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Chapter 1

General Introduction

The human histaminergic system in health and neuropsychiatric disorders

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Manuscript in preparation
Chapter 1

Abstract

Histaminergic neurons in the human brain are exclusively located in the tuberomamillary nucleus of the posterior hypothalamus and project all over the central nervous system. The neuronal histaminergic system is involved in a number of basic physiological functions, such as the sleep-wake cycle, energy and endocrine homeostasis, sensory and motor functions, cognition, attention, learning and memory, all of which are severely affected in neuropsychiatric disorders.

However, the fundamental properties of the histaminergic system differ for experimental animals and humans. In addition, recently histamine-3-receptor antagonists/inverse agonists have been making their way into the clinic as a potential treatment for Alzheimer’s and Parkinson’s disease and schizophrenia.

Here we therefore mainly review the histaminergic system alterations of postmortem tissue from patients with neuropsychiatric disorders.

1. Introduction

The neuronal histaminergic system is involved in a number of basic physiological functions, such as the sleep-wake cycle, energy and endocrine homeostasis, sensory and motor functions, cognition, attention, learning and memory (Haas et al., 2003, Haas et al., 2008). These functions are often gender, age and time-of-the-day- dependent and are severely affected in a number of brain disorders, including Parkinson’s disease (PD), Huntington’s disease (HD), Alzheimer’s disease (AD), Multiple sclerosis (MS), depression and schizophrenia (Haas et al., 2003, Haas et al., 2008). Recently, a dense Tourette syndrome pedigree appeared also to have a mutation in the HDC gene encoding L-histidine decarboxylase, the rate-limiting enzyme for the synthesis of histamine (Ercan-Sencicek et al., 2010). However, HDC knockout mice with a functional mutation of the same gene had a phenotype that was only partly comparable to these patients (Ercan-Sencicek et al., 2010), which illustrates that human studies are critical for understanding the neuronal histaminergic system in our species.

Histamine in the central nervous system (CNS) has been the subject of a number of animal experimental reviews (Haas et al., 2003, Haas et al., 2008). The present review is aimed to bridge the gap between fundamental properties of the histaminergic system in experimental animals and the
recently observed alterations in postmortem tissue of patients with neuropsychiatric disorders. This topic seems to be especially timely since histamine-3-receptor antagonists/inverse agonists are advancing into the clinics as a potential treatment for AD, PD and schizophrenia (Brioni et al., 2010, Passani et al., 2011), while the insights on alterations in histamine receptors, obtained recently from postmortem studies, seem to reveal crucial information on the potentials of these compounds.

2. Neuronal histaminergic system in the brain

2.1 Tuberomamillary nucleus

Anatomy

The tuberomamillary nucleus (TMN), located in the posterior hypothalamus (Figure 1 A) consists of large, irregularly bordered neurons that have an intensely stained endoplasmic lipofuscin-laden reticulum. These neurons surround the lateral tuberal nucleus (NTL), the fornix in its final descending course, and the mamillary body (Figure 1 B). The pronounced Nissl substance is situated in the periphery of the cytoplasm, interrupted by typical irregularities in the cell membrane (Figure 1 C and D). The TMN can already be distinguished at 34 weeks of gestation (Koutcherov et al., 2003). An earlier study in 3 subjects without a clear neurological disease reported the presence of about 32000 large and multipolar histaminergic neurons on each side of human hypothalamus (Airaksinen et al., 1991). We have recently also found a comparable number of TMN neurons (37052 ± 5181) based upon 9 controls (Shan et al., 2012b).

Recent tracing and pharmacological studies in rodents have shown that histaminergic neurons are organized in functionally distinct circuits that influence different brain areas (Giannoni et al., 2009, Lee et al., 2008, Miklos et al., 2003, Sergeeva et al., 2002). Although histaminergic fibers have been reported in the prefrontal cortex (PFC), thalamus and substantial nigra (SN) of the human brain (Anichtchik et al., 2000b, Jin et al., 2002, Panula et al., 1990), information on the regional TMN origin of the histamine innervation is, in our species, however, lacking - for obvious reasons.

Co-transmitters of TMN neurons

Neuronal histamine is exclusively synthesized from the amino acid histidine by HDC in the TMN (Ericson et al., 1987, Panula et al., 1990, Panula et al., 1984) (Figure 2, 3, 4). In the human TMN, the
histaminergic neurons are characterized by HDC expression (Panula et al., 1989, Trottier et al., 2002), while most HDC positive neurons co-localize gamma-aminobutyric acid (GABA), characterized by its synthesizing enzyme glutamic acid decarboxylase (GAD) (Trottier et al., 2002) (Figure 3). In addition, acetylcholinesterase (Saper et al., 1987), monoamine oxidase (Nakamura et al., 1991), and the food-regulating neuropeptide cocaine and amphetamine-regulated transcript-positive (Hurd et al., 2000) neurons have been described in the human TMN. It should be noted that, although in a previous study TMN was negative for galanin staining (Trottier et al., 2002), a recent paper with novel galanin antibody showed galanin-positive neurons in the TMN (Garcia-Falgueras et al., 2011).

**HDC expression in the TMN**

HDC is the key enzyme for histamine production (Watanabe et al., 2002). Knock-out or pharmacological manipulation of HDC significantly decreases histamine production in rodents (Watanabe et al., 2002).

In order to study neuronal histamine production in formalin-fixed, paraffin-embedded archival postmortem human brain tissue, we optimized in situ hybridization conditions to quantify HDC-mRNA expression (Liu et al., 2010) (Figure 4).

In our group, too, the improved protocol has yielded favorable results for other low-to-moderate abundant genes that previously were also found to demonstrate background problems, such as corticotropin-releasing hormone (CRH)-mRNA, tyrosine hydroxylase, neuropeptide Y, agouti-related protein, and thyrotropin-releasing hormone expression in the human hypothalamic, which supports the general applicability of this procedure. In our studies, HDC-mRNA expression levels in the postmortem human TMN appeared to be a useful indicator for neuronal histamine production since the same direction of changes between HDC-mRNA expression levels in the TMN and histamine or the histamine metabolite, tele-methylhistamine (t-MeHA), were found in the cerebrospinal fluid (CSF). These indicators for central histamine neurotransmission (Soya et al., 2008) were found to show similar changes of HDC expression during diurnal fluctuations (see below), in HD (Prell et al., 1991a, van Wamelen et al., 2011), PD (Prell et al., 1991b, Shan et al., 2011) and AD (Motawaj et al., 2011, Shan et al., 2012b) (Table 1).

**Sex and age-dependency of the histaminergic system**

TMN neurons are sensitive to sex hormones. Estrogen receptors (ER)-α and β are expressed in TMN neurons (Kruijver et al., 2003). In addition, a stronger cytoplasmic ER-β staining was observed in women (Kruijver et al., 2003), possibly targeted by the fluctuating estrogen levels in females.

In a small sample size, the total number of TMN neurons was slightly, but not significantly, higher (32%) in 4 females than in 5 male subjects, which is in line with the HDC-mRNA expression showing significantly higher (46%) levels in females than in males (Shan et al., 2012b). A slightly but not significantly higher histaminergic system activity in healthy females was also revealed in the higher (about 20%) levels of histamine metabo-
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Not only histamine production and metabolites showed higher activity in females, the HRs, too, were reported to have higher binding densities in females. A positron emission tomography (PET) study demonstrated a higher \(H_1R\) binding potential in females compared to age-matched males (Yoshizawa et al., 2009).

Our group’s previous work has shown that TMN cell size, an indicator for neuronal metabolism, increases during aging in males but not in females (Ishunina et al., 2003). Literature showed that the CSF t-MeHA levels were significantly increased with age in either females or males (Motawaj et al., 2011, Prell et al., 1988). However, we did not observe significant age or sex-related changes in HDC-mRNA levels in the TMN (Figure 5) (Shan et al., 2012c). This discrepancy might relate to our smaller group size of control subjects \((n = 35, \text{female} = 11, \text{male} = 24)\), compared to the t-MeHA studies \((n = 97, \text{female} = 47, \text{male} = 50)\) (Motawaj et al., 2011). However, the alternative, a higher activity of histamine metabolite production with age, should also be considered.

Diurnal differences

In many species, neuronal histamine displays a diurnal rhythm with high levels during the waking period and low levels during sleep. An increase...
in histamine release, higher c-fos expression in the TMN and increased neuronal activity in the TMN are shown during the dark period in nocturnal animals, e.g., in rodents (Ko et al., 2003, Mochizuki et al., 1992, Steininger et al., 1999, 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). We are the first to demonstrate that the total expression of HDC-mRNA in the human TMN exhibits higher levels between 8:01-20:00 and lower levels in 20:01-8:00, which supports a role for neuronal histamine in the regulation of day-night patterns (Shan et al., 2012c) (Figure 6). It should be noted that recently some systematic experiments have shown that the circadian rhythm of histamine in the CSF of a diurnal mammal, i.e. squirrel monkey, reached acrophase values at 17:49 (Zeitzer et al., 2011), which fits very well with the maximum values of HDC-mRNA we observed in the human TMN around 18:09 (Figure 6 B). This similarity also supports the reliability of our postmortem data.

Our results, which indicate more HDC-mRNA expressed in the TMN during daytime, are also in agreement with previous findings of a diurnal variation of the main histamine metabolite t-MeHA in the CSF of rhesus monkeys (Prell et al., 1989) and human beings (Kiviranta et al., 1994). Concluding, our observation supports the proposed ‘flip-flop’ hypothesis of the sleep switch with evidence that, also in humans, TMN neurons may promote wakefulness (Saper et al., 2001).

**Interaction with the suprachiasmatic nucleus/biological clock**

Diurnal histamine fluctuations are crucial for the modulation of the circadian rhythmicity of the sleep–wake cycle (Lin, 2000, Saper et al., 2001). The hypothalamic suprachiasmatic nucleus (SCN) is the central circadian pacemaker and shows, in the human brain, circadian fluctuations in the number of neurons expressing vasopressin and vasoactive intestinal peptide (Hofman et al., 1994). The modulation of SCN functions by means of histamine is crucial. In rodents, chronic depletion of histamine results in abolished circadian rhythmicity of cortisol (Itowi et al., 1989). Since the SCN induces circadian fluctuations of cortisol secretion via inhibition of CRH and by modulation of adrenal responsiveness to corticotropin (ACTH) through a polysynaptic, sympathetic, pathway (Kalsbeek et al, 2010), these findings indicate that histamine is capable of influencing the circadian rhythmicity of corticosteroids via different mechanisms. The influence of histamine on circadian rhythms is further illustrated by the observation that the TMN and SCN are reciprocally connected (Abrahamson et al., 2001, Jacobs et al., 2000).

In addition, histamine-containing fibers were found in the pineal gland, where the circadian hormonal messenger melatonin is produced (Cassone et al., 1986, Moller et al., 2002, Wu et al., 2007). The observations that the human SCN contains melatonin receptor 1 (Liu et al., 1997) and 2 (Wan et al., 1999) and that TMN expresses melatonin receptor 1 (Wu et al., 2006), while melatonin receptor 2 is absent in this nucleus (L. Shan unpublished observations) (Figure 7) indicated that melatonin provides an alternative mechanism for the interaction between the SCN and TMN.

Moreover, the SCN provides long-lasting inhibition of the sleep-promoting center in the rat ventro-
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lateral preoptic nucleus (VLPO) (Saint-Mleux et al., 2007), which closely interacts with the TMN (Gallopin et al., 2000, Liu et al., 2011).

Interestingly, recent evidence shows that HDC- or H1R- knockout mice have disturbed clock gene expression in many brain areas (e.g. cortex and striatum) but not in the SCN (Abe et al., 2004, Masaki et al., 2006), all of which implies that the diurnal fluctuations we have observed in the histaminergic system (Shan et al., 2012c) may play a crucial role in the modulation of circadian functions in the SCN and other brain areas.

2.2 Histamine 1-4 receptors, metabolite enzyme and histamine releasing factor

Histamine receptor 1 (H1R)

Classic antihistamines have strong sedative properties - they induce sleepiness and cognitive deficits via H1R (Mochizuki et al., 2002, Reiner et al., 1994). The severity of these side effects is correlated with the amount of antihistamine that penetrates the human cerebral cortex (Tashiro et al., 2002).

In postmortem human brain material, the highest binding density of H1R is observed in the internal layers (lamina V and VI) of the neocortex. In addition, the claustrum, hippocampal formation and thalamus and the two segments of the globus pallidus also show high levels of H1R-binding (Martinez-Mir et al., 1990). This distribution is in keeping with mapping results from PET (Yanai et al., 1992).

Figure 7.
Immunocytochemical staining of melatonin receptors (A, MT1) - positive staining in the cytoplasm of tuberomammillary nucleus (TMN) neurons (Wu et al., 2006), (B) negative MT2 staining in TMN. Scale bar indicates 5 µm. (unpublished results).

Figure 6.
A: Box plots show the median, 25th-75th percentiles and the total range of radioactivity in arbitrary units. The total amount of radioactivity of histidine decarboxylase (HDC)-mRNA expression is given for control subjects between daytime (08:01-20:00; n = 18) and nighttime (20:01-08:00; n= 15) on the left side, and for neurodegenerative diseases group (NDD, daytime n = 20, nighttime n = 11) on the right side. Note that there is a significant difference (P = 0.004) between daytime and nighttime in control subjects, but not in NDD (P = 0.410). B and C: Raw data of HDC-mRNA expression plotted along the clock time of death. The nonlinear periodic functions describe the circadian cycles. The model in controls (open dots) reach an estimated maximum at the end of the afternoon (Tmax = 18:09 h) and a minimum shortly after midnight (Tmin = 1:09 h; B). The model in the NDD group (block dots) reaches an estimated maximum in the morning (Tmax = 8:56 h) and a minimum in the afternoon (Tmin = 14:43 h). The horizontal lines indicate the 24 hours mean of HDC-mRNA expression in controls and NDD group respectively. (modified from (Shan et al., 2012c) Figure 1).
Details on the functions of H1Rs come from the phenotypes of H1R deficient mice. H1R knockout mice show late-onset obesity, associated with a disturbance of the circadian rhythm of food intake (Masaki et al., 2006) and of locomotor activity (Inoue et al., 1996). The H1R knockout mice also show lower hypocretin/orexin levels (Lin et al., 2002), which synergistically functions with histamine in sleep-wake cycle modulation (Mochizuki et al., 2011, Saper et al., 2001). Combined H1R and H2R deficient mice exhibit impaired cognition, which is in line with decreased long-term potentiation in the cornu ammonis (CA1) (Dai et al., 2007). In addition, H1R knockout mice display a lower pain threshold (Haas et al., 2003). Moreover, H1R-binding showed sex and age-dependency in CNS in human. A previous PET study demonstrated a marked decrease of H1R-binding potential during aging in human (Higuchi et al., 2000). By the same ligand, females showed higher binding potential compared with age-matched males (Yoshizawa et al., 2009).

**Histamine receptor 2 (H2R)**

H2R-binding shows a high density in the basal ganglia, amygdala, hippocampus and cerebral cortex in both primates and rodents (Martinez-Mir et al., 1990, Ruat et al., 1990, Vizuete et al., 1997). Unlike H1R, the distribution of H2R in the human cerebral cortex is denser in the superficial layers (I and II) (Martinez-Mir et al., 1990), where there is a denser histaminergic innervation (Panula et al., 1990). The H2R distribution is thus consistent with the histamine projection in the cortex in both humans and rodents (Ruat et al., 1990, Vizuete et al., 1997). A close functional relationship between these two components of the histaminergic system is also supported by the observation that H2R expression is significantly lower in HDC knockout mice brain, (Fitzsimons et al., 2001).

Interestingly, neither the H1R knockout nor H2R knockout mice, but only the combined H1R and H2R knockout mice show suppressive roles of histamine on methamphetamine-induced behavioral sensitization (Ogawa et al., 2009). In addition, histamine increases excitability of rat spinal motoneurons via either H1R or H2R, (Wu et al., 2012). Both observations imply that H1R and H2R are synergistically functioning in locomotion.

**Histamine receptor 3 (H3R)**

H3R was first discovered in 1983, by the group of J.C. Schwarts, as a presynaptic auto receptor that regulated the synthesis and release of histamine (Arrang et al., 1983). 15 years later, this receptor was cloned (Lovenberg et al., 1999). Subsequently, H3R was found to consist of a large number of receptor isoforms with different distribution and pharmacological profiles (Coge et al., 2001a, Lovenberg et al., 2000).

Immunohistochemical studies in mice and radioligand binding studies in rat revealed high H3R expression levels in the deep layers of the cerebral cortex, dentate gyrus and subiculum of hippocampal formation (Chazot et al., 2001, Pollard et al., 1993). H3R radioligand binding sites were observed in the middle layers (III, IV) of the cerebral cortex and in the thalamus in human post-mortem tissue (Jin et al., 2002, Jin et al., 2005). Our qPCR and immunohistochemistry results show that both H3R-mRNA and H3R protein expression levels are higher in putamen than in SN or caudate, which is in agreement with a previous report showing that both H3R-mRNA and protein levels are higher in the striatum than in the SN in both rodents and human (Anichtchik et al., 2001, Chazot et al., 2001, Pillot et al., 2002a). The H3R knockout mouse shows decreased spontaneous locomotor activity, wheel running behavior and body temperature (Toyota et al., 2002), a metabolic syndrome with hyperphagia, late-onset obesity associated with hyperinsulinemia and leptinemia, (Tokita et al., 2006, Yoshimoto et al., 2006) and increased severity for neuroinflammatory diseases (Teuscher et al., 2007). Moreover, histamine modulates core body temperature by acting on the mice anterior hypothalamic neurons that express H1R and H3R, respectively (Lundius et al., 2010). These data indicate the involvement of H3R in a variety of brain functions, including arousal, locomotor activity, thermoregulation and food intake.
Stimulation of H₃R activates several intracellular pathways, including Gᵢ/o dependent inhibition of adenylate cyclase (Clark et al., 1996), activation of phospholipase A₂; Akt and mitogen-activated kinase (Bongers et al., 2007b) as well as inhibition of the Na⁺/H⁺ exchanger and of K⁺-induced Ca²⁺ mobilization (for references see (Bongers et al., 2007a)).

H₃R is not only localized on somata, dendrites and axons of the TMN as an autoreceptor. Activation of H₃R as a heteroreceptor located in a number of brain areas, inhibits the release of various neurotransmitters (see review (Leurs et al., 1998, Sander et al., 2008)). In animal models, inverse agonists/antagonists of H₃R increase the release of neuronal histamine, acetylcholine (ACh), norepinephrine (NE) and dopamine (DA) in different brain areas by synaptic signals that are not yet well understood (Giannoni et al., 2010, Pilot et al., 2002b, Schlicker et al., 1999). Therefore, several H₃R antagonist/inverse agonist have been introduced in preclinical trials to treat different symptoms of neurological diseases, such as sleep-wake disorders in PD and narcolepsy, and cognitive disorders in AD and attention-deficit hyperactivity disorder (Brioni et al., 2010, Lin et al., 2011). However, according to our postmortem brain material study findings (Shan et al., 2012a, Shan et al., 2012b, Shan et al., 2011), so far, the application of H₃R antagonist/inverse agonist in PD and AD deserves careful consideration (see below). It should also be noted that in a recent clinical pilot study one H₃R-antagonists/inverse agonist did not show positive effects in AD treatment (Egan et al., 2012).

Histamine receptor 4 (H₄R)

The novel receptor H₄R has been reported to be functionally expressed in the human brain (Coge et al., 2001b, Connelly et al., 2009). It was found that cortical neurons are directly hyperpolarized via H₄R, as opposed to H₁R, which indirectly depolarizes cortical neurons by closing the leak potassium currents (Connelly et al., 2009, Reiner et al., 1994).

A high expression of H₄R was found by immunohistochemistry in the deep layers of the human cortex (lamina VI and V) (Connelly et al., 2009). Although H₄R knockout mice have been documented (Hofstra et al., 2003), the behavior phenotype of this animal warrants further study.

Since H₄R shares a high sequence similarity with H₃R, it is not surprising that H₄R is targeted by various imidazole-containing H₃R ligands (Lim et al., 2005). Therefore, some of the previous H₃R pharmacology and binding studies urgently require revaluation by more specific ligands. Moreover, we have reported, for the first time, an increase in H₄R-mRNA expression in the striatum in PD (Shan et al., 2012a), which warrants further studies to uncover its functional relevance and clinical implications.

HMT

Histamine in the brain is inactivated by HMT, which transfers a methyl group from S-adenosyl-L-methionine to the nitrogen atom of the imidazole ring, yielding tele-methylhistamine and S-adenosyl-L-homocysteine (Schwartz et al., 1991). It was unclear from the literature in which compartment HMT was located. On the one hand, HMT immunocytochemistry shows positive staining of HMT in bovine brain neurons, endothelial cells (Nishibori et al., 2000) and rodent synaptosomal membrane (Barnes et al., 2002), on the other hand, according to pharmacological studies in rodent the glia compartment is thought to play a major role in histamine inactivation (Huszti et al., 1990).

In our study on postmortem human PFC, in spite of the positive correlation between HMT-mRNA and the astrocyte marker GFAP in both controls and AD patients, HMT appeared to be localized only in the neurons and there was no co-localization of HMT-mRNA and GFAP in astrocytes in the human PFC (Shan et al., 2012b) (Figure 8). The X-ray crystallographic structures of two human HMT polymorphic forms have been identified (Horton et al., 2001). Following up a polymorphisms study that showed a higher activity form, Thr105Ile, HMT was found to be associated with PD (Agundez et al., 2008, Ledesma et al.,...
2008), although another study could not replicate this finding (Keeling et al., 2011). In addition, Thr105Ile HMT was reported not to be associated with AD (Marasovic-Susnjara et al., 2011).

**Translationally controlled tumor protein**

The translationally controlled tumor protein was discovered in Ehrlich ascites tumor cells and was characterized by MacDonald as a histamine-releasing factor (HRF, see review of (Telerman et al., 2009)). Little is known about the function of this novel protein. The HRF-mRNA level was found to be decreased in the hippocampus in schizophrenia (Chung et al., 2003), and the protein level of HRF was found to significantly decrease in both Down syndrome and AD temporal cortex (Kim et al., 2001). Therefore, HRF was proposed to be associated with cognitive deficits (Chung et al., 2003).

3. Neuronal histaminergic system in neuropsychiatric disorders

3.1. PD

There were conflicting opinions about the nature of alterations of the neuronal histaminergic system in PD. On the basis of the abundant accumulation of the characteristic neuropathological PD lesions, i.e. Lewy bodies (LBs) and Lewy neurites (LNs) in the TMN of PD patients, a severe destruction of this nucleus was presumed to occur in the course of this disorder (Braak et al., 1996, Braak et al., 2003). In contrast, 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 the SN (Liu et al., 2008). In addition, 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). Consequently an increased histamine activity was presumed to occur in PD (Anichtchik et al., 2001, Anichtchik et al., 2000a).

In contrast to the presumed degeneration of the TMN in PD, we observed no clear quantitative changes in TMN HDC-mRNA in PD (Shan et al., 2011). This is in line with the intact number of histaminergic neurons (Nakamura et al., 1996), as well as with the unchanged enzyme activity of HDC (Garbarg et al., 1983) and with the unaltered t-MeHA levels in the CSF in PD (Prell et al., 1991b). The unchanged TMN HDC-mRNA in PD showed for the first time that the accumulation of LBs and LNs in the TMN was not influencing HDC-mRNA expression. Moreover, not only in clinical PD, but also in preclinical PD did we find an unchanged HDC-mRNA expression, while there was no or little accumulation of LBs and LNs in the TMN (Shan et al., 2011).

Furthermore, we found a significant decrease of H3R-mRNA in the SN in PD (Shan et al., 2012a). Immunocytochemistry in the SN of the same subjects revealed a nearly exclusive localization of H3R in the large neuromelanin-containing neurons. The lower density of these neurons in PD thus offers an explanation for the decreased H3R expression levels in the SN in PD.

There was an increase in the density of histaminergic fibers in the SN of PD patients (Shan et al., 2012a). This observation supported the possibility that, in PD, the histamine levels may be increased in brain areas such as the putamen and SN (Rinne et al., 2002). Our recent study showed that in the same brain areas of PD patients there was an up-regulation of HMT-mRNA (Shan et al., 2012a), which may act as a protective mechanism by metabolizing enhanced histamine levels in these areas. Such a protective effect might be of importance, since 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). In addition, we observed an inverse correlation between HMT-mRNA expression and disease duration in the SN of PD patients, suggesting that the more serious (thus the shorter lasting) the disease, the more HMT-mRNA is expressed, which further supports such a compensa-
tory mechanism. This also means that special attention should be paid to PD with regard 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 the degeneration of neurons, e.g. in the putamen or SN in PD.

### 3.2. AD

Alterations in the histaminergic system in AD are controversial. On the one hand it is known that the accumulation of neurofibrillary tangles (NFT) takes place in the TMN in 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 high performance liquid chromatography (HPLC) results, showing that histamine levels diminish in different brain areas in AD, including the hippocampus, frontal and temporal cortex (Mazurkiewicz-Kwilecki et al., 1989, Panula et al., 1998) and with the decreased neuronal metabolic activity of the TMN observed in AD (Nakamura et al., 1993, Salehi et al., 1995). In contrast, however, several other reports claim that the histaminergic system may be hyperactive both in aging (Prell et al., 1988) and in the course of AD (Cacabelos et al., 1989, Fernandez-Novoa et al., 2001). Increased histamine levels have been reported not only in the frontal cortex, basal ganglia and hippocampus (Cacabelos et al., 1989), but also, together with its metabolites, in the CSF of AD patients (Fernandez-Novoa et al., 2001). It should be noted that the differences in putative confounding factors, such as postmortem delay (PMD), gender and age, may have contributed to the varying results (Panula et al., 1998).

Interestingly, although we confirmed again that the TMN neurons were significantly (57%) lost in AD, the total HDC-mRNA expression level was not significantly decreased (24%) in these patients (Shan et al., 2012b). A slightly but not significantly lower level of the histamine metabolite t-MeHA (22%) in the CSF of AD patients fully supported our finding (Motawaj et al., 2011). It implies that the significant (57%) loss of large TMN neurons in AD patients is largely compensated. The mechanism underlying such a functional compensation of the TMN in AD certainly deserves further study. In addition, increased H₃R and HMT-mRNA expression was found in the PFC of AD patients, but only in females. Moreover, a significant positive correlation was observed in females between the H₃R-mRNA and HMT-mRNA levels on the one hand and AD Braak-stages on the other. There was no co-localization of HMT-mRNA and GFAP in astrocytes in the human PFC. In fact, HMT-mRNA was only present in neurons in the PFC (Figure 8) although there was a positive correlation between HMT-mRNA and the astrocyte marker GFAP-mRNA in both controls and AD patients (Shan et al., 2012b).
In general, our present data may also provide a rationale for the use of $H_3$R-antagonists, in particular in female AD patients, since these compounds increase the release of histamine, acetylcholine, noradrenalin and dopamine, and may in this way modulate cognitive processes in PFC. However, regarding the small $H_3$R-mRNA increase we observed, together with insignificant changes of binding density in this area (Medhurst et al., 2009), the positive effects of $H_3$R-antagonists are expected to be modest. In a recent clinical pilot study one of the $H_3$R-antagonists/inverse agonists appeared to be ineffective as far as improving cognitive function was concerned in mild to moderate AD patients who were under concomitant symptomatic AD treatment (Egan et al., 2012). In addition it should be noted that, because the activity of the remaining TMN neurons is already higher, the administration of an $H_3$R-antagonist should be done with some degree of reticence, if degeneration of these neurons is to be prevented.

### 3.3 HD

The TMN shows the highest frequency of both nuclear and cytoplasmatic inclusions of mutant huntingtin, the neuropathological hallmark of HD (Aziz et al., 2008) (Figure 9). An involvement of the histaminergic system in HD was thus presumed. In postmortem binding studies, both $H_2$R (Martinez-Mir et al., 1993) and $H_3$R (Goodchild et al., 1999) appeared to be decreased in many brain regions, especially in the striatum. $H_1$R, on the other hand, was reported to be increased in cortical areas of HD patients (Whitehouse et al., 1985).

We found an increase in HDC-mRNA levels in the TMN, an increase in HMT, $H_1$R and $H_3$R-mRNA levels in the inferior frontal gyrus (IFG) of HD patients (van Wamelen et al., 2011). Moreover, we observed a negative correlation in the IFG between the age at onset of disease and HMT-mRNA, suggesting that the more serious (thus the shorter lasting) the disease was, the more HMT-mRNA was expressed. Since the levels of histamine metabolites in CSF have also been shown to be increased in HD patients, an enhanced activity of the histaminergic system seems to be present in HD.

PET scanning revealed a loss/dysfunction of dopamine 2 receptor binding in the hypothalamus of clinical HD patients and pre-manifest HD patients (Politis et al., 2008). Interestingly, a recent rodent study showed that activation of the dopamine 2 receptor on TMN neuron increased its firing and histamine release (Yanovsky et al., 2011). Therefore, a decreased availability of dopamine 2 receptors on TMN neurons may contribute to the HDC-mRNA increase that we reported (van Wamelen et al., 2011).

All these findings suggest a functional increase of brain histaminergic signaling which may contribute to sleep disturbances (Yanovsky et al., 2011), weight loss and neuronal loss in HD patients. Our findings provide a rationale for the use of $H_3$R-agonists in HD patients.

### 3.4 Multiple sclerosis (MS)

MS is an inflammatory disorder of the central nervous system associated with chronic and extensive neurodegeneration (Compston et al., 2008). For MS therapy, there are five possible mechanisms by which histamine may have a positive effect: 1) increased histamine levels in the subnormal cerebral tissue, 2) improved electrical function of demyelinated fibers, 3) increased cerebral blood...
flow, 4) suppressed autoimmune responses, and 5) remyelination stimulation (Gillson et al., 2000).

It was observed that the experimental allergic encephalomyelitis (EAE), one of the experimental MS models, is more severe in HDC knockout, histamine-deficient mice, with diffuse inflammatory infiltrates containing a prevalent granulocytic component in the brain (Musio et al., 2006). In addition, an EAE model made with H₃R knockout mice showed more severe neuroinflammation compared with an EAE model made with wild type animals (Teuscher et al., 2007). These findings strongly support the possibility that the histaminergic system may play an important role in limiting the extent of immune damage to the CNS (Musio et al., 2006). However, it should be noted that histamine can change the permeability of the blood brain barrier, which may lead to elevation of infiltrating cells in the CNS and neuroinflammation (Haas et al., 2008). Although one may hypothesize that TMN neuron numbers will decrease due to the severe axonal loss and cortical involvement in MS patients (Schirmer et al., 2012), this has never been studied yet. It should be noted that the results of histamine and t-MeHA levels in CSF in MS are contradictory. Relatively low CSF levels of histamine and histamine metabolites were indeed recently reported in MS (Bassetti et al., 2010). However, unchanged histamine and t-MeHA CSF levels had been observed earlier, both in chronic progressive MS and relapsing-remitting MS (Rozniecki et al., 1995). In contrast, another study even showed a CSF histamine level that was 60% higher in MS than in controls (Tuomisto et al., 1983). Again, age, sex and diurnal fluctuations are putative confounders regarding these contradictory results. Furthermore, in one study, H₁R expression was found to increase 4.6-fold in chronic silent MS lesions as compared to acute/active lesion of MS (Lock et al., 2002). It would be worth following up the observation that the use of H₁R-blockers is associated with decreased MS risk (Alonso et al., 2006). To pave the road for histamine-related therapeutic strategies, the putative alterations in neuronal histamine production, release, and breakdown, and in receptors should be systematically studied in MS patients.

3.5 Depression

Since hypothalamic histamine significantly increases when animals are exposed to acute stress (Mazurkiewicz-Kwilecki et al., 1986), and 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). This possibility was supported by the observation that histamine, when infused intracerebroventricularly in the rat, increased by way of H₁R and H₂R, the mRNA expression of stress-related neuropeptides such as CRH, arginine vasopression, and oxytocin in the paraventricular nucleus and oxytocin in the supraoptic nucleus (Kjaer et al., 1994). In addition, a PET scanning experiment showed a decrease in H₁R-binding that was correlated with severity of depression symptoms in living depression patients (Kano et al., 2004). We found no difference in the HDC-mRNA expression in the TMN in depression. Furthermore, a lack of correlation between HDC-mRNA and the number of CRH-expressing neurons was observed (Chapter 8). The idea that changes in the histaminergic system play a key role in the activation of the hypothalamo-pituitary-adrenal (HPA) axis is thus not supported. The latter system is considered to be a key factor in the pathogenesis of depression (Bao et al., 2008). These seemingly discordant results suggest that the animal models used may not elucidate relevant aspects of the etiology and pathophysiology of depression-related disorders (Neumann et al., 2011). Systematic validation of results obtained with animal models in patients and postmortem material studies is thus crucial. Interestingly, our data also point to the possibility that the diurnal fluctuations of histamine receptors in the cortex are diminished in depression. This is in agreement with a disorder of the day-night fluctuation observed in the biological clock, the SCN, in depression (Zhou et al., 2001). These preliminary data warrant further study.
3.6 Schizophrenia

Schizophrenia affects about 1% of the total world population. Cognitive impairment is a core feature of this disorder (Lewis et al., 2000). It was reported that the mamillary body, which is surrounded by the TMN neurons, is larger in schizophrenia patients and more so in women than in men (Goldstein et al., 2007). Both PET studies and postmortem brain samples show decreased H₁R-binding sites in the frontal and cingulate cortex in schizophrenia (Iwabuchi et al., 2005, Nakai et al., 1991). In addition, famotidine, a H₂R-antagonist, reduces negative symptoms in schizophrenia, as supported by several open-label clinical trials (Kaminsky et al., 1990, Oyewumi et al., 1994, Rosse et al., 1996). In a postmortem study, increased H₃R radioligand binding was found in the dorsolateral PFC of schizophrenic patients (Jin et al., 2009). Moreover, elevated levels of t-MeHA, the major histamine metabolite, were found in the CSF which indicates a higher central histaminergic activity in patients with chronic schizophrenia (Prell et al., 1996, Prell et al., 1995). Our pilot study showed a significant increase of HMT-mRNA in the superior frontal cortex of schizophrenic patients, thus supporting the presence of an elevation of histamine turnover (Shan et al. Chaper 9). This observation is of potential clinical importance because the H₃R-inverse agonist/antagonist may thus be used as intervention in this disorder (Brioni et al., 2010).

4. Conclusions

First of all, we established a reliable measurement to quantify the mRNA level of the key enzyme for histamine production, histidine decarboxylase (HDC), in formalin-fixed, paraffin-embedded archival postmortem human brain tissue. The level of HDC-mRNA expression is known to be a sensitive indicator for histamine levels in the brain. We observed diurnal fluctuations of histamine production in control subjects without neuropsychiatric disorders, with the highest levels around 18:09. This fluctuation was strongly altered in a group of patients with neurodegenerative diseases, including PD, AD, and HD.

Different alterations in the neuronal histaminergic system shown as expression levels of HDC, HMT, H₁₄R and HRF were found in various neuropsychiatric disorders (Table 1). Discrepancies between animal models and postmortem human brain material study results have made it clear that the validation of animal models is necessary and that studies on patients and human postmortem material are essential to understand the changes occurring in neuropsychiatric disorders.

On the basis of available data one may hypothesize that the neuronal histaminergic system is a potential target for the treatment of the circadian rhythm disorders in PD, AD, and HD. H₃R-antagonists could thus be a valuable adjunct treatment for these diseases. Due to their potential side effects, such as the induction of degeneration of dopaminergic neurons in the SN or the over-activation of the remaining TMN neurons in AD, the administration of H₂R-antagonist in PD and AD should be under strict control.

The augmentation of histamine in both HD and schizophrenia may provide a rationale to apply an H₃R-agonist.

Scope of the present thesis

The overall aim of this thesis is to investigate the human neuronal histaminergic system in health and its changes in neuropsychiatric diseases. The TMN in the posterior hypothalamus is the exclusive source of neuronal histamine is involved in a number of basic physiological functions, such as the sleep-wake cycle, energy and endocrine homeostasis, sensory and motor functions, cognition, attention, learning and memory (Haas et al., 2008). In addition, activation of H₃R as a heteroreceptor located in different brain areas inhibits the release of various neurotransmitters (see reviews (Leurs et al., 1998, Sander et al., 2008)). In animal models, inverse agonists/antagonists of H₃R increase the release of neuronal histamine, acetylcholine, norepinephrine and dopamine (Gianonii et al., 2010, Pillot et al., 2002b, Schlicker et al., 1999) in different brain areas by synaptic signals that are not yet well understood. A study of the histaminergic system in neuropsychiatric
disorders appears to be especially timely, since H₃R-antagonists/inverse agonists are making their way into the clinic for potential treatment of AD, PD and schizophrenia (Brioni et al., 2010, Passani et al., 2011), without taking into account the recent information on alterations in HRs and histamine production as reviewed in the present thesis (Chapter 1).

It has been known for a while that the use of radioactive in situ hybridization to quantitatively determine low-to-moderately abundant mRNA expression in formalin-fixed, paraffin-embedded postmortem human brain and other tissues is often hampered by non-specific deposits, visible as speckles. Therefore, in Chapter 2 we report on the optimization of the hybridization conditions, which allows adequate signal quantification of HDC-mRNA, the key enzyme of histamine production in TMN. In our studies (Chapter 3, 4, 6 and 7), HDC-mRNA expression levels in the postmortem TMN have shown to be a useful indicator of histamine production, since the same direction of changes was found between HDC-mRNA expression levels in the TMN and histamine or histamine metabolites, t-MeHA, in the CSF - both indicators of central histamine neurotransmission (Soya et al., 2008).

Histamine levels show diurnal rhythms in rodents and play a major role in the maintenance of vigilance (Mochizuki et al., 1992). No data are available on their diurnal fluctuation in the human brain, either in health or in neurodegenerative disorders such as PD, AD or HD, all of which are characterized by sleep-wake disturbances. In Chapter 3 we examine the diurnal fluctuations in HDC-mRNA expression in control patients and neurodegenerative disorders. HDC-mRNA levels in controls were found to be significantly higher during the daytime than at night. This day-night fluctuation was markedly disturbed in patients with neurodegenerative diseases.

Animal experiments have shown that histamine induces or accelerates the degeneration of dopaminergic neurons in substantia nigra pars compacta (SNpc) (Liu et al., 2007, Vizuete et al., 2000). In the classic PD model of 6-OHDA-lesioned rat, an increase of endogenous histamine enhanced the apomorphine-induced turning behavior and increased the loss of tyrosine hydroxylase-expressing neurons in the SNpc, while a decrease of endogenous histamine as a result of an inhibition of the HDC strongly reduced the rotation behavior and prevented the loss of TH-expressing cells in an early stage of the 6-OHDA lesion (Liu et al., 2007). Furthermore, in PD patients, it was known that Lewy bodies/Lewy neurites (LBs/LNs) accumulated strongly in the TMN. However, there were controversial ideas about the functional consequences on histamine production. In Chapter 4, we demonstrate that there is no relationship between LBs and LNs aggregation in TMN and HDC-mRNA expression in the course of PD. To further understand whether the histaminergic system is crucially involved in the pathology of PD, we measured the changes of HRs and HMT-mRNA levels, together with immunocytochemical localization H₃R, in the histaminergic projection areas including the SN and the striatum in Chapter 5.

In Chapter 6 we show that both histamine production in the TMN and the expression of histamine-related genes are upregulated in HD patients in the IFG. These findings indicated a general increase in brain histaminergic signaling in HD. The reports on alterations in the histaminergic system in AD are equivocal, probably because of the influence of putative confounding factors from previous studies, such as PMD, gender, and age (Panula et al., 1998). In Chapter 7 we study the possible alterations of neuronal histamine production in AD by relating the changes of TMN neuron numbers to histamine production as determined by quantitative in situ hybridization of HDC-mRNA expression in the TMN in the last stage of AD (Braak 6) (Braak et al., 1991). In addition, in one of the major neuronal histamine projection areas, the PFC, we used real time quantitative polymerase chain reaction to assess the mRNA levels of the four major HRs (H₁-₄R) and of HMT, as well as of several glia markers. Furthermore, double-labeling of HMT-mRNA in situ hybridization and glial fibrillary acidic protein (GFAP)-immunocytochemistry was performed to explore
the localization of histamine inactivation in the human PFC.

Since hypothalamic histamine significantly increases when animals are exposed to acute stress (Mazurkiewicz-Kwilecki et al., 1986), and both acute and chronic stress increase histamine turnover (Ito, 2000), it was proposed that the histaminergic system may be involved in the pathogenesis of depression (Jin et al., 2009). In Chapter 8 we therefore studied the histamine production in depression TMN and histaminergic genes in PFC and anterior cingulated cortex.

Chapter 9 deals with a pilot study we performed, to determine the alterations of the histaminergic system in schizophrenia.

In the last chapter of my thesis (Chapter 10), the clinical significance of our studies is discussed and future experiments are proposed.

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## Postmortem Histamine Projection brain areas

<table>
<thead>
<tr>
<th>Disorders</th>
<th>Histamine production</th>
<th>Histamine Projection brain areas</th>
<th>Spinal CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson’s Disease</td>
<td>– († LB, LN)</td>
<td>SN (HMT+51%; H₄R-40%)</td>
<td>–/ † (-22%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PU (HMT+51%; H₄R-23%; H₃R +4.2 folds)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN (H₄R +6.3 folds)</td>
<td></td>
</tr>
<tr>
<td>Alzheimer’s Disease</td>
<td>†(1NFT)</td>
<td>SPFC (HMT and H₄R †)</td>
<td>–/ † (-22%)</td>
</tr>
<tr>
<td>Huntington’s Disease</td>
<td>–(Mutant Hutingin)</td>
<td>IFG† (H₄R+133%; H₃R+84%; HMT+83%)</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>†(+63%)</td>
<td>CN† (H₄R+96%; H₃R+100%)</td>
<td>†</td>
</tr>
<tr>
<td>Depression</td>
<td>–</td>
<td>– DLPFC; – ACC</td>
<td>?</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>?</td>
<td>SPFC †(HMT; HRF)</td>
<td>†</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 1 overview of alterations of brain histaminergic system in neuropsychiatric disorders