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The roles of histamine and its receptor ligands in central nervous system disorders: an update

Weiwei HU; Zhong Chen*

Department of Pharmacology, Key Laboratory of Medical Neurobiology of the Ministry of Health of China, School of Basic medical sciences, College of Pharmaceutical Sciences, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310058, P. R. China

*Correspondence to:
Zhong Chen, Ph.D., Professor
Tel & Fax: +86-571-88208228
Email address: chenzhong@zju.edu.cn
Abstract
The neurotransmitter histamine receives less attention compared with other biogenic amines, because of its moderate action in the central nervous system (CNS). However, recent evidence suggests that histamine plays an important role in multiple CNS disorders including insomnia, narcolepsy, Parkinson’s diseases, schizophrenia, Alzheimer's disease, and cerebral ischemia. New insights are emerging into the potential roles of histamine receptors as targets for the treatment of these diseases. Although some histamine related agents have failed in clinical trials, current preclinical studies suggest that this neurotransmitter may still have extensive applications in treating CNS disorders, however, advanced studies are warranted. This review summarizes findings from animal models and clinical research on the role of histamine and its receptor ligands in the brain for treatment of CNS disorders. The development of novel histamine receptor ligands and gaining an in-depth understanding of their potential mechanisms are necessary stepping stones to unlock their wide-ranging applications in the clinical arena.

Keywords: Alzheimer's disease, cerebral ischemia, histamine, histamine receptor, Parkinson's diseases, sleep disorders, and schizophrenia

Abbreviations: AD, Alzheimer's disease; Arch, aequorin; BBB, brain blood barrier; cyclic adenosine monophosphate (cAMP); CBF, cerebral blood flow; ChR2, channelrhodopsin-2; CREB, cAMP response element-binding protein; CSF, cerebrospinal fluid; CNS, central nervous system; GPe, globus pallidus; GPi, internal segments of the globus pallidus; GS, glutamine synthetase; HCRT, hypocretin; HDC, histidine decarboxylase; HNMT, histamine N-methyltransferase; IP3, inositol trisphosphate; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCAO, occlusion of the middle cerebral artery; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MSNs, medium-spiny projection neurons; NMDA, N-methyl-d-aspartate; NSCs, neural stem cells; 6-OHDA, 6-hydroxydopamine; PD, Parkinson’s disease; PFC, prefrontal cortex; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PLA2, phospholipase A2; rCBF, regional cerebral blood flow; rtPA, recombinant tissue plasminogen activator; SNc,
substantia nigra pars compacta; SNr, substantia nigra pars reticulate; STN, subthalamic nucleus; SVZ, subventricular zone; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus.

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1. Introduction

In contrast with other biogenic amines, histamine has garnered less attention in the context of neuroscience. This is likely due to its relatively moderate action to fine tune other principal neurotransmitters, such as excitatory glutamate and inhibitory GABA. However, the direct regulation of those excitatory and inhibitory neurotransmitters in cerebral disorders may elicit severe side effects, which impede their extensive use for medical applications. Therefore, the pharmacological application of histamine related agents may bring forth new prospects for the therapy of several CNS disorders. Taken together, it is imperative to expand our knowledge regarding the role of histamine in the central nervous system. In this review, we provide an overview of the action of histamine and its receptor ligands in animal studies and clinical trials of several CNS disorders, with a focus on cerebral ischemia, which receives less attention. This review also summarizes the prospects of further preclinical studies in this field.

2. Histamine in the brain

Histamine is produced in peripheral tissues by mast cells and basophils found in nearby connective tissues to participate in allergic and inflammatory responses. Another important site of histamine storage and release is the enterochromaffin-like cells in the stomach, which control gastric acid release. The presence of histamine in the brain was first mentioned by Kwiatkowski in 1943 (Kwiatkowski, 1943), and was one of the last organs where histamine receptors were identified. The histaminergic neuron is the main source of histamine production in the brain, the soma of which is located in the tuberomammillary nucleus (TMN) of the hypothalamus (Haas and Panula, 2003). Approximately 64,000 histaminergic neurons are found in the tuberomammillary nucleus in humans (Airaksinen et al., 1991), while only 4,600 are localized in the brains of rats (Ericson et al., 1987). Although the location of histaminergic cell body is limited to a small area, its projections are widely spread throughout the brain, including the cerebrum, cerebellum, posterior pituitary, and the spinal cord. The synthesis of histamine relies on the action of histidine decarboxylase (HDC), an enzyme that catalyzes the oxidative decarboxylation of L-histidine. HDC knockout mice are often used to study the role of
histamine in the brain (Watanabe and Yanai, 2001). The newly generated HDC-Cre mice have provided a superior approach to selectively modulate histaminergic neurons through cross-breeding with conditional knockout mice or by conditional expression of channelrhodopsin-2 (ChR2) to activate specific neurons (Williams et al., 2014; Yanovsky et al., 2012). The synthesized histamine is carried into vesicles by the vesicular monoamine-transporter VMAT-2. After release, histamine is metabolized by histamine N-methyltransferase (HNMT) into inactive tele-methylhistamine in the postsynapses or glia. The turnover rate for neuronal histamine is rapid (half-life <1 h); therefore histamine levels in brain tissue can be used as good approximations of the release of neuronal histamine. However, the turnover rate may be altered under certain conditions (Schwartz et al., 1991), so direct measurement of extracellular histamine levels by microdialysis is a more precise method to detect the status of its release. Mast cells also contain a significant amount of brain-derived histamine (Grzanna and Shultz, 1982), but are limitedly distributed in the thalamus, hypothalamus, dura mater, leptomenings, and choroid plexus (Ibrahim, 1974). Other possible sources of histamine in the brain may include microglia and microvascular endothelial cells (Katoh et al., 2001; Yamakami et al., 2000). However, the action of nonneuronal histamine remains elusive.

3. Histamine receptors and ligands

Four types of histamine receptors have been identified in brain. Histamine H1 and H2 receptors (H1R and H2R) are found postsynaptically in all parts of the brain, including the cortex, hippocampus, striatum, and hypothalamus. The H1R (486-491 amino-acids) is coupled to the Gq/11 protein and phospholipase C, which is known to promote inositol trisphosphate (IP3)-dependent Ca\(^{2+}\) release from intracellular Ca\(^{2+}\)-stores, and is also directly involved in diacylglycerol formation. The latter, in turn, activates protein kinase C, which phosphorylates intracellular proteins. H1R also activates AMP-kinase, nuclear factor kappa B, nitric oxide synthases, and phospholipase A2 (PLA2), which induces arachidonic acid formation (Haas et al., 2008). H2R (359 amino-acids) is coupled to Gs and stimulates adenylylcyclase, thereby increasing intracellular cyclic adenosine monophosphate (cAMP), which in turn activates protein kinase A (PKA) and the
transcription factor cAMP response element-binding protein (CREB). H2R activation also blocks Ca\(^{2+}\)-activated potassium conductance and inhibits both PLA2 and the release of arachidonic acid. Although the expression levels and location of H1R and H2R are comparable in the brain (Haas and Panula, 2003), H1R is the predominant histamine receptor in the brain in terms of function, leading to many open questions for researchers.

The histamine H3 receptor (H3R; 326-445 amino-acids) is located on histaminergic neuron somata, dendrites and axons, as well as on the axons of other neurons, providing negative feedback to inhibit histamine synthesis and the release of histamine or other transmitters, including glutamate, acetylcholine, and GABA. However, vast majority of H3Rs are postsynaptically present in the basal ganglia and especially within the dorsal and ventral striatum. H3R is coupled to G\(\text{G}_i/\text{G}_o\) to inhibit adenylyl cyclase and the high voltage activated Ca\(^{2+}\) channels that are responsible for regulation of histamine synthesis and the neurotransmitter release. Other signaling pathways have been reported to be involved in H3R activation, such as the inhibition of the Na\(^+\)/H\(^+\) exchanger (Karmazyn, 1999), the enhancement of G protein-gated inwardly rectifying K\(^+\) channels (Sahlholm et al., 2012), phospholipase C activation (Coge et al., 2001), the mitogen-activated protein kinase (MAPK) pathway (Drutel et al., 2001), and the phosphatidylinositol 3-kinase (PI3K) pathway (Bongers et al., 2007).

The histamine H4 receptor (H4R) was recently discovered, and is predominantly expressed in immune cells, including mast cells, eosinphils and dendritic cells. In the brain, functional H4R is present in microglia, however its function in these cells remains mysterious. Thus far, there has been no convincing evidence confirming the expression of H4R in neurons, which is due to flawed methods used for detection (Schneider and Seifert, 2016). Further studies from independent research groups will be necessary with more robust techniques including the use of monoclonal anti-H4R antibodies, qPCR experiments, and concentration dependent stimulation of H4R by agonists. H4R shares ~40% homology with H3R, and belongs to the class of Gi/Go-coupled GPCRs that function to reduce cAMP accumulation. In addition, activation of the H4 receptor has also been shown to increase Ca\(^{2+}\) mobilization and activate kinases (ERK, PI3K, and MAPK) and transcription factor activating protein-1 (Dong et al., 2014; Jemima et al., 2014).
Despite their importance, H1R or H2R agonists have not been directly applied in clinic due to their broad and largely undesirable peripheral action. The first generation of H1R antagonists, including diphenhydramine, promethazine, tripelennamine, and chlorphenamine, have been shown to penetrate the brain blood barrier (BBB), and often show strong sedative effects. Also, anticholinergic activities were often found in first generation histamine H1R antagonists. The second generation of H1R antagonists (cetirizine, terfenadine, astemizole, and loratadine, et al.) has displayed long-term action, but showed weak sedative and anticholinergic effects with a relatively poor capacity for BBB penetration (Yanai et al., 1995a; Yanai et al., 1995b). Among these second generation antagonists, terfenadine and astemizole have both been withdrawn from the market due to their cardiotoxicity. Third generation H1R antagonists are active enantiomers (levocetirizine) and metabolites (desloratadine and fexofenadine) of the second generation H1R antagonists. Fexofenadine has been associated with a decreased risk of cardiac arrhythmia compared to terfenadine. In contrast, there is almost no advantage of levocetirizine or desloratadine, compared to cetirizine or loratadine, respectively, so the term “third generation” should be used with caution (Camelo-Nunes, 2006; Kalpaklioglu and Baccioglu, 2012). The sedative effects of H1R antagonists were initially recognized as side effects for the treatment of allergic rhinitis, but such action is responsible for the use of the induction of sleep. Due to blockage of H1R in the emetic center, H1R antagonists are also often used for the treatment of motion sickness. However, a recent study emphasized the need for caution in the application of such drugs in older hospitalized patients because there have been putative links to the onset of delirium (Rothberg et al., 2013), and H1R antagonists may also affect cognitive functioning to different extents and increase the susceptibility of patients to seizures (Hu et al., 2012; Simons et al., 1996). Moreover, H1R is found to be occupied by other drugs, such as antidepressants mirtazapine and antipsychotics olanzapine and quetiapine from positron emission tomography study in humans (Sato et al., 2015; Sato et al., 2013). Therefore, further evidence is needed to elucidate the role of H1R in CNS disorders, however, this knowledge will be used to guide the development of a new generation of H1R antagonists.
The H2R antagonist cimetidine (Tagamet®) became the first billion-dollar drug in the 1980s for the treatment of peptic ulcer disease. Other H2R antagonists include ranitidine, famotidine, nizatidine, and zolantidine, the latter showing good BBB penetration with a steady-state brain/blood ratio of 1.4 (Young et al., 1988). Despite the widespread use of H2R antagonists for control of gastric acid release, they are rarely considered as CNS drugs. There are some relevant preclinical research studies highlighting their potential to enhance the effects of opiate analgesics (Nalwalk et al., 1995) and for treatment of psychotic disorders (Kaminsky et al., 1990; Mehta and Ram, 2014).

H3R has been viewed as a potential pharmacological target for the treatment of cerebral disorders, because it is primarily expressed in the brain (Nuutinen and Panula, 2010). Due to the high constitutive activity of H3R, both the agonists and the antagonists have received much attention. All H3R agonists, including N\textsuperscript{a}-methylhistamine, \((R)-\alpha\)-methylhistamine, imetit, immepip, methimmepip, proxyfan, and GT-2331, contain the imidazole moiety, which is derived from histamine. Antagonists of H3R are classified into imidazole-based and non-imidazole-based antagonists. Most imidazole-based H3R antagonists, including thioperamide, clobenpropit and ciproxifan, have the disadvantage of poor BBB penetration, interaction with cytochrome P450 proteins, hepatic and ocular toxicities, and incidence of off-target activity at H4R and other receptors (Berlin et al., 2011). To avoid these problems, non-imidazole-based H3R antagonists, with high affinity and selectivity, have been developed. These include ABT-239, pitolisant, JNJ-5207852, NNC 381049 and GSK189254. Additionally, a H3R antagonist/inverse agonist has been reported to have potential value in the treatment of wake-sleep disorders, cognitive impairment in Alzheimer’s disease (AD), and schizophrenia. In March 2016, pitolisant (Wakix®) was approved by the European Commission for the treatment of narcolepsy after a decade of intensive studies, which brings new prospects for the development of H3R ligands. However, it is noteworthy that various isoforms of the H3R have been identified in several different species including humans. The long isoform is largely predominant, however, several shorter functional isoforms have been identified including those with deletions in the third intracellular loop (Coge et al., 2001). The autoreceptors that have been shown to modulate histaminergic neurons are short isoforms (Gbahou et
Importantly, H3R isoforms differ markedly in their pharmacological profiles, for example the stereoselectivity of N’Me-αClMeHA enantiomers differs between the short and long isoforms of H3R (Gbahou et al., 2012). It points out the necessity for clarifying the role of H3R in specific cell types and brain regions, in addition to understanding how protein-ligand interactions vary between different H3R isoforms.

Many H3 receptor ligands, especially imidazole-containing compounds, also possess significant affinity for the H4R, including (R)-α-methylhistamine, imetit, immepip, and clobenpropit acting as agonists, and thioperamide acting as inverse agonist of H4R. To date, 4-methylhistamine is the most selective H4R agonist, with more than 100-fold selectivity over the other histamine receptors (Lim et al., 2005). The indole carboxamide compound, JNJ-7777120, is the first selective and widely used non-imidazole-based H4R antagonist, displaying high selectivity over other histamine receptors (Thurmond et al., 2004). A 2-Aminopyrimidine compound, ZPL-3893787 (formerly PF-3893787), has completed phase I studies and is being developed as an oral treatment for atopic dermatitis (Liu, 2014). However, H4R ligands are not currently being evaluated to treat CNS disorders due to the enigmatic role of this receptor in the brain.

4. The action of histamine and the receptor related ligands in CNS disorders

In the CNS, histamine is known to regulate sleep and wakefulness, learning and memory, feeding, and energy. Here, we address its actions in relation to CNS disorders, and emphasize potential clinical applications of histamine receptor ligands (Table 1). In addition, as the effect of histamine on neuroprotection is often ignored, we spend considerable space to review those actions that are predominantly under the context of cerebral ischemia, which broadens the clinical applications of histamine related ligands.

4.1 Sleep disorders

Histamine system is known to play a significant role in the modulation of sleep and wakefulness (Lin et al., 1989; Panula and Nuutinen, 2013). During the transition from wakefulness to sleep, histaminergic neurons stop firing and then remain silent during slow-wave and rapid eye movement (REM) sleep. In contrast, they begin firing after the
transition from sleep to wakefulness (Takahashi et al., 2006). Histaminergic neuron activity is lowest during quiet waking, moderate during active waking and maximal during high vigilance. HDC knockout mice display sleep fragmentation and increased REM sleep during the light period, along with significant wakefulness deficits at the onset of darkness (Parmentier et al., 2002). Accordingly, central administration of H1R agonists increases wakefulness, whereas administration of H1R antagonists promotes sleep (Thakkar, 2011). To evaluate the effects of histamine on wakefulness and sleep, several actions have been described that involve regulating neural circuit activity (Fig.1). The ventrolateral preoptic nucleus (VLPO) has been implicated in promoting sleep. Recently, Williams, et al. used an optogenetic approach to demonstrate that histamine indirectly inhibits VLPO neurons through the activation of the GABAergic interneuron, which also reciprocally disinhibits histaminergic neurons to favor the wake states (Lin et al., 1989; Williams et al., 2014). In addition, histaminergic axons in the neocortex co-release paracrine GABA to prevent over-activation from histamine, regulating the wakefulness levels (Yu et al., 2015). Histamine can also directly modulate glutamatergic neurons of the thalamus, causing a general excitation effect in multiple brain regions (Groenewegen and Berendse, 1994; Matsumoto et al., 2001). Moreover, studies have shown that histamine induces cortical activation, not only through activating the cholinergic neurons in the basal forebrain (Vu et al., 2015; Zant et al., 2012) or in the mesopontine tegmentum (Lin et al., 1996) and activating the serotonergic neurons in the dorsal raphe nucleus, but also by direct projections to the cortex (Haas and Panula, 2003). Additionally, the hypocretin (HCRT) system, closely linked to narcolepsy, has been reported to maintain wakefulness largely via histaminergic neurons. In situ hybridization study has confirmed that HCRT neurons and histaminergic neurons are adjacent to each other in the human hypothalamus (Krolewski et al., 2010), and their projections are largely overlapped (Oh et al., 2014). HCRT neurons have been shown to directly excite histaminergic neurons though the HCRT receptor 2 (Yamanaka et al., 2002). Further, histamine also regulates HCRT neurons possibly via the H1R (Sundvik et al., 2011). H1R antagonist pyrilamine has been shown to inhibit HCRT-induced arousal in rats (Yamanaka et al., 2002) and H1R knockout mice do not show HCRT-induced increase of wakefulness (Huang et al., 2001).
Insomnia is one of the most common disturbances of the sleep-wake cycle. Currently, insomnia is often treated with short-lived benzodiazepines. H1R antagonists are well known to cross the BBB and cause drowsiness as a side effect (Lieberman, 2009). Indeed, several H1R antagonists, such as diphenhydramine and doxylamine have been used as over-the-counter sleep aids (Krystal et al., 2013), but they cause several side effects including constipation, dry mouth, and blurred vision due to their antagonism of muscarinic receptors (Table 1). In addition, protracted exposure of the CNS to the H1R antagonist diphenhydramine may cause next-day cognitive or psychomotor performance impairments (Kay et al., 1997). Therefore, it is necessary to seek more selective H1R antagonists with fewer side effects. The FDA has approved doxepin, as Silenor, for the treatment of insomnia characterized by difficulty with sleep maintenance, which has been shown to have no anticholinergic side effects (e.g., dry mouth) or memory impairment in elderly adults (Lankford et al., 2012).

Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness, cataplexy, and narcoleptic episodes. Modafinil and classical psychostimulants such as amphetamine, are currently used as a treatment for primary narcolepsy and other sleepiness disorders of various causes. Their effects do not rely on the histaminergic system, since their actions known to be preserved in HDC KO mice (Parmentier et al., 2007). However, the histaminergic system has been reported to be involved in narcolepsy. Despite reports of either lower cerebrospinal fluid (CSF) histamine (Nishino et al., 2009) or unchanged CSF histamine (Dauvilliers et al., 2012) in patients with narcolepsy, a compensatory increase in the number of histaminergic neurons was found in the TMN (John et al., 2013; Valko et al., 2013). The H3R is also a promising target for the treatment of hypersomnia because its antagonists are able to promote wakefulness through a histamine-dependent mechanism. Several reports have indicated that H3R antagonists/inverse agonists are efficacious for the treatment of narcolepsy (Dauvilliers et al., 2013; Inocente et al., 2012; Lin et al., 2008). The H3R antagonist pitolisant (previously called BF2.649) has been reported to improve symptoms of sleepiness and decrease the abnormal short REM sleep latency in HCRT KO mice (an animal with narcolepsy) by enhancing histamine activity (Lin et al., 2008). In a double-blind, randomized,
parallel-group controlled trial in patients with narcolepsy pitolisant, administered at doses up to 40 mg, ameliorates excessive daytime sleepiness in patients with narcolepsy to a similar degree to the of modafinil (Dauvilliers et al., 2013). More importantly, pitolisant is well tolerated compared with modafinil and has the advantage of possessing no obvious withdrawal symptoms and abuse liability, unlike other psychostimulants (Dauvilliers et al., 2013; Uguen et al., 2013). Thus, pitolisant may be viewed as a new and ideal drug for patients with narcolepsy, and has been approved by the European Commission in March, 2016. Preclinical studies also reported the effects of other H3R antagonists, such as GSK-189254, in mouse models of narcolepsy (Guo et al., 2009), but there are some discrepancies on wakefulness and narcoleptic episodes following acute and repeat dosing with GSK-189254.

4.2 Parkinson’s disease (PD)

It is now widely acknowledged that motor symptoms, such as bradykinesia, tremors and gait impairment, are the main manifestations of PD, which is attributable to midbrain dopaminergic cell loss within the substantia nigra pars compacta (SNc). Postmortem brain tissues of patients with PD show that histamine levels increase selectively in the substantia nigra, putamen, and globus pallidus (Rinne et al., 2002). Moreover, increases in the density of histaminergic fibers and alterations of its morphology in the substantia nigra have been described in the brains of PD patients. However, the activity of HDC and the number of histaminergic neurons remains unaltered in patients with PD (Garbarg et al., 1983; Nakamura et al., 1996; Shan et al., 2012c). Hence, the most likely source of these abnormally high histamine levels in the substantia nigra may be caused by the alteration of the activity of histamine metabolizing enzyme HNMT. This hypothesis is in agreement with a genotyping study which shows that lower frequencies of the HNMT<sup>Thr105Ile</sup> allele are associated with decreased enzymatic activity in patients with PD (Agundez et al., 2008; Palada et al., 2012). Further, in patients with PD and rats treated with intracerebral 6-hydroxydopamine (6-OHDA), the H3R radioligand binding is strongly increased in the striatum and substantia nigra and but the H3R mRNA expression is decreased in the substantia nigra (Anichtchik et al., 2001; Ryu et al., 1994; Shan et al., 2012a).
together, evidence suggests that the histaminergic system may have a place in PD therapy.

In PD, the degeneration of the SNc dopaminergic cells leads to a depletion of dopamine in the striatum. The D1 receptor (D1R) and D2 receptor (D2R) expressing GABAergic medium-spiny projection neurons (MSNs) are the main projection neurons of the striatum. They respond to cortex and thalamus glutamatergic afferents, giving rise to the direct and indirect pathways, respectively (Fig.2). In the direct pathway, D1R-expressing MSNs project to the substantial substantia nigra pars reticulata (SNr) and internal segments of the globus pallidus (GPi), and are involved in motor activation. In the indirect pathway, D2R-expressing MSNs project into the SNr and GPi via the external part of the globus pallidus (GPe) and the subthalamic nucleus (STN), and are involved in motor inhibition (Bolam and Ellender, 2016). In PD, the activity of D1R-positive direct pathway MSNs is reduced, while the activity of D2R-positive indirect pathway MSNs is increased, eventually leading to increased firing of GABAergic nigrothalamic neurons and reduced activity of the thalamocortical pathways. A recent analysis has confirmed that more than 85% of D1R and D2R-expressing MSNs in the dorsal and ventral striatum contain H3R (Ellenbroek and Ghiabi, 2014). H3R and D2R can potentiate each other, whereas H3R can inhibit the effects of D1R (Arias-Montano et al., 2001; Pillot et al., 2002a). In addition, H3R can form heterodimers with both D1R and D2R; however, the dimerization with D2R merely seems to lower the affinity of dopamine for the receptor, whereas dimerization with D1R can inhibit, rather than stimulate, the production of cAMP when D1R is activated (Ferrada et al., 2008; Ferrada et al., 2009). It would be expected that stimulation of H3R decreases the hyperactivity of the indirect pathway similar to the stimulation of the D2R, which may offer a beneficial effect. Although H3R activation may further suppress the D1R-positive direct pathway, the reduced excitation of the D1R-positive direct pathway appears to not be critical for PD. On the other hand, histamine can selectively activate microglia, leading to the increased inflammation that is characteristic of PD pathology, and damage dopaminergic neurons of SNc (Anichtchik et al., 2000; Rocha et al., 2016). This lends further support to the beneficial effects of H3R agonists and their inhibition of histamine production. We previously found that the H3R
agonist immepip relieves apomorphine-induced turning behavior in 6-hydroxydopamine (6-OHDA) lesioned rats (Liu et al., 2008). Utilization of either immepip or imetit, co-administered with L-DOPA, significantly relieves L-DOPA-induced dyskinesia or chorea, but not dystonia (Gomez-Ramirez et al., 2006). However, intranigral injection of immepip increases turning performance following systemic apomorphine administration in rats (Garcia-Ramirez et al., 2004). Likewise, in 6-OHDA-lesioned rats, the H3R antagonist thioperamide alleviates apomorphine-induced stereotyped behavior (Nowak et al., 2009).

In a single-blind trial, the H3R antagonist pitolisant alleviates excessive diurnal sleepiness of patients with PD, but not impacts motor performance (Schwartz, 2011). The elusive action of H3R in PD makes it hard to postulate the actual value of H3R ligands. Although the levels of striatal histamine H2R are unaltered in patients with PD (Martinez-Mir et al., 1993), H2R antagonist famotidine and ranitidine enhance the anti-parkinsonian actions provided by L-DOPA in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD in macaques and in 6-OHDA model in rats (Cui et al., 2014; Johnston et al., 2010), so H2R antagonists may serve as adjuncts for the treatment of PD.

Although the exact role of histamine and H3R in PD is unknown, several lines of evidence for the interaction between histaminergic and dopaminergic systems or striatal synaptic transmission should be taken into account for further study (Fig.2). A new group of dopaminergic neurons in TMN has been identified that share electrophysiological properties with histaminergic neurons (De Luca et al., 2016). H3Rs are expressed on GABAergic input terminals from the SNr, and stimulation of these receptors leads to a decrease in GABA release, which induces an increased excitation of the dopaminergic cells within the SNC (Ellenbroek and Ghiabi, 2014; Garcia et al., 1997). Moreover, histamine but not H3R antagonists, inhibit dopamine release in the mouse striatum via H3R (Schlicker et al., 1993). By combined electrophysiological and optogenetic experiments, Ellender et al. demonstrated that through action on presynaptic H3R, histamine inhibits both the cortical and the thalamic excitatory projections to MSNs, yet is able to selectively modulate the dynamics of thalamostriatal synapses to drive a facilitation of thalamic input (Ellender et al., 2011). Meanwhile, histamine also depolarizes both classes of MSNs through action of the H2R, and suppresses lateral feedback
inhibition between MSNs by the H3R or H2R. The action through H2R may be indirectly caused by the activation of the H2R in cholinergic interneurons to enhance striatal acetylcholine release (Prast et al., 1999). This implies that the histaminergic system is intimately linked to the dopaminergic systems or striatal synaptic transmission, but extensive studies are still needed to investigate the role of histamine in the context of PD.

4.3 Schizophrenia

Schizophrenia is a chronic and severe mental illness with a prevalence of 0.5–1% of the population. It usually manifests with positive symptoms including delusions, hallucinations, and disordered thoughts; negative symptoms including blunted affect, speech poverty, social withdrawal and anhedonia; and cognitive impairments. Accumulating evidence suggests that histamine is involved in the pathophysiology of schizophrenia. The level of tele-methylhistamine, the major histamine metabolite, is elevated in the CSF of patients with schizophrenia, especially in those with predominantly negative symptoms, which reflects increased histamine release and turnover (Prell et al., 1996; Prell et al., 1995). Many atypical antipsychotics have been reported to increase histamine turnover in the brain and show a marked affinity for histamine receptors, which may offer therapeutic effects despite its side-effect of drowsiness. However, low H1R binding was observed in the frontal and prefrontal cortices and cingulate cortex in the brains of people with schizophrenia (Iwabuchi et al., 2005; Nakai et al., 1991). Although decreased H1R binding is could be a consequence of increased histamine levels and turnover, the exact role of the histamine H1R in schizophrenia is not fully understood.

In the 1990s, the effect of an H2R antagonist was evaluated in patients with schizophrenia. H2R antagonist famotidine and ranitidine have been shown to have a positive therapeutic effect on the negative symptoms of schizophrenic patients (Kaminsky et al., 1990; Mehta and Ram, 2014). A randomized, double-blind, placebo-controlled study with famotidine on patients with treatment-resistant schizophrenia showed significant improvement in both the positive and negative syndrome scales (Meskanen et al., 2013), providing a new alternative for the treatment of this disorder. Moreover, clinical trials have showed the efficacy of nizatidine in weight reduction in patients with schizophrenia.
(Cavazzoni et al., 2003). Although such an effect seems to be unrelated to its antipsychotic action, further studies will be imperative to understand the roles and mechanisms of the H2R in schizophrenia.

Histamine H3R antagonists have also been proposed to have potential therapeutic effects in schizophrenia. H3R binding is increased in the dorsolateral prefrontal cortex, but remains unchanged in the temporal cortex of patients with schizophrenia (Jin et al., 2009). The H3R antagonist ciproxifan showed a positive therapeutic effect on cognitive impairment induced by MK-801 (Bardgett et al., 2010). Moreover, the H3R antagonists thioperamide and ciproxifan were also reported to enhance the pre-pulse inhibition of startle (Browman et al., 2004), but did not relieve the pre-pulse inhibition impairment induced by MK-801 (Bardgett et al., 2009), a hallmark of schizophrenia. However, the action of imidazole H3R antagonists on locomotion remains contradictory (Akhtar et al., 2006; Brabant et al., 2009; Mahmood et al., 2016). For example, thioperamide has been found to reduce amphetamine-induced hyperactivity and apomorphine-induced climbing in mice (Akhtar et al., 2006), but it is also shown to enhance the hyperactivity induced by cocaine (Brabant et al., 2009). More and more non-imidazole based compounds have attracted attention in schizophrenia, including BF2.649, GSK207040, ABT-239, and A-431404. These compounds have showed desired therapeutic effects on cognitive deficits (Brown et al., 2013; Southam et al., 2009), impaired sensory motor gating (Ligneau et al., 2007; Southam et al., 2009), and even locomotor hyperactivity (Ligneau et al., 2007) in animal models. Despite the successes observed using animal, three important clinical trials with H3R antagonists MK-0249, ABT-288, and GSK239512 failed to ameliorate the cognitive impairments in patients with schizophrenia (Haig et al., 2014; Jarskog et al., 2015; M et al., 2013). CEP-26401 (Irdabisant), a novel antipsychotic agent, is still in phase I clinical trials. The failure in clinical trials has dampened the confidence in developing H3R antagonists, but existing data from preclinical studies still implies that H3R antagonists may show promising therapeutic potential by selectively alleviating cognitive impairments and negative symptoms. In addition, weight gain is a common side effect of antipsychotic drugs, and H3R antagonists have been reported to reduce weight gain caused by antipsychotic drugs (Barak et al., 2016; Poyurovsky et al., 2013).
The elucidation of the role of histamine and its receptors in schizophrenia will promote the development of a new group histamine related drugs for the treatment of this disorder. It has been proposed that an increase in subcortical mesolimbic dopamine release causes the positive symptoms via the D2R, while reduced mesocortical dopamine release in the prefrontal cortex results in the negative symptoms and cognitive impairment via the D1R. In addition, abnormalities in dopamine transmission may result from a persistent alteration in glutamatergic transmission in the prefrontal cortex (PFC). Schizophrenics have been shown to have aberrant dopaminergic and glutamatergic systems, which may open the door for the use of H3R antagonists. First, H3R antagonists/inverse agonists enhance the release of dopamine in the PFC but not in the striatum (Fox et al., 2005; Ligneau et al., 2007), and the reduced dopamine release in the PFC has been linked to negative symptoms and cognitive impairment. Moreover, activation of presynaptic H3Rs on glutamatergic terminals in the striatum results in a reduction of glutamate release (Ellender et al., 2011; Molina-Hernandez et al., 2001). In contrast, administration of H3R antagonists increases glutamate release from corticostriatal terminals (Doreulee et al., 2001), which may rescue the glutamatergic hypoactivity in schizophrenic patients. Finally, a preclinical study showed that the H3R antagonist ciproxifan strongly potentiates haloperidol-induced encephalin, c-fos, neurotensin mRNA, and locomotor hypoactivity (Pillot et al., 2002b). Because H3R and D2R are co-expressed in striatopallidal neurons, the synergic effects of ciproxifan and haloperidol imply that there is a direct, functional H3/D2-receptor interaction in these neurons, and H3R antagonists might be helpful tools to improve the symptomatic treatment of schizophrenia (Mahmood, 2016; Pillot et al., 2002b).

4.4 Alzheimer's disease

Major pathological markers of AD include cholinergic neurodegeneration, an aggregation of extracellular Aβ plaque, and deposition of intracellular neurofibrillary tangles due to the hyperphosphorylation of the tau protein in the brain. Alterations of the histaminergic system were found in patients with AD. Increased CSF histamine and histamine metabolite levels in the frontal cortex, temporal cortex, basal ganglia, and hippocampus
were detected in AD patients (Cacabelos et al., 1989; Fernandez-Novoa and Cacabelos, 2001), whereas others reported reduced histamine levels in the hippocampus, frontal, hypothalamus and temporal cortex of AD patients (Panula et al., 1998). AD patients typically display degeneration of histaminergic neurons in the rostral TMN (Nakamura et al., 1993; Shan et al., 2015). The amount of H1R binding is also decreased in the frontal and temporal areas of AD patients, which is significantly correlated with the severity of their cognitive symptoms (Higuchi et al., 2000). In addition, mRNA expression of H3R and HNMT was remarkably increased in the PFC of female AD patients (Shan et al., 2012b). H3 antagonists alleviate the cognitive deficiencies in APPTg2576 AD transgenic mice and reverse tau hyperphosphorylation in the spinal cord and hippocampus of TAPP (tau×APP) AD transgenic mice (Bardgett et al., 2011; Bitner et al., 2011).

Patients suffering from AD show evidence of evident cholinergic dysfunction. So, the protection of H3R antagonists may be due to the upregulation of acetylcholine release, by working on heteroreceptors (Galici et al., 2009). On the other hand, the promotion of histamine synthesis and release can also be the underlying mechanism of the effect of H3R antagonists, since histamine is recognized to play important roles in cognitive functions. HDC deficient mice exhibit specific changes in task related learning and memory: displaying improved performance in passive avoidance and fear memory, but showing gender relied deficiency or enhancement in water-maze and novel location recognition (Acevedo et al., 2006a; Acevedo et al., 2006b; Liu et al., 2007). It has proposed that histamine plays an important role in learning and memory via H1R and H2R. Pharmacological blockade of H1R impairs spatial memory in Barnes maze test and radial maze test (Chen et al., 2001; Chen et al., 1999), consolidation of object recognition memory (da Silveira et al., 2013) and avoidance memory (Fabbri et al., 2016; Kamei and Tasaka, 1993). H2R antagonists block consolidation of object recognition memory and inhibitory avoidance memory (da Silva et al., 2006; da Silveira et al., 2013). Histamine ameliorates the both the spatial working and reference memory functions by H1R and H2R in the radial maze task after scopolamine induced memory deficits (Xu et al., 2009). Moreover, H1R or H2R knockout mice show impairments in object recognition and acquisition of spatial memory, but improvement in auditory and contextual freezing (Dai et
H1R knockout mice also exhibits seriously impaired temporal order memory (Zlomuzica et al., 2013). The above evidence suggests that histamine H1R and H2R may participate in pathological process of AD and the action of H3R antagonists. H3R antagonists have shown to have profound protective effects in various cognitive impairments in Y-maze, object recognition, passive avoidance, radial arm maze, and water maze tests (Huang et al., 2004; Orsetti et al., 2001; Zlomuzica et al., 2016). It has been reported that recognition enhancement by H3R antagonist ciproxifan is at least in part dependent on H1R and H2R (Pascoli et al., 2009). Given that cognitive impairments are often the first symptom of AD patients, the powerful improvements on learning and memory shown by mice treated with H3R antagonists suggests that they may also alleviate cognitive impairment in patients with AD. Some other potential actions such as the amelioration of Aβ induced neuron death (Fu et al., 2010) and promotion of neurogenesis may also contribute to the protective action of H3R antagonists (Bernardino et al., 2012).

In recent years, a series of phase II clinical studies have been carried out to investigate the efficacy and safety of H3R antagonists to treat cognitive symptoms in AD patients, including ABT-288, MK-0249 and GSK239512. In a trial of a small group of patients with AD, over 4 weeks administration of GSK239512 was found to be well tolerated and was shown to improve cognitive function in those with mild to moderate symptoms when the patient was administrated with an ascending dose titration regimen (Nathan et al., 2013). In another double-blind, randomized, placebo-controlled study, patients with mild-to-moderate AD treated for 16-weeks with GSK239512 showed improved episodic memory performance, but no improvements on executive function/working memory, or other sides of cognition (Grove et al., 2014). At the same time, other clinical studies regarding the efficacy of the H3R antagonist ABT-288 and the inverse agonist MK-0249 failed to show any significant effects in mild-to-moderate AD patients (Egan et al., 2012). Pitolisant (BF2.649), the first H3R antagonist (inverse agonist) is currently being evaluated to enhance cognitive effects in patients suffering from schizophrenia. Although these clinical trials for H3R antagonists in AD are not so promising, a titration regimen appears feasible route to provide modest and selective
benefits on cognitive function. Additionally, extensive evaluation of novel H3R ligands in AD animal models, not only in cognitive deficit models, and understanding its mechanism of action are required.

4.5 Cerebral ischemia

Cerebral ischemia results from a reduction of cerebral blood flow (CBF), which results in damage to the entire brain or a localized region. Although more than a hundred neuroprotective drugs are shown effective in animal experiments, they are often found to be virtually ineffective in the treatment of human ischemia. At present, the most widely accepted treatment for ischemic stroke, caused by a permanent or transient reduction of CBF in a localized region, still is reperfusion with recombinant tissue plasminogen activator (rtPA) combined with the newly developed mechanical embolectomy (Caplan, 1998; Lo, 2008). However, both treatments have certain limitations, such as the narrow therapeutic time window and the risk of hemorrhage (Davis and Donnan, 2009). The major disease progress following cerebral ischemia happens through excitotoxicity, programmed cell death, inflammation and cerebral reconstruction, which not only involve the neurons but also the glia (astrocytes, microglia and oligodendroglia) and vascular cells (endothelia, vascular smooth muscle cells and pericytes) (George and Steinberg, 2015; Zhou et al., 2017). Therefore, protective agents that act on multiple targets have potential value for treatment.

Histamine in the brain has been suggested to protect against cerebral ischemic injury (Hu and Chen, 2012). The release of histamine in the cerebral cortex and striatum gradually increases as early as 2h after occlusion of the middle cerebral artery (MCAO), which is a classical experimental model of focal cerebral ischemia (Adachi et al., 1992). It has been proposed that the source of increased histamine may largely come from the histaminergic neurons (Adachi et al., 1992). However, abundant mast cells are subjected to degranulation after global cerebral ischemia in rats (Hu et al., 2004), and mast cells are accumulated in the ipsilateral hemisphere after focal cerebral ischemia (McKittrick et al., 2015). The expression of histamine receptors are also modulated after acute cerebral ischemia (Lozada et al., 2005). H1R mRNA expression increases, but H2R binding
density and protein expression decreases in the caudate-putamen 48 h after transient global ischemia. H3R mRNA expression increases in the caudate-putamen, but reduces in the thalamus and the globus pallidus. Abundant evidence has shown that histamine offers neuroprotection against cerebral ischemic injury. Intraperitoneal administration of histidine, a precursor of histamine, at the early stage following reperfusion, remarkably alleviates infarction after MCAO (Adachi et al., 2005). Our recent study indicated that histidine provides long-term protection in terms of neurological score, cognitive ability, and infarct area, even at 2 months after cerebral ischemia (Liao et al., 2015). Direct intracerebroventricular administration of histamine was found to relieve the delayed ischemic damage in hippocampal CA1 pyramidal cells after transient forebrain ischemia (Fujitani et al., 1996). The underlying mechanism of the observed protection from histamine and the receptor ligands may be related to the pathological progress after cerebral ischemia (Fig.3).

4.5.1 The regulation of excitotoxicity at the acute period

Excitotoxicity takes place within minutes after cerebral ischemia due to redundant extracellular glutamate. Histamine alleviates the neuronal excitotoxicity induced by N-methyl-d-aspartate (NMDA) via H2R and cAMP/PKA pathway (Dai et al., 2006). Although H1R is less referred to in the context of ischemia, blockage of H1R enhances the excitotoxic response of NMDA, which is abrogated by histamine (Diaz-Trelles et al., 2000). On the other hand, reduction of glutamate release as well as increase of its clearance through the upregulation of uptake by the glutamate transporter GLT-1 and conversion into glutamine by glutamine synthetase (GS) in astrocytes may be an alternative to relieve excitotoxicity after cerebral ischemia (Bak et al., 2006). It was reported that pre-ischemic administration of histamine suppresses the upregulation of glutamate levels during ischemia (Hamami et al., 2004). In addition, we found that histamine upregulates the GLT-1 mediated current and its expression via H1R, which provides protection against cerebral ischemic injury in MCAO models (Fang et al., 2014). Histamine also upregulates GS expression in cultured astrocytes exposed to OGD, which is abrogated by H1R antagonists (Wang et al., 2013). Therefore, histamine is found to relieve excitotoxicity
following cerebral ischemia through multiple pathways involving both neurons and astrocytes (Adachi, 2005; Juric et al., 2016).

Carnosine, a dipeptide highly expressed in the CNS, has been assigned many putative roles, such as anti-inflammation, radical scavenging, and mobile organic pH buffering. We found that carnosine protects differentiated PC12 cells, a neuronal cell line, against NMDA induced excitotoxicity through the carnosine-histidine-histamine pathway and inhibition of glutamate release (Shen et al., 2007a; Shen et al., 2007b). In addition, carnosine confers protection for brain microvascular endothelial cells against oxidative stress injury through histamine H1R and H2R (Zhang et al., 2012), and rescues the GS expression and prevents the damage of astrocytes through H1R (Shi et al., 2017). From experimental ischemic stroke, carnosine exhibits robust protection in terms of infarct volume and neurological score (Davis et al., 2016; Park et al., 2014). However, using both the in vitro and in vivo experiments, we demonstrated that carnosine suppressed the production of mitochondrial reactive oxygen species to preserve GLT-1 expression in astrocytes and reduce excitotoxicity through a histamine independent pathway (Shen et al., 2010). This type of histamine independent action for carnosine on ischemic injury was also confirmed by Bae, et al. (Bae and Majid, 2013). This discrepancy may be due to different mechanism on different cell types, whereas carnosine itself can be viewed as a candidate for the treatment of cerebral ischemia.

Regional cerebral blood flow (rCBF) is critical for neuronal damage either during ischemia or during reperfusion. Histaminergic fibers have been shown to innervate cerebral blood vessels (Karlstedt et al., 2001; Takagi et al., 1986), in the endothelium and smooth muscle cells where H1R and H2R are located (Karnushina et al., 1980; Stanimirovic et al., 1994). It has been reported that histamine and the H3R antagonist clobenpropit significantly increase rCBF in the hippocampus through H1R and H2R mediated action, but reduces the rCBF in the parietal lobe through H1R (Chen, 2001; Suzuki et al., 1999; Yang et al., 2010). However, in our study using the mice MCAO model, no change in rCBF was observed after histamine treatment, or in HDC knockout mice (Fan et al.). More detailed evaluation of rCBF with different doses of histamine or the receptor ligands, combined with multiple region evaluation and long-term measurements,
should be undertaken to determine the effect of histamine on rCBF after cerebral ischemia.

4.5.2 Regulation of autophagy and inflammation in the subacute period

Autophagy is an evolutionarily conserved process that eventually triggers the degradation of intracellular proteins and organelles in lysosomes. In the context of cerebral ischemia, autophagy is initiated within hours after ischemia in several models such as global ischemia, focal ischemia, and hypoxia-ischemia (Yuan et al., 2015). The inhibition of autophagy is thought to reduce neuronal death during permanent focal cerebral ischemia (Xing et al., 2012). However, we previously found that induction of mitochondrial autophagy by pharmacological intervention or genetic manipulation reduces neuronal injury during reperfusion after focal cerebral ischemia (Zhang et al., 2013; Zhang et al., 2014). Activation of mitochondrial autophagy by acidic post-conditioning extends the thrombolytic time window for cerebral ischemia therapy (Shen et al., 2017). Our recent study demonstrated that H3R antagonism by H3R antagonists or via genetic knockout of H3R provided a neuroprotective effect during reperfusion after cerebral ischemia (Yan et al., 2014). Interestingly, H1R and H2R antagonists, α-FMH, and HDC knockout fail to compromise the protection by H3R antagonism, suggesting that the protection is achieved through a histamine-independent mechanism. Furthermore, we found that the protection from H3R antagonism is generated through an inhibition of H3R/CLIC4 binding and subsequent induction of autophagy. Our study reveals a novel biological action of the H3R, which we have shown should be considered as a potential therapeutic target for the treatment of cerebral ischemia.

At the subacute period, inflammation is initiated by microglia activation and the release of proinflammatory cytokines, followed by the infiltration of neutrophils, macrophages, and monocytes. It may result in beneficial or deleterious effects depending on the timing or the contribution of inflammatory factors and cells. Histamine is known to augment inflammatory reactions via H1R in the periphery during bronchial asthma and anaphylaxis. However, stimulation of the H2R shows an inhibitory effect on inflammation through downregulation of proinflammatory cytokines combined with the chemotactic
responsiveness of leukocytes (Akdis and Simons, 2006; Azuma et al., 2001; Takahashi et al., 2002). After MCAO reperfusion, facilitation of histaminergic activity suppresses inflammatory cell infiltration via the H2R (Hiraga et al., 2007). Recently, microglia was found to constitutively express all four histamine receptors (H1R, H2R, H3R and H4R). Histamine induces TNF-alpha and IL-6 release from activated microglia via H1R and H4R (Dong et al., 2014; Zhu et al., 2014). However, the microglial response to challenge with lipopolysacchariade (LPS) is potentiated in HDC knockout mice (Frick et al., 2016). In addition, both histamine and the H3R agonist imetit dose-dependently inhibit microglial chemotaxis, phagocytosis, and LPS-induced cytokine production (Iida et al., 2015). Thus far, the exact action of histamine on microglia activation and the outcome of inflammation following cerebral ischemia awaits further detailed studies. Notably, the responses of microglia to ischemic insult may evolve at the different time-points, and different histamine receptors may mediate distinct actions.

Moreover, it has been reported that simultaneous administration of thioperamide with L-histidine completely prevents edema formation after MCAO (Irisawa et al., 2008). On the other hand, the H2R antagonist ranitidine attenuates water accumulation and the maldistribution of ions in the brain in a bilateral common carotid artery occlusion model (Tosaki et al., 1994). The precise role of histamine on edema, and whether it results from the control of inflammation or the alleviation of endothelial cell damage, remains unknown. The use of mice with selective histamine receptor depletion in immune or endothelial cells may shed light on this mechanism in the future.

4.5.3 Regulation of glial scar formation and neurogenesis at the late period

At the late period of focal cerebral ischemia, the reactive astrocytes, their released various extracellular matrix molecules, and microglia all contribute to glial scar formation, which impedes axonal regeneration and neurological recovery (Silver and Miller, 2004). Recently, we found that treatment with histidine yields remarkable long-term neuroprotection at the late period after cerebral ischemia (Liao et al., 2015). Interestingly, this protection results from a facilitation of astrocyte migration towards the infarct core, inhibiting glial scar formation through the H2R. The promotion of astrocyte migration, but
not its proliferation or activation, has been confirmed through both *in vivo* and *in vitro* experiments. In addition, for the first time, we established an *in vitro* glial scar model induced by oxygen-glucose deprivation. This model can now be employed to explore the fundamental mechanism of action of histamine or its receptor ligands in this process (Wang et al., 2012).

Efficient neurogenesis is achieved not only through increased proliferation of neural stem cells, but also via the successful differentiation and functional integration of newborn neurons, which are critical for functional recovery (Zhang et al., 2005). Adult neural stem cells (NSCs) are localized in the subgranular zone of the hippocampus and the subventricular zone of the brain (Palmer et al., 1997; Reynolds and Weiss, 1992). Using a combination of RT-PCR and Western blot analysis, both the proliferating and differentiated neural stem cells were shown to express H1R, H2R and H3R (Molina-Hernandez and Velasco, 2008). Treatment with histamine not only increases the proliferation of neural stem cells through the H2R, but also promotes their differentiation by activation of the H1R, with downregulation of a proportion of the astrocytes in cultured neural stem cells. Administration of histamine in the lateral ventricle increases the production of new neuroblasts in the subventricular zone (SVZ), which ultimately reach the olfactory bulb (Eiriz et al., 2014). H1R knockout mice exhibit reduced adult hippocampal neurogenesis, compared with controls (Ambree et al., 2014). Our unpublished data suggest that H3R antagonists promote the differentiation and successful integration of NSCs through the upregulation of histamine and activation of the H1R during experimental traumatic brain injury. A prominent increase of neuroblasts are observed after histidine treatment at late periods after transient MCAO in rats (Liao et al., 2015); therefore, it is imperative to investigate the fate of those newborn neurons and their contribution to neural circuits. Nevertheless, histamine-related agents have potential value in cerebral reconstruction, since histamine may have dual benefits both in limiting glial scar formation, and stimulation of the intrinsic growth potential of adult NSCs.

Taken together, histamine displays multiple actions by targeting neurons, glial cells, and vascular cells. Such multifaceted protective actions at the different periods following cerebral ischemia indicate great potential for the clinical application of histamine ligands.
Clinical trials should be encouraged for the study of histamine related agents in ischemic stroke patients, especially the evaluation of H3R antagonists.

5. Prospect for the study of histamine in the CNS

It is the exciting time for translational histamine research, since preclinical studies show potential value for histamine receptor ligands. As reviewed above, there are several concerns for future studies of histamine and its role in the CNS. 1) Because of the availability of HDC-Cre mice (Williams et al., 2014; Yanovsky et al., 2012), histaminergic neuron specific modulation can be achieved through the use of optogenetic or chemogenetic approaches. This has the potential to rapidly advance exploration the role of histaminergic circuits in CNS disorders. Optogenetics uses light-responsive cellular elements, such as ChR2 or archaerhodopsin (Arch), to produce stimulation or inhibition of neurons. One major advantage of this tool is the ability to generate rapid kinetic models, leading to more precise temporal and geographic targeting (Deisseroth, 2015). On the other hand, the chemogenetic approach is an alternative route towards targeted and temporal increase or suppression of neuronal excitability via designer receptors exclusively activated by designer drugs (Roth, 2016). A merit for this approach is that the modulation of neuron firing can persist for hours or even days. In addition, since other neurotransmitters, such as GABA or dopamine, may be co-released from histaminergic neurons (Yanovsky et al., 2011; Yu et al., 2015), the action of other neurotransmitters is needed to be concerned, when the activity of histaminergic neurons is manipulated. 2) The inconsistent actions of histamine receptors have been observed, which is likely due to the discrepant actions of histamine receptors in different cell types. The use of conditional knockout mice is an optimal approach to shed light on the cell type selective action of histamine receptors. In addition, most actions of histamine in the brain are mediated by the H1R, but not the H2R, making the role of the H2R a bit mysterious. This is due to the fact that it is expressed in the same location and in comparable amounts as the H1R. Investigation of the action of the H2R in specific cell types in the brain may provide new targets for the treatment of CNS disorders. Recently, we found that the H2R in oligodendrocytes regulates differentiation to improve remyelination and cognitive abilities.
in postnatal mice exposed to hypoxia/ischemia (unpublished data). 3) Several diverse histamine receptor isoforms are present in the CNS, and the ligands may show different activity for different isoforms. For example, (R) sopromidine is an antagonist at the short isoform of H3R, but a full agonist at the long isoform of H3R (Gbahou et al., 2012). Therefore, it is important to identify both the isoform and the function of the histamine receptors present in a particular the cell type. Based on the aforementioned studies, development of drugs that selectively target certain receptors in specific cell-types might provide precise therapeutic approaches with enhanced efficacy and/or decreased incidence of side effects. 4) The expression and role of the H4R need to be evaluated in more detail and under different pathologic settings, which may play a critical role in neuroinflammation and also serve as a therapeutic target. More rigorous experiments should be undertaken for such studies; this is especially important since H3R ligands may also act on H4R, and H4R ligands may display different pharmacological properties in different species and under different experimental conditions (Panula et al., 2015). For example, JNJ-7777120 may display partial inverse agonistic activity on human the H4R, whereas it displays partial agonistic activities on rat, mouse, and canine species specific H4R variants (Schnell et al., 2011). 5) Due to the high constitutive activity of H3R and H4R, receptor antagonists may also have histamine independent effects. The functional mechanisms that are modulated through direct binding of proteins to the receptors are found for the downstream pathway of histamine receptors, such as the binding of CLIC4 with the H3R for the induction of autophagy (Yan et al., 2014) and the direct binding dopamine D1R or D2R with the H3R for the modulation of MSN firing (Ferrada et al., 2008; Ferrada et al., 2009). Therefore, exploring the role of the proteins that directly bind the histamine receptors may help to understand the mechanism of the action of histamine receptors. 6) Mast cell-derived histamine is suggested to affect hypothalamic neurons, and is involved in endocrine control and homoeostatic regulation, but its relative limited distribution leads to less attention for it (Kawakami et al., 2000). Recently, cerebral mast cells have been suggested to participate in some CNS disorders, including cerebral ischemia, AD, traumatic brain injury, and multiple sclerosis (Conti and Kempuraj, 2016; Hendrix et al., 2013; Shaik-Dastthagirisah and Conti, 2016). Although selective
depletion of histamine in mast cells is not available at this moment, using mast cell deficient W/W \(^v\) mice helps to elucidate the role of histamine in mast cells. Moreover, other possible nonneuronal sources of histamine, including that from microglia and microvascular endothelial cells (Katoh et al., 2001; Yamakami et al., 2000), may also have potential action in CNS disorders.

6. Conclusion

In light of present and past studies, the modulatory neurotransmitter histamine is suggested to be involved in various CNS disorders. Thus, it is an exciting time for more extensive future studies of its role in regulating neural circuit activity and in CNS disorders using cutting-edge technology. Moreover, due to the promising effects of histamine related ligands in preclinical experiments for some diseases, the development of novel agents and double-blind, randomized, multi-center clinical trials should be encouraged. It may result in the identification of a new group of agents for clinical therapy that have fewer side effects.

Acknowledgments

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Figure legends

Fig. 1 A schematic diagram of the action of the histaminergic system in wakefulness through interaction with other neurons in different brain regions. ACH, cholinergic neurons; BF, basal ganglia; DRN, dorsal raphe nucleus; GABA, GABAergic neurons; GLU, glutaminergic neurons; HA, histaminergic neurons; HCRT, hypocretin neurons; 5-HT, 5-hydroxytryptamine-expressing (serotonergic) neurons; LDT/PPT:
laterodorsal/pedunculopontine tegmental nuclei; LH, lateral hypothalamic; TMN, tuberomammillary nucleus; and VLPO, ventrolateral preoptic nucleus.

Fig. 2 The effect of histaminergic neurons on the basal ganglia in Parkinson’s disease. The simplified connections between the basal ganglia and their associated structures are shown in A. In the striatum, D1R positive MSNs project to the SNr and GPi, while D2R positive MSNs project into the SNr and GPi via the GPe and the STN. The GABAergic neurons in SNr and GPi are connected to thalamocortical pathways. Histaminergic neurons regulate the D1R positive MSNs or D2R positive MSNs, and glutaminergic cortical or thalamic inputs in the striatum. The synaptic connections in the striatum are shown in B. In the striatum, histaminergic neurons act on D1R positive MSNs or D2R positive MSNs via the H3R or H2R. Activation of H3R can potentiate D2R inhibitory effect, but inhibit the D1R’s response in MSNs. Meanwhile, histamine also depolarizes both classes of MSNs through action of the H2R. This activation of H2R in cholinergic interneurons enhances striatal acetylcholine release. Through presynaptic H3R, histamine inhibits both the cortical and the thalamic excitatory projections to MSNs. The synaptic connections in the SNc are shown in C. Within the SNc, histamine inhibits GABAergic inputs from SNr via presynaptic H3R to induce an increased excitation of dopaminergic cells. On the other hand, histamine can activate microglia via H1R to damage dopaminergic neurons. ACH, cholinergic projections; D1R+ MSN, dopamine D1 receptor positive medium-spiny projection neuron; D2R+ MSN, dopamine D2 receptor positive medium-spiny projection neuron; DA, dopaminergic projections in A or dopamine in B; GABA, GABAergic projections in A or γ-Aminobutyric acid in B; GLU, glutamatergic projections in A or glutamate in B; GPe, external part of globus pallidus; H1R, histamine H1 receptor; H2R, histamine H2 receptor; H3R, histamine H3 receptor; HA, histaminergic projections in A or histamine in B; MSN, medium-spiny projection neuron; SNc, substantia nigra pars compacta; SNr, substantial substantia nigra pars reticulate; STN, subthalamic nucleus; and TMN, tuberomammillary nucleus; +, positive effect; −, negative effect.
Fig. 3 Schematic representation of the multitarget-directed protective activity of histamine and its receptors at different time-points following cerebral ischemia. Histamine reduces excitotoxicity via the H2R, promoting glutamate metabolism in astrocytes via the H1R at the acute period after cerebral ischemia. Meanwhile, during the subacute period, histamine may be involved in neuroinflammation and cerebral edema, whereas H3R antagonists provide histamine-independent protection through induction of autophagy. During the late period of cerebral ischemia, histamine still has a beneficial effect through suppression of glial scar formation and promotion of neurogenesis. CBF, cerebral blood flow; and HA, histamine.

Fig. 4 Systematic approach for investigating the role histamine in CNS disorders. This experimental platform is comprised of four major elements: 1) selective manipulation of histaminergic neuron activity by optogenetics or chemogenetics in mice expressing opsin or DREADD in histaminergic neurons; 2) selective depletion of histamine receptors in particular cell types (CaMKIIα+ glutamatergic neuron; ChAT+ cholinergic neuron; or Vgat+ GABAergic neuron, et al.) in the brain using conditional histamine receptor knockout mice; 3) evaluation of electrophysiological, histological, and behavioral alterations based on above manipulations; 4) development of drugs that selectively target certain receptors in specific cells to obtain enhanced efficacy and/or decreased incidence of side effects.

CaMKIIα, calmodulin-dependent protein kinase IIα; ChAT, choline acetyltransferase; DREADD, designer receptors exclusively activated by designer drugs; GABA, γ-aminobutyric acid; H1R, histamine H1 receptor; H2R, histamine H2 receptor; H3R, histamine H3 receptor; H4R, histamine H4 receptor; and Vgat, vesicular GABA transporter.

Table 1 The characteristics and CNS clinical applications of histamine receptor ligands

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<th>Ligands</th>
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<th>Molecular Formula</th>
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<th>Kp*</th>
<th>Ki for other receptors (nM)</th>
<th>CNS clinical trials (phase, status)</th>
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ACCEPTED MANUSCRIPT
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<td>16.25(R)</td>
<td>Migraine(I,VC); Sleep(I,VC); Pain; Postoperative(I,VC); Depression(I,VC); Restless Legs Syndrome (II, C); Specific Sedation; Hypnotic(IV,VC); Acute Cerebrovascular Accident; Cerebral Edema(II,JC); Autoimmune Failure; Orthostatic Hypotension (III,RC)</td>
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<tr>
<td>Promethazine (First generation)</td>
<td>H1R (inverse agonist)</td>
<td>C₂₂H₂₄N₂O₂</td>
<td>284.421</td>
<td>20.00 (Rb)</td>
<td>Migraine(III,LC); Psychotic Disorders; Assault (III, C); Conscious Sedation (II, LC); Labor Pain (III, C); Hyperemesis Gravidarum (III, LC); Nausea; Vomiting (IV,VC); Vestibular (IV,VC);</td>
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<tr>
<td>Triptenamine (First generation)</td>
<td>H1R (inverse agonist)</td>
<td>C₂₄H₂₅N₃</td>
<td>345.391</td>
<td>34.0±9.0</td>
<td>Nausea; Vomiting (IV, VC); Feeling Anxious (II, LC); Pain (IV, VC);</td>
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<tr>
<td>Mepyramine (First generation)</td>
<td>H1R (inverse agonist)</td>
<td>C₃₁H₃₂O₂N₂</td>
<td>388.892</td>
<td>3.83(M)</td>
<td>H1R (7.8)</td>
</tr>
<tr>
<td>Chlorpheniramine (First generation)</td>
<td>H1R (inverse agonist)</td>
<td>C₂₃H₂₄NO₂Cl</td>
<td>274.792</td>
<td>8.6±0.1</td>
<td>34.0±9.0</td>
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<tr>
<td>Hydroxyzine (First generation)</td>
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<td>C₁₅H₂₁NO₂Cl</td>
<td>374.90</td>
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<td>Doxepin (First generation)</td>
<td>H1R (inverse agonist)</td>
<td>C₂₁H₂₅NO</td>
<td>279.383</td>
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<td>Cetirizine (Second generation)</td>
<td>H1R (inverse agonist)</td>
<td>C₂₉H₃₁NO₄</td>
<td>388.89</td>
<td>8.2±0.8</td>
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<td>Loratadine (Second generation)</td>
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<td>C₁₅H₂₃C₅N₂</td>
<td>282.388</td>
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<td>Mizolation (Second generation)</td>
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<td>Terfenadine (Second generation)</td>
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<td>C₂₁H₂₆NO₄</td>
<td>471.665</td>
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<td>Fexofenadine (Third generation)</td>
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<td>C₂₅H₂₃NO₄</td>
<td>501.667</td>
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<td>Levocetirizine (Third generation)</td>
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<td>Cimetidine</td>
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<td>252.34</td>
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<td>Ranitidine</td>
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<td>(R)-o-Methylhistamine</td>
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<td>H4R(7.13)</td>
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<tr>
<td>Imetit</td>
<td>H3R, H4R (agonist)</td>
<td>H1R(&lt;5)</td>
<td>H2R(&lt;4)</td>
<td>H3R(8.6)</td>
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<td>Imepip</td>
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<td>Proxyfan</td>
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<td>GT-2331</td>
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<td>H2R(4.27)</td>
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<td>Thioramamide</td>
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<td>H4R(7.98)</td>
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<td>VUF-6684</td>
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### Table

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<td>ZPL-3893787</td>
<td>H4R (antagonist)</td>
<td>C_{13}H_{22}N_{6}</td>
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<td>1.12</td>
<td>6.73</td>
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</table>

*+: Kp for blood/brain ratio tested from mice (M), rats (R) or rabbit (Rb); #: Ki for muscarinic receptors (M), dopamine D1 or D2 receptor, or serotonin 5-HT3 receptor (nM); &: clinical trials in completed (C); recruiting (R); unknown (U); terminated (T) status according to clinicaltrials.gov.


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Figure 1
Figure 2
Figure 3
Figure 4