet after precipitation first in water instead of in hybridization buffer (HBF), (3) increasing the dithiothreitol (DTT) concentration during hybridization, and (4) increasing the stringencies during hybridization and post-hybridization washes by increasing the temperature (Chapter 2). It should be noted that this optimized protocol is still very sensitive to the quality of the radioactive label. Since the labels we obtain from the industry are often of low quality, we routinely tested them, prior to a large HDC experiment, for high abundant genes such as vasopressin.

Using the optimized quantitative radioactive in situ hybridization protocol, we investigated the neuronal histaminergic system in non-neuropsychiatric subjects in relation to age, sex, and day-night fluctuations, and in neuropsychiatric disorders including Parkinson’s disease, Alzheimer’s disease, Huntington’s disease, depression and schizophrenia.
1. Physiology of neuronal histaminergic system in brain

1.1. Age dependency

The function of the neuronal histaminergic system has been reported to be age-dependent. An age-related decline in histamine 1 receptor (H1R) binding in the normal human brain was reported by a positron emission tomography (PET) study, especially in the prefrontal, temporal, cingulate and parahippocampal regions (Yanai et al., 1992). Judging by the levels of the main histamine metabolite tele-methylhistamine (t-MeHA), the neuronal histaminergic system showed a developmental pattern with peak level in infants (Kiviranta et al., 1994), after which the levels of t-MeHA in cerebrospinal fluid (CSF) decreased to near-adult values in adolescent subjects. This study was done in 81 children with ages ranging from 3 months to 14.6 years (Kiviranta et al., 1994). In elderly subjects (97 non-demented controls, age range 34-101 years), the t-MeHA levels were found to be positively correlated with age (Motawaj et al., 2011, Prell et al., 1988).

However, we did not observe any age-related changes in the level of HDC-mRNA expression in the TMN for the combination of all our control subjects (n = 35, age range 44-93, see General Introduction, Figure 5). It should thus be investigated whether the age-dependent changes of the neuronal histaminergic system are exhibited exclusively in the event of inactive histamine in the brain.

1.2. Sexual dimorphism

As mentioned above, the levels of CSF-t-MeHA reach peak levels in infants, then decrease to near-adult values in adolescent subjects. In adult cases a higher level of t-MeHA was observed in women than in men (Motawaj et al., 2011, Prell et al., 1988). These data suggest a similar developmental pattern of the neuronal histaminergic system to e.g. that of the sexually dimorphic nucleus of the preoptic area, in which cell numbers reached peak values at the postnatal age of 5 years, after which a sex difference arises (Swaab et al., 1988).

Because our group has found that both estrogen receptor (ER)-α and -β are expressed in human TMN neurons (Kruijver et al., 2003), it appears that the TMN is sensitive to sex hormones. In addition, a stronger cytoplasmic ER-β staining was observed in women than in men (Kruijver et al., 2003), which may be targeted by the fluctuating estrogen levels in women. Moreover, the histamine receptors showed sexually dimorphic expression levels. A PET study showed higher H1R binding potential in human females compared to age-matched males (Yoshizawa et al., 2009). Furthermore, also in the rat cerebral cortex H1R and H2R binding were higher in females than in males (Ghi et al., 1991).

We reported a slightly but not significantly higher (32%) TMN neuron number in 4 females compared with 5 male subjects. This was in agreement with HDC-mRNA expression, which showed significantly higher (46%) levels in female than in male subjects (Chapter 7). It should, however, be noted that we did not observe such sexual dimorphism of HDC-mRNA expression in a larger group (n = 35, female = 11, male = 24) not matched for brain weight or pH (Chapter 3). A slightly but not significantly higher histaminergic system activity in females was also revealed in the higher (about 20%) levels of histamine main metabolite t-MeHA levels in CSF (n = 97, female = 47, male = 50) (Motawaj et al., 2011, Prell et al., 1988). Apparently there are no large sex differences in the histaminergic system in controls.

Interestingly, we found significant sex differences in the neuronal histaminergic system in Parkinson’s disease (Chapter 4). For the pooled PD group, which included clinical PD and preclinical PD patients, female PD patients showed significantly lower HDC-mRNA expression than the pooled male PD patients (Mann Whitney U test P = 0.004), and lower than the pooled female controls (P = 0.035) (Figure 1). There was no significant difference in HDC-mRNA expression between the pooled male PD patients and the pooled male controls (P = 0.753) (Figure 1). The pooled data of female PD and female control subjects and the pooled data of male PD and male control subjects were still
well-matched (all $P \geq 0.165$) for age, postmortem delay, fixation time, Braak stage for Alzheimer’s disease (van de Nes et al., 1998) and clock time of death. A pooled data comparison was also performed. However, because this study was not aimed at researching sex differences, the validation of the sex difference we found needs to be performed in a larger and well-matched group.

1.3. Diurnal rhythmicity of HDC-mRNA

Accumulating results show that drugs that reduce histamine signaling, including traditional antihistamines such as the H1R antagonists diphenhydramine and pyrilamine, and low doses of doxepin may increase sleep (Krystal et al., 2010, Lin et al., 1988, Monti et al., 1986, Roehrs et al., 1984). In addition, mice lacking histamine show less wakefulness (Parmentier et al., 2002). These data suggest that the histaminergic system may be important for maintenance of wakefulness (Espana et al., 2012).

In many species, neuronal histamine displays a diurnal rhythm with high levels during the waking period and low levels during the sleeping period (Haas et al., 2008, Lin, 2000). Increased histamine release, TMN neuronal electrical activity, and higher c-fos expression were shown during the dark period in the hypothalamus of nocturnal rodents (Mochizuki et al., 1992, Prast et al., 1992, Takahashi et al., 2006). In addition, microdialysis and quantitative radioenzymatic assays revealed a considerably higher histamine concentration in the cat preoptic/anterior hypothalamic area during the waking stage as compared to the sleep stage (Strecker et al., 2002). One report showed that t-MeHA levels in lumbar puncture CSF of febrile children and infants are higher during the day time (06:00-20:00 hr) than at night (20:00-06:00 hr) (Kiviranta et al., 1994). Moreover, sleep-wake perturbations are frequently observed in people using antihistaminergic medication (Espana et al., 2012) and patients suffering from neurodegenerative disorders such as PD, AD and HD (Arnulf et al., 2002, Aziz et al., 2011, Riemersma-van der Lek et al., 2008).

Our findings showed that HDC-mRNA expression was significantly higher during the daytime (08:00-20:00 hr) than at night (20:00-08:00 hr) in the control group (Chapter 3) which agrees well with the rat data of Mochizuki et al. (Mochizuki et al., 1992), who showed that histamine release increased in the active period (20:00-08:00 hr) as compared to the sleep period (08:00-20:00 hr). The estimated acrophase of HDC-mRNA expression in controls at 18:09 hr in our study agrees also very well with the recent finding of an acrophase for the histamine rhythm at 17:49 hr in diurnal nonhuman squirrel monkeys (Zeitzer et al., 2011). In addition, this day-night fluctuation was found to be markedly different in patients with neurodegenerative diseases, including Parkinson’s disease, Alzheimer’s disease and Huntington’s disease patients with an acrophase at 8:56 hr (Chapter 3).

Future Perspectives

1) Investigation of the development and sexual dimorphism of the histamine production system in the human brain, will require larger groups and bigger age ranges from both genders.

2) Investigation of whether the neuronal histaminergic system undergoes an age-dependent change and to study these changes in health and disease, histamine or histamine metabolite levels in the CSF must be measured. The Netherlands Brain Bank has collected a large number of CSF samples from people with neuropsychiatric disorders and from controls, including the subjects whose HDC-mRNA we measured. It would be of significance to study the relation between the histamine production and the CSF-histamine levels. However, it should be noted that, due to the fact that CSF-histamine levels are very low (at some pg/ml concentration levels), the standard high-performance liquid chromatography (HPLC) could not offer a convenient and reliable measurement. It is expected that HPLC combined with mass spectrum detector may be of assistance here.
3) To confirm the disturbed diurnal rhythm of the neuronal histaminergic system in neurodegenerative disorders, a larger group of patients with individual diseases have been collected and should be studied.

2. Histaminergic system in neuropsychiatric disorders

2.1. Parkinson’s disease (PD)

PD patients frequently show symptoms other than their motor problems, such as mood disorder, sleep problems, and cognitive decline (Boeve et al., 2007, Braak et al., 2006, Quelhas et al., 2009, Riedel et al., 2010), all of which involve functions that are at least partly regulated by histamine (Haas et al., 2003). In addition, the abundant accumulation of the characteristic neuropathological PD lesions, Lewy bodies (LBs) and Lewy neurites (LNs), in the TMN of PD patients presumed a destruction of this nucleus in the course of the disease (Braak et al., 1996, Braak et al., 2003). Moreover, animal studies showed that endogenous histamine might play an important role to accelerate the course of disease. In the 6-hydroxodopamine (6-OHDA)-lesioned rat, a classic PD model, an increase of endogenous histamine appeared to enhance the apomorphine-induced turning behavior and to increase the loss of tyrosine hydroxylase (TH) in the SN (Liu et al., 2008). A decrease of endogenous histamine by injection of α-fluoromethylhistidine, an irreversible inhibitor of HDC, strongly reduced rotation behavior and prevented the loss of TH-expressing cells in an early stage of the 6-OHDA lesion in the rat (Liu et al., 2007).

In contrast to the presumed degeneration of the TMN in PD, no clear changes of HDC-mRNA were observed in the TMN in PD in our studies (Chapter 4). This is in agreement with other studies in PD patients showing an intact number of histaminergic neurons (Nakamura et al., 1996), stable HDC enzyme activity (Garbarg et al., 1983) and unaltered t-MeHA levels in the CSF (Prell et al., 1991) in PD. Our observations also showed - for the first time - that the accumulation of Lewy bodies and Lewy neurites in the TMN did not influence HDC-mRNA expression during the course of PD (Chapter 4). In fact, there is increasing evidence that the presence of these compact inclusions represents a protective mechanism on the part of the surviving cells (Tanaka et al., 2004, Tompkins et al., 1997) and protects the cell, e.g., by the uptake of misfolded and non-functional proteins (Olanow et al., 2004, Shults, 2006), whereas evidence that the α-synuclein aggregates in PD are neurotoxic is lacking (Lee et al., 2008). In addition, studies have reported on cell and animal models with different neurodegenerative diseases developing inclusion bodies that, conversely, correlate with cell death (Ross et al., 2005). We found an unchanged HDC mRNA expression, not only in clinical PD, but also in preclinical PD (i.e. patients who did not have clinical PD symptoms but in whom PD pathology was beginning to develop (Braak et al., 1996, Braak et al., 2003)), while there was no accumulation of LBs and LNs in the TMN. There seems, therefore, to be no enhancement of histamine production in the early stages of PD, as has been presumed on the basis of animal experiments (Liu et al., 2007).

It is very meaningful that our study (Chapter 5) showed that there was an up-regulation of HMT-mRNA in the putamen and SN of PD patients, which was in line with the increased histamine levels that were reported in the same brain areas of PD patients (Rinne et al., 2002). These results plead for the possibility that histamine stimulates the PD process in its projection brain areas. The up-regulation of HMT-mRNA may act as a protective mechanism by metabolizing enhanced levels of histamine in these brain areas. Such a protective effect might be crucial, because previous animal experiments have shown that increased histamine levels in the SN may cause degeneration of dopaminergic neurons (Liu et al., 2007, Vizuete et al., 2000). The significant inverse correlation between HMT-mRNA expression and disease duration in the SN of PD patients suggests that the more serious (and thus the shorter lasting) the disease, the more expression of HMT-mRNA, which may be explained as a brain mechanism to protect it against PD.
**Future Perspectives**

1) To investigate the functional relevance of the increased HMT-mRNA in the SN and striatum of PD patients, measurement of histamine and/or histamine metabolite concentration in this brain tissue may be of help, and may provide a better insight into local histamine alterations.

2) In the 6-hydroxydopamine (6-OHDA)-lesioned rat, a classic PD model, an increase of endogenous histamine appeared to enhance the apomorphine-induced turning behavior and to increase the loss of tyrosine hydroxylase (TH) in SN (Liu et al., 2008). Furthermore, a decrease of endogenous histamine by injection of α-fluoromethylhistidine (α-FMH), an irreversible inhibitor of HDC, strongly reduced rotation behavior and prevented the loss of TH-expressing cells in an early stage of the 6-OHDA lesion in the rat (Liu et al., 2007). Although we showed that neuronal histamine production in TMN was stable in an early stage of PD, it may also be possible that local augmentation of histamine transmission in SN and putamen in PD happened in an early stage of PD. Is so, it might contribute to SN degeneration in the early stages of PD. To understand alterations of the histaminergic system in an early stage of Parkinson’s disease, postmortem tissue from the SN and putamen should be collected and the expression of histaminergic genes should be measured.

3) Histamine receptor-4 expression was considerably higher - 6.3-fold in caudate nucleus and 4.3-fold in putamen in PD compared to matched controls. However, only limited information is available on the function of H₄R in the brain (Connelly et al., 2009). To find out whether the H₄R-mRNA upregulation plays a role in the pathology of PD in this brain area, the distribution and the compartments that contribute to the H₄R up-regulation in caudate and putamen must be investigated.

### 2.2. Huntington’s disease (HD)

Of all the nuclei in the hypothalamus, the TMN shows the highest frequency of both nuclear and cytoplasmic inclusions of mutant huntingtin, the neuropathological hallmark of HD (Aziz et al., 2008) (General introduction Figure 7), so an involvement of the histaminergic system in HD was presumed.

**We found an increase in HDC-mRNA levels in the TMN and an increase in HMT-, H₁R- and H₃R-mRNA levels in the inferior frontal gyrus (IFG) of HD patients. In addition, we observed a significant negative correlation between age at onset of the disease and HMT-mRNA, which suggests that the more serious (thus the shorter lasting) the disease, the more HMT-mRNA is expressed. Since the levels of histamine metabolites in CSF have also been shown to be increased in HD patients, it all points to an enhanced activity of the neuronal histaminergic system in HD (Chapter 6).**

These findings suggest a functional increase in brain histaminergic signaling in HD, which may contribute to sleep disturbances (Yanovsky et al., 2011), weight loss or neuronal loss (Liu et al., 2007, Vizuete et al., 2000). In addition, our findings provide a rationale for the use of H₄R agonists in HD patients, since it may inhibit histamine production. Recently Yanovsky et al. reported that dopamine could activate TMN neurons to increase histamine release via Dopamine-2 receptor (Yanovsky et al., 2011). It is thus of interest to notice that PET scanning revealed a loss/dysfunction of Dopamine-2 receptor binding reported in the hypothalamus of clinical HD patients and premanifest HD patients (with abnormal CAG repeat numbers but no clinical symptoms yet) (Politis et al., 2008). These findings provide a potential explanation for the up-regulation of HDC-mRNA in HD TMN.

**Future Perspectives**

1) In order to correlate the cognitive impairment, sleep disturbances and weight loss in the last few years of a HD patient’s life with hypothalamic alterations, thorough documentation of these parameters in donors is needed. Subsequently the causality of such correlations and the effect of histaminergic compounda may be
studied. Unfortunately such physiological parameters were not documented in the clinical histories of the HD patient whose post-mortem material is available. The Netherlands Brain Bank has therefore begun to document the patients’ bodyweight at autopsies.

2) It would be of interest to study the mechanism of histamine augmentation in the HD brain, for which it is imperative to set up reliable and valid HD animal models that show at least the changes that are similar to neuronal histaminergic changes. This is not the case with the current mouse models (Williams et al., 2012).

3) Based upon a recent acute rodent study that dopamine can activate TMN neurons to increase histamine release via the Dopamine-2 receptor (Yanovsky et al., 2011), PET scanning revealed a loss/dysfunction of Dopamine-2 receptor binding in the hypothalamus of both clinical HD patients and premanifest HD patients (with abnormal CAG repeat numbers but no clinical symptoms yet) (Politis et al., 2008). This points to a potential mechanism for the HDC-mRNA upregulation we observed in the TMN of HD and premanifest HD patients. To investigate this, having access to HDC-mRNA expression in postmortem premanifest HD TMN would be of importance.

2.3. Alzheimer’s disease (AD)

The accumulation of neurofibrillary tangles (NFT) in the TMN takes place in the early stages of the AD process, i.e. in Braak stage 3 (Braak et al., 1993). In addition, a loss of large histaminergic neurons has been described in the rostral TMN in AD (Nakamura et al., 1993). These observations are in accordance with HPLC results showing that histamine levels, along with the decreased neuronal metabolic activity of the TMN, diminish in a number of brain areas, including the hippocampus, frontal and temporal cortex, in AD (Mazurkiewicz-Kwilecki et al., 1989, Panula et al., 1998).

Surprisingly, our study showed that, although there was a significant (57%) overall loss of TMN neurons in AD, the total HDC-mRNA expression level was only slightly (24%) and non-significantly decreased in the same patients (Chapter 7). Interestingly, this seems to be in full agreement with the previous finding of a slightly but not significantly lower level of the histamine metabolite t-MeHA (22%) in the lumbar CSF of AD patients (Motawaj et al., 2011). Our findings suggest that the remaining TMN neurons in AD compensate for the loss in histamine neurons. Only in females did we find a significantly increased H_3R-, HMT-mRNA expression in the PFC, which is one of the major projection areas of histamine produced in the TMN (Haas et al., 2008), together with increased glial fibrillary acidic protein (GFAP)-, Vimentin (VIM) and Proteolipid protein (PLP)-mRNA levels. Our findings indicate that the histaminergic system as far as the PFC is concerned is especially affected in female AD patients. In addition, although we observed a significant positive correlation between HMT, GFAP and VIM-mRNA levels, both in controls and AD patients, HMT-mRNA was exclusively located in neurons in the human PFC (Chapter 7), and not in astrocytes, contrary to expectation (Husztí et al., 1990, Nishibori et al., 2000, Rafalowska et al., 1987).

It is known that H_3R antagonists may increase the release of histamine, acetylcholine, noradrenaline and dopamine, and it was therefore proposed to modulate the cognitive processes in the PFC of AD patients. Regarding the rather small and female-specific H_3R-mRNA increase we observed in the PFC in AD patients, together with the finding of insignificant changes of H_3R binding density in this area (Medhurst et al., 2009), the positive effect of H_3R antagonists was, however, expected to be modest. This idea has recently been confirmed by a clinical pilot study with one of the H_3R antagonists/inverse agonists that appeared to be ineffective in improving cognitive functions in mild to moderate AD patients (Egan et al., 2012). Furthermore, it should be noted that, as the activity state of the remaining TMN neurons is supposed to be increased, a conservative application of H_3R antagonists is in order if further degeneration of these neurons is to be prevented.
Future Perspectives

1) The mechanism of the functional compensation of TMN neurons in AD is at present unknown. My hypothesis is that a reduced GABAergic inhibition (Gallopin et al., 2000) may be the basis for the activation of TMN neurons. A similar neuronal compensation has been reported in AD in the locus coeruleus (LC) where the small proportion of remaining noradrenergic neurons is hyperactive (Hoogendijk et al., 1999, Raskind et al., 1999, Szot et al., 2006), indicating this may be a general mechanism in AD. The possible source of GABAergic projections for the TMN and locus coeruleus in such compensatory mechanisms is not clear, while the ventrolateral preoptic nucleus (VLPO) (Chou et al., 2002, Steininger et al., 2001), which sends GABAergic projections both to the TMN and to the LC in rat could be a clue. It has been proposed that rat VLPO is homologous to the human intermediate nucleus/sexually dimorphic nucleus of the preoptic area (SDN-POA)/interstitial nucleus of the anterior hypothalamus-1 (INAH1), because this nucleus is galanin positive (Gaus et al., 2002, Saper et al., 2001). However, the proposal that this human nucleus is homologous to the rat VLPO (Gaus et al., 2002, Saper et al., 2001) appears to be at odds with other observations (Garcia-Falgueras et al., 2012). The rat VLPO was localized much more ventrolaterally, i.e., lateral of the optic chiasm (Gaus et al., 2002), than the human intermediate nucleus. The marker galanin is found throughout the hypothalamus. A specific marker for the intermediate nucleus is necessary to solve the problem regarding the possible homology with the rat VLPO.

2) In mature mast cell lines and in an erythroblast cell line it was found that demethylation in the HDC promoter area increased HDC-mRNA expression and histamine content (Kuramasu et al., 1998, Suzuki-Ishigaki et al., 2000). It may therefore be of interest to investigate whether HDC expression is regulated by methylation in the TMN. Recently a report showed that decrements in DNA methylation status as measured by 5-methylcytosine and 5-methylcytidine, as well as the immunoreactivity of all eight methylation stabilization factors were decreased in entorhinal cortex layer II in AD compared with matched controls (Mastroeni et al., 2012). If such a decline of DNA methylation activity also happened in the TMN, this would greatly contribute to the higher HDC-mRNA expression in AD. Such an epigenetic mechanism seems a worthwhile subject for future study.

3) We found that the H₃R and HMT in PFC were upregulated in both the preclinical (Braak stage III-IV) and clinical stages (Braak stage V-VI) of AD patients. This result, together with the NFT accumulation in the TMN in Braak stage III (Braak et al., 1993), seemed to imply that alteration of histamine production in TMN might start in an early stage of AD. Counting TMN neurons and measuring HDC-mRNA in an early stage of AD TMN should be performed to test this possibility.

4) Significantly increased H₃R, HMT and mRNA expression in the course of AD was only found in the female PFC. The sex differences we reported deserve further study, due to possible clinical implications. Astrocyte markers, GFAP, VIM-mRNA and oligodendrocyte markers, and PLP-mRNA showed sex differences in PFC in AD as well. These findings need to be confirmed on the protein level by means of Western-blot and, if possible, by CSF measurements of astrocyte and oligodendrocyte markers.

5) The novel histamine receptor H₄R was recently found to be functionally expressed in the human cortex (Connelly et al., 2009). We have reported for the first time that the H₄R-mRNA tended to be higher in the PFC in AD. The functional meaning of such an up-regulation should be elucidated with pharmacological studies in an animal model or cell line. This receptor should also be studied in other disease (see in PD) to gauge the quality/quantity of the changes.
2.4. Multiple sclerosis (MS)

MS is an inflammatory disorder of the central nervous system associated with chronic and extensive neurodegeneration (Compston et al., 2008). It should be noted that histamine can change the permeability of the blood brain barrier, which may lead to an elevation of infiltrating cells in the CNS as well as to an increased risk of neuroinflammation (Michelsen et al., 2005).

In addition, histamine has shown to have positive effects in MS therapy by five possible mechanisms: 1) augmentation of subnormal cerebral tissue levels of histamine, 2) improved electrical function of demyelinated fibers, 3) increased cerebral blood flow, 4) suppression of autoimmune responses, and 5) stimulation of remyelination (Gillson et al., 2000). Although the effects of histamine in MS patients are not yet clear, animal studies (Musio et al., 2006) and human observations (Alonso et al., 2006, Lock et al., 2002) both show that alterations in the histaminergic system may be involved in the progression of MS.

Future perspective

To pave the road for the development of histamine-related therapeutic strategies, putative alterations in neuronal histamine production, release, and breakdown, as well as in its receptors, should be systematically studied in multiple sclerosis patients. The requirements for such studies i.e. material from the Netherlands Brain Bank and techniques are in place.

2.5. Depression

Due to the fact that hypothalamic histamine significantly increases when animals are exposed to acute stress (Mazurkiewicz-Kwilecki et al., 1986), and as both acute and chronic stresses increase histamine turnover (Ito, 2000), it was proposed that the histaminergic system may be involved in the pathology of depression (Jin et al., 2009). The observation that intracerebroventricularly infused histamine increased the expression of stress-related neuropeptides, such as CRH, arginine vasopression-mRNA and oxytocin-mRNA in the PVN and oxytocin-mRNA expression in the SON of the rat hypothalamus via both H₁R and H₂R, supported this possibility (Kjaer et al., 1994).

However, our study on postmortem human brain material did not reveal any significant changes in the neuronal histaminergic system, either in the rate-limiting enzyme, HDC, involved in its production or in its receptors and breakdown enzyme in the two main projection sites, the anterior cingulate cortex and the dorsolateral prefrontal cortex (Chapter 8).

Future perspective

1) Larger numbers of patients in the bipolar disorder and major depression groups are needed in order to see whether there is a change in the day-night fluctuations in HDC-mRNA in depression in the TMN. Measurements of histamine or histamine metabolite levels in CSF could also be instrumental here.

2.6. Schizophrenia

Famotidine, a H₂R antagonist, was shown in several open-label clinical trials to have antipsychotic effects and to reduce negative symptoms in schizophrenia (Kaminsky et al., 1990, Oyewumi et al., 1994, Rosse et al., 1996). For this reason, the histaminergic system has been thought to be involved in the pathophysiology of this disorder (Tandon, 1999).

Superior frontal gyrus (SFG) dysfunction has been implicated in the pathophysiology of schizophrenia (Smee et al., 2011). Our pilot study has shown, for the first time, that the histamine inactivating enzyme HMT-mRNA is up-regulated twofold in the SFG of schizophrenia patients, whereas the histamine releasing factor HRF-mRNA tended to be elevated (Chapter 9). In addition, the expression levels of the astrocyte-associated genes, including GFAP, VIM, were higher in schizophrenia, which confirmed earlier findings (Nanitsos et al., 2005, Rothermundt et al., 2009, Toro et al., 2006). The data obtained so far imply an increased release and turnover of histamine in this brain area of schizophrenia patients.
**Future perspectives**

1) Our pilot provides a rationale for the exploration of histamine production in the hypothalamus in schizophrenia and the levels of histamine and its metabolites in the CSF.

2) HRF was first discovered as the translational controlled tumor protein in Ehrlich ascites tumor cells and MacDonald characterized it as a histamine-releasing factor (see reviewed in (Telerman et al., 2009)). Since a down-regulation of HRF protein expression was observed in Alzheimer’s disease and Down’s syndrome, it has been proposed that reduced HRF is associated with cognitive and memory deficits (Kim et al., 2001). The previously reported down regulation of HRF-mRNA (1.4-1.9 fold) in the hippocampus of schizophrenic patients (Chung et al., 2003), and our finding that HRF tended to be upregulated in the PFC, supports the possibility of its involvement in cognition. It seems therefore of importance to determine this novel compound also in the PFC of neurodegenerative disorders. Furthermore, in schizophrenia, but not in controls, we observed a positive correlation between HRF and glia markers such as GFAP and VIM. This may indicate that the upregulation of HRF comes largely from the glia compartment. These preliminary data thus ask for a localization of HRF at the messenger and protein level in the brain. To delineate, in addition, the function of HRF in the brain, a HRF knock-out model is needed.

3. **Histamine receptor-3 antagonists/inverse agonists: clinical implications**

Recently a phase III Alzheimer’s disease trial with Dimebon (latrepirdine), originally an antihistamine compound used for allergy treatment, failed to show any significant improvement in primary or secondary outcomes in 598 patients (Bezprozvanny, 2010). The failure of Dimebon is presumed to be largely due to an insufficient understanding of its mechanism (Jones, 2010). This topic, therefore, seems to be especially timely, since histamine-3 receptor (H₃R) antagonists/inverse agonists are now making their way into the clinic for potential treatment of Alzheimer’s disease, Parkinson’s disease and schizophrenia (Brioni et al., 2010, Passani et al., 2011), without taking into account the recent information on alterations in the histaminergic system.

Regarding Parkinson’s disease (Chapters 4 and 5), we paid special attention to the ongoing Phase III clinical trials of H₃R antagonist/inverse agonists (Benarroch, 2011, Passani et al., 2011), which may potentially increase the histamine release and thus accelerate degeneration of neurons, e.g. in the putamen or substantia nigra.

In Alzheimer’s disease (Chapter 7), because H₃R antagonists increase the release of histamine, acetylcholine, noradrenalin and dopamine, they may in this way modulate cognitive processes in the PFC of AD patients (Brioni et al., 2010, Passani et al., 2011). However, regarding the small and female-specific H₃R-mRNA increase we observed, together with insignificant changes of binding density in this area (Medhurst et al., 2009), the positive effects of H₃R antagonists are expected to be modest. This presumption has already been supported by a recent clinical pilot study, which showed that one of the H₃R antagonists/inverse agonists appeared to be ineffective with regard to improving cognitive function in mild to moderate AD patients (Egan et al., 2012). In addition, it should be noted that, because the activation state of the remaining TMN neurons is already higher, the application of the H₃R antagonist should be on the conservative side in order to prevent accelerated degeneration of these neurons.

As far as schizophrenia was concerned (Chapter 9), our pilot study showed a significant increase of HMT-mRNA in the superior frontal cortex of schizophrenic patients, thus supporting the notion of an elevation of histamine (Prell et al., 1996, Prell et al., 1995). Of course, a cohort has to be included for the histamine production site TMN to warrant future studies. However, the current pilot study, may already give arguments against clinical application of H₃R inverse agonist/antagonist in this disorder (Brioni et al., 2010, Passani et al., 2011), because such compounds may potentially further increase histamine production and release (Brioni et al., 2010, Passani et al., 2011). In addition, a
recent preclinical pharmacological study failed to show antipsychotic-like properties of H$_3$R inverse agonist/antagonists (Burban et al., 2010). In general, our post-mortem data supported that - as an adjunct form of medication - H$_3$R antagonists might modulate the circadian rhythmicity of neuropsychiatric disorders (Chapter 3). However, for improving cognitive impairment or locomotion, the effects of H$_3$R antagonists were modest or indirect, and warrants careful future studies. Last but not least, for this complex medication to be used properly, the effects of H$_3$R antagonists on the modulation of histamine and other neurotransmitters in the human brain must be studied with some urgency.

4. Limitations of the thesis

The other source of brain histamine, mast cells, is not discussed in the current thesis because the localizations are not well-documented in the human brain. However, it might be so that up to 50% of whole brain histamine levels are made up of mast cells (Goldschmidt et al., 1985). In addition, some medication showed interaction with histamine receptors (Appl et al., 2011, Jin et al., 2009) or influenced the histamine levels in lumbar CSF (Kanbayashi et al., 2009, Zeitzer et al., 2011). For the study it was also therefore virtually impossible to exclude the effects of medication.

References


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Summary

The histaminergic system is involved in many brain functions, such as the sleep-wake cycle, energy and endocrine homeostasis, sensory and motor functions, cognition, attention, learning and memory. My interest in the functions of the histaminergic system in the brain was awakened when I noticed that traditional antihistamines, such as the histamine receptor antagonists diphenhydramine, pyrilamine and doxepin, which increase sleepiness and decrease attention, came with a caution about strong side effects.

The posterior part of the hypothalamic tuberomamillary nucleus (TMN) is the exclusive source of neuronal histamine, produced by the key enzyme histidine decarboxylase (HDC). Four types of G-protein coupled HRs, i.e., H1-4R, have been found in the human brain. And histamine methyltransferase (HMT) is the inactivation enzyme for histamine in the brain.

In the Introduction to this thesis (Chapter 1) we reviewed the data on the functions of the histaminergic system that are often gender and age-dependent and are disturbed in neuropsychiatric disorders such as Parkinson’s disease (PD), Huntington’s disease (HD), Alzheimer’s disease (AD), Multiple Sclerosis (MS), depression and schizophrenia. In addition, in this chapter we tried to bridge the gap between the fundamental features of the histaminergic system in experimental animals and the recently observed alterations in postmortem tissue of patients with neuropsychiatric disorders. We consider this a matter of some urgency, because histamine-3-receptor antagonists/inverse agonists are making their way into the clinic as a potential treatment for AD, PD and schizophrenia, while the insights on alterations in histamine production, breakdown and receptors recently obtained from postmortem studies yield crucial information on the potential effects and side effects of these compounds.

Chapter 2 deals with an experiment in which a number of alterations were introduced into an otherwise routine protocol in order to create optimal in situ hybridization conditions for quantification of the messenger ribonucleic acid (mRNA) HDC in formalin-fixed, paraffin-embedded archival postmortem human brain tissue by radioactive probe.

One of the most pronounced effects of neuronal histamine is its crucial role in maintaining wakefulness, also in human. In various species a diurnal fluctuation of histamine has been described, with high levels during the waking stage and low levels during the sleeping period. However, such information is still lacking for humans, both in physiology and in neurodegenerative disorders, where sleep-wake perturbations are a common feature.

In Chapter 3, we showed - for the first time - diurnal fluctuations in human histamine production, i.e. higher HDC-mRNA levels during the wake stage (08:00-20:00 hr) than during the sleep stage (20:00-08:00 hr) in control subjects. The estimation of the acrophase of HDC-mRNA expression in human healthy controls, i.e. at 18:09 hr, corresponded very well with the acrophase for the histamine rhythm at 17:49 hr in diurnal nonhuman squirrel monkeys. In addition, this day-night fluctuation was found to be strongly changed in patients with neurodegenerative diseases, i.e. in PD, AD and HD patients with an acrophase at 8:56 hr. Our observations thus add weight to the proposed ‘flip-flop’ hypothesis of the sleep switch, which says that TMN neurons may promote wakefulness in humans, too. Moreover, the inverted profile in neurodegenerative diseases may be involved in the restless nights and listless days that are so typical of these disorders.

Previous animal studies have shown that in the 6-hydroxydopamine (6-OHDA)-lesion rat, a classic PD model, inhibition of endogenous histamine production in an early stage of the disorder put a halt to dopaminergic neuron degeneration. In agreement with this possibility and on the basis of the abundant accumulation of the characteristic neuropathological PD lesions, i.e. Lewy bodies (LBs) and Lewy neurites (LNs), a severe destruction of the histaminergic system was presumed to occur in the TMN of PD patients. However, surprisingly, we did not observe any quantitative changes in TMN HDC-mRNA
in PD, which tallied with the intact number of histaminergic neurons, as well as with the unchanged enzyme activity of HDC and with the unaltered tele-methylhistamine (t-MeHA) levels in the CSF in PD. Our observation showed an unchanged TMN HDC-mRNA, not only in late stage PD, but also in a preclinical stage of this disease (Chapter 4). Furthermore, we observed that the expression of the histamine receptor-3 (H₃R), which appeared to be localized immunocytochemically in the large pigmented neurons, was significantly decreased in the substantia nigra (SN) in PD (Chapter 5). In Chapter 5 we also showed that there was an up-regulation of HMT-mRNA in the SN and putamen of PD patients, which may act as a protective mechanism as it metabolizes enhanced histamine levels in these areas. Because animal experiments have shown that increased histamine levels may cause degeneration of dopaminergic neurons in the SN, such a protective effect might be of importance. Moreover, an inverse correlation between HMT-mRNA expression and disease duration was observed in the SN of PD patients, suggesting that the more serious (and thus the shorter lasting) the disease, the more HMT-mRNA was expressed, which further supports the notion of such a compensatory mechanism.

Of all the nuclei in the hypothalamus, the TMN shows the highest presence of both nuclear and cytoplasmic inclusions of mutant huntingtin, the neuropathological hallmark of HD. Nevertheless, we found an increase in HDC-mRNA levels in the TMN and an increase in HMT-, H₁R- and H₃R-mRNA levels in the inferior frontal gyrus (IFG) of HD patients. In addition, we observed a significant negative correlation between age at onset of the disease and HMT-mRNA, which suggests that the more serious (and thus the shorter lasting) the disease, the more HMT-mRNA is expressed. Since the levels of histamine metabolites in CSF have also been shown to be increased in HD patients, all observations point to an enhanced activity of the neuronal histaminergic system in HD (Chapter 6).

It is known that the accumulation of neurofibrillary tangles (NFT) in the TMN takes place in an early stage of the AD process, i.e. in Braak stage 3. Also, a loss of large histaminergic neurons has been reported in this nucleus in AD. Our study showed that the total HDC-mRNA expression level was only slightly (24%) and non-significantly decreased in the same patients, despite a significant (57%) overall loss of TMN neurons in AD (Chapter 7). Our findings suggest that the remaining TMN neurons in AD compensate for the loss of histamine neurons. Interestingly, when the TMN was divided into 3 parts, a significant reduction in the number of neurons was found in all 3 TMN sub-regions in AD patients, while a significantly lower HDC-mRNA expression was only found in the middle part of the TMN, and not in the rostral or caudal part. In addition, only in females did we find a significantly increased H₃R-, HMT-, mRNA expression in the prefrontal cortex, which is one of the major projection areas of histamine produced in the TMN, together with increased glial fibrillary acidic protein (GFAP)-, vimentin (VIM) and proteolipid protein (PLP)-mRNA levels. Moreover, and contrary to expectation, HMT-mRNA was exclusively located in neurons (Chapter 7), and not in astrocytes in the human PFC. However, we did observe a significant positive correlation between HMT, GFAP and VIM-mRNA levels in controls and AD patients.

Our study on postmortem human depression brain material (Chapter 8) did not reveal any significant changes in the neuronal histaminergic system, either in the rate-limiting enzyme for histamine production, HDC, or in its receptors and breaking-down enzyme in the two main projection sites, the anterior cingulate cortex and the dorsolateral prefrontal cortex, despite the fact that animal experimental models for depression suggest the presence of changes in the histaminergic system.

In schizophrenia (Chapter 9) we have shown, for the first time, that the histamine inactivating enzyme HMT-mRNA is up-regulated twofold in the superior frontal gyrus of schizophrenia patients, whereas the expression of a novel gene, the histamine-releasing factor (HRF), tended to be elevated (Chapter 9). HRF was proposed to be associated with cognitive deficits and histamine release.

In the General discussion (Chapter 10), we showed alterations in the histaminergic system in day-night fluctuations and neuropsychiatric disorders. Ongoing clinical trials with H₃R antagonists/inverse ago-
nists in PD patients deal with the possibility that these (ant)agonists may potentially increase histamine release and thus accelerate the degeneration of neurons, e.g. in the putamen or substantia nigra in PD. In AD, the histamine-3 receptor antagonists are expected to be ineffective, or at best modestly effective, with regard to improving cognitive function in AD patients, since we have not seen any substantial alterations in the TMN or in the prefrontal cortex for H3R-mRNA. Histamine-3 receptor antagonists may, however, cause further degeneration of the remaining TMN neurons, because they are already hyper-activated. Our current schizophrenia study, which shows activation of the histaminergic system, may provide us with arguments against clinical application of H3R inverse agonist/antagonist in this disorder, because such compounds could potentially further increase histamine production and release. In general, our post-mortem data supported our supposition that H3R antagonists, as an adjunct form of medication, might modulate the circadian rhythmicity of neuropsychiatric disorders. However, when it comes to improving cognitive impairment or locomotion, the effects of H3R antagonists are modest or indirect.

Last but not least, we discussed the limitations of our work and proposed topics for future research.