Histamine neuronal system as a therapeutic target for the treatment of cognitive disorders

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Much has been learned over the past 20 years about the role of histamine as a neurotransmitter. This brief article attempts to evaluate the progress accomplished in this field, and discusses the therapeutic potential of the H_3 receptor (H_3R). All histaminergic neurons are localized in the tuberomammillary nucleus of the posterior hypothalamus and project to almost all regions of the CNS. Histamine exerts its effect via interaction with specific receptors (H_1R, H_2R, H_3R and H_4R). Antagonists of both H_1R and H_2R have been successful as blockbuster drugs for treating allergic conditions and gastric ulcers. H_4R is still awaiting better functional characterization, but the H_3R is an attractive target for potential therapies of CNS disorders. Indeed, considerable interest was raised by reports that pharmacological blockade of H_3Rs exerted procognitive effects in a variety of animal tasks analyzing different types of memory. In addition, blockade of H_3Rs increased wakefulness and reduced bodyweight in animal models. Such findings hint at the potential use of H_3R antagonists/inverse agonists for the treatment of Alzheimer's disease and other dementias, attention-deficit hyperactivity disorder, obesity and sleep disorders. As a result, an increasing number of H_3R antagonists/inverse agonists progress through the clinic for the treatment of a variety of conditions, including attention-deficit hyperactivity disorder, cognitive disorders, narcolepsy and schizophrenia. Moreover, the use of H_3R antagonists/inverse agonists that weaken traumatic memories may alleviate disorders such as post-traumatic stress syndrome, panic attacks, specific phobias and generalized anxiety. The use of H_3R ligands for the treatment of neurodegenerative disorders is demonstrated in several studies, indicating a role of the histamine neurons and H_3Rs in neuroprotection. Recently, direct evidence demonstrated that histaminergic neurons are organized into functionally distinct circuits, impinging on different brain regions, and displaying selective control mechanisms. This could imply independent functions of subsets of histaminergic neurons according to their respective origin and terminal projections. The possibility that H_3Rs control only some of those functions implies that H_3R-directed therapies may achieve selective effects, with minimal side effects, and this may increase the interest regarding this class of drugs.

Histamine is a neurotransmitter

The first indication of the functional importance of histamine in the CNS can be traced back to the 1930s, when it was observed that centrally penetrating histamine H_1 antagonists had marked sedative properties. However, no attention was given to histamine receptors as sites of action for these unwanted effects [1]. Indeed, the role of histamine as a neurotransmitter has been neglected for many years, in spite of early reports of its presence in the brain [2] and suggestions that this amine has central functions [3]. The delay in searching for a histaminergic neuronal system, in comparison to the exploration of other aminergic neurotransmitter systems, may rest on the methods available for their visualization. The distribution of the catecholaminergic and serotonergic neurons in the brain became known using a fluorescent immunohistochemical analysis with o-phthalaldehyde as a tracer [4]. However, the same method was not suitable for visualizing histamine owing to strong interference with the ubiquitous spermidine [5,6]. The first direct evidence for the existence of histaminergic neurons did not occur until the 1980s, with the development of immunohistochemistry using antibodies against histamine [7] and histidine decarboxylase [8]. All histaminergic neurons are localized in the tuberomammillary nucleus (TMN) of the posterior hypothalamus [7,8], which is also the location of histidine decarboxylase (HDC) immunoreactivity [9], an

Keywords

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essential determinant of brain histamine levels [10,11]. They project to almost all regions of the CNS [12], mostly unmyelinated fibers that, with the exception of the mesencephalic trigeminal nucleus [13], do not form synaptic contacts but present diffuse varicosities containing synaptic vesicles [14,15]. This peculiarity suggests that histamine may act as a local hormone, affecting not only neuronal but also glial activity, and blood vessel tone [16]. Consistently, cultured astrocytes from rat cerebral cortex display histamine receptors identical to those present on neuronal cells [17,18]. This morphological feature (a compact cell group with widely distributed fibers) resembles that of other biogenic amine systems, such as norepinephrine or serotonin, thus suggesting that histaminergic neurons may regulate several central functions.

Histamine receptors as therapeutic drug targets

Histamine exerts its effect by interacting with specific receptors: H_1R [19], H_2R [20], H_3R [21] and H_4R [22], as well as with the polyamine-binding site on the NMDA-receptor complex [23]. All four histaminergic receptor subtypes belong to the rhodopsin-like family of G-protein-coupled receptors (GPCRs) [24,25], and are functionally expressed on neurons in the mammalian CNS [6,26]. The first two members of the histamine receptor family, H_1R and H_2R, are well-established drug targets, and antagonists of these receptors have been successfully used as blockbuster drugs for treating allergic conditions and gastric ulcers. The H_4R is primarily distributed in immune cells, where it mediates immune and inflammatory responses [27]. However, the recent description of a functional expression of H_4R on human and rodent neurons [26,28] is still waiting for a better functional characterization. The discovery of the H_3R by Jean-Charles Schwartz and his group in Paris, France, has been a real breakthrough in histamine research [21]. This receptor is largely confined to the nervous system [29], where it acts as a presynaptic autoreceptor that restricts histamine release, as well as synthesis both *in vitro* [21] and *in vivo* [30–33]. The H_3R is located also on histaminergic somata, where it provides a tonic inhibition of firing [34]. Moreover, the presence of the H_3R is not restricted to histaminergic neurons [35–37]. Accordingly, H_3Rs also act as heteroreceptors, modulating the release of several neurotransmitters [6], including acetylcholine (Ach) [38,39], dopamine [40], norepinephrine [41] and serotonin [42,43] from brain regions crucial for the maintenance of alertness or the storage of information [1]. H_3R signaling is mediated through $G_{i/o}$ proteins, negative coupling to adenylyl cyclase, and also through other signaling cascades, such as the activation of phospholipase A2, as well as protein kinase and PI3K pathways, which activate extracellular signal-regulated kinases and Akt and, subsequently, inhibit the action of glycogen synthase kinase 3β [29,44].

Network analyses of the brain and its dysfunction suggest that agents with multiple and complementary modes of action are more likely to show broad-based efficacy against core and comorbid symptoms. Thus, the regulatory role in the release of histamine and other neurotransmitters makes the H_3R an attractive target for therapies of CNS disorders, and H_3R ligands are good therapeutic candidates for their simultaneous exploitation of multiple neuronal systems [45,46]. Consistent with the widespread distribution throughout the entire CNS of histaminergic fibers [47,48], brain histamine is, directly or indirectly, involved in a variety of basic homeostatic and higher brain functions, such as the sleep–wake cycle, appetite, nociception, cognition and emotion [1,6]. H_3R antagonists/inverse agonists have been shown to increase wakefulness, improve cognitive performances and reduce bodyweight in animal models [6]. Such findings hint at the potential use of these compounds for the treatment of Alzheimer's disease (AD) and other dementias, attention-deficit hyperactivity disorder (ADHD), cognitive deficits in schizophrenia, obesity and sleep disorders [45,49–51]. Thus, it is not surprising that much effort is focused on the development of clinically suitable H_3R antagonists/inverse agonists by academic and industrial laboratories [45,51,52]. As a result, more and more H_3R antagonists/inverse agonists, such as ABT-239 [4-(2-{2-[(2R)-2-methylpyrrolidinyl] ethyl}-benzofuran-5-yl)benzonitrile]; BF2.649 (tiprolisant/pitolisant; 1-{3-[3-(4-chlorophenyl) propoxy]propyl}piperidine, hydrochloride; GSK-239512 (structure not disclosed); JNJ-17216498 (structure not disclosed); MK-0249 (structure not disclosed) and PF-03654746 (trans-*N*-ethyl-3-fluoro-3-[3-fluoro-4-(pyrrolidin-1-ylmethyl)phenyl]cyclobutanecarboxamide), progress through the clinic for a variety of conditions, including ADHD, cognitive disorders, hyperalgesia, narcolepsy and schizophrenia [45].

Characteristics of the H_3R

The H_3R is largely confined to the nervous system, and the highest levels were found in the cerebral cortex, hippocampus, basal ganglia and

hypothalamus [36,37]. This receptor has multiple splice variants. Not all isoforms appear to be functional, and some of them might regulate functional isoforms by associating with them [53,54]. H_3Rs are members of the seven-transmembrane receptor superfamily [55] and couple to $G_{i/o}$ proteins [56]. Their stimulation restricted the influx of calcium ions [57], inhibited adenylate cyclase [55], and increased extracellular signal-related kinase (ERK) phosphorylation in receptor-transfected cells [58]. All histamine receptors displayed a high degree of constitutive (agonist-independent) activity, which occurred in human, rat and mouse recombinant receptors expressed at physiological concentrations [59–62]. Of note, constitutive activity of native H_3Rs seems one of the highest among the GPCRs in the brain [63]. Constitutively active H_3Rs presumably regulate the release of neuronal histamine [61]; therefore, several H_3R antagonists (e.g., clobenpropit, thioperamide and ciproxifan) that block constitutive activity are being reclassified as inverse agonists, a concept that may have clinical relevance. Indeed, either inverse agonists or neutral antagonists may be favorable for different therapeutic applications.

Therapeutic potential of H_3R antagonists/inverse agonists in cognitive & emotional disorders

Considerable interest was raised by reports that pharmacological blockade of H_3Rs exerted procognitive effects in a variety of animal tasks analyzing different types of memory, which should be taken as proof of concept. In the social memory [64,65], rats treated with H_3R antagonists/inverse agonists performed better than controls in the five-trial inhibitory avoidance task [66,67] and the five-choice serial reaction-time test [68]. Further studies indicated that both imidazole and nonimidazole H_3R antagonists/inverse agonists exerted procognitive effects in cognitively impaired animals, as observed in senescence-accelerated mice or scopolamine-impaired rats challenged in a passive-avoidance response [69,70], scopolamine-impaired rats tested in object recognition [49,70,71] or the elevated plus-maze paradigm [72], and MK-801-treated rats evaluated in the radial maze [73]. Administration of nonimidazole H_3R antagonists/inverse agonists, A-304121 or A-317920, improved cognitive performances in spontaneously hypertensive rat pups that were normotensive during early development, but exhibited many cognitive impairments [66,67]. Certainly, such a model is clinically very relevant, as deficits are genetic in origin and do not require pharmacological or surgical intervention. Although another report provided some contrasting data, as H_3R antagonists/inverse agonists impaired object recognition in wild-type and *Apoe*$^{-/-}$ mice [74], these findings may be relevant to predict the potential of H_3R antagonists/inverse agonists in ameliorating cognitive dysfunctions in humans [67]. In this regard, the presence of [^{3}H]GSK189254-labeled H_3Rs in hippocampal and cortical sections from patients with advanced AD is important [75], and suggests the persistence of H_3Rs, even in severe AD.

If cognitive deficits are related to reduced availability of ACh in the synaptic cleft [76], increase of ACh release in the prefrontal cortex exerted by H_3R antagonists/inverse agonists could account for the procognitive effects produced by these compounds, at least in short-term memory paradigms with important cortical cholinergic components, such as object recognition [77] and a passive-avoidance response [78]. Indeed, H_3R ligands modulate cortical ACh release in a bimodal fashion, and modify the expression of memories accordingly. Stimulation of cortical H_3Rs inhibited local ACh release, and impaired object recognition and a passive-avoidance response [39]. Conversely, TMN perfusion with GSK189254 significantly increased the release of cortical ACh in freely moving rats, and counteracted amnesic effects produced by scopolamine administration in rats, as measured in object recognition [71]. Cortical ACh increase can be a consequence of the augmentation of histamine release in the nucleus basalis magnocellularis (NBM) elicited by intra-TMN administration of GSK189254 [71]. Indeed, histamine, by activating H_1Rs, depolarized the cell membrane and increased the tonic firing of NBM cholinergic neurons [79], which provide all cholinergic innervation to the cortex [80]. These findings are in keeping with the report that perfusion of the NBM with H_3R antagonists/inverse agonists increases cortical ACh release [81]. H_3R antagonists/inverse agonists also augment NBM histamine release by blocking local H_3-autoreceptors (Figure 1) [71,82]. A comparable enhancement of cortical ACh was also observed in response to systemic administration of several nonimidazole H_3R antagonists/inverse agonists, such as ABT-239 [65], BF2.649 [83] or GSK189254 [84]. Neuronal alterations associated to cognitive deficits are not restricted to the cholinergic systems, as many neurotransmitter systems, including dopamine, contribute to specific aspects of cognition. Therefore, it is important to point out that systemic administration

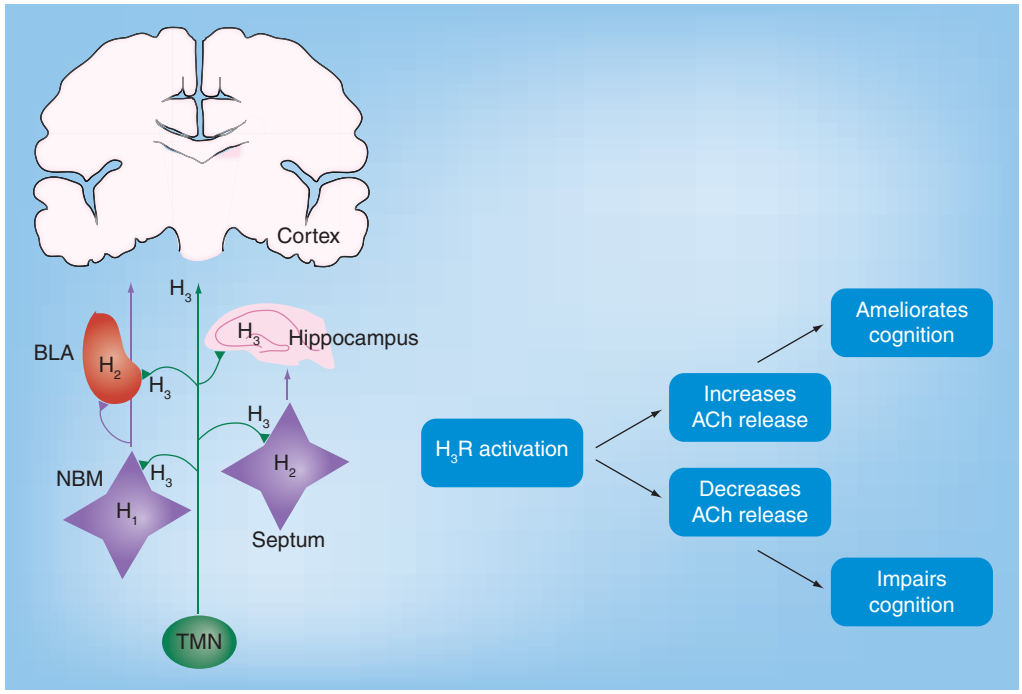

Figure 1. Cholinergic neurotransmission represents an essential neurophysiological component in attentional information processing. H_3 receptors modulate ACh release with different modalities in different brain regions.
ACh: Acetylcholine; BLA: Basolateral amygdala; NBM: Nucleus basalis magnocellularis; TMN: Tuberomamillaris nucleus.

of ABT-239 [65] or BF2.649 [83] also increases the release of cortical dopamine. However, H_3R antagonists/inverse agonists failed to increase dopamine release from other regions, such as the striatum [65] or the nucleus accumbens [71], and these observations may provide the rational basis for clinical indication in disorders such as schizophrenia or ADHD.

Interactions between the histaminergic and cholinergic systems serve as one of the physiological correlates for learning and remembering; however, H_3Rs modulate ACh release with modalities that differ according to regional architectural constraints, to their role as auto- or hetero-receptors, and to the distinct actions that histamine exerts by activating different receptor subtypes (Figure 1). It is of note that basolateral amygdala (BLA) perfusion with H_3R agonists increases, whereas with H_3R antagonists/inverse agonists decreases, ACh release from the BLA [85,86]. These drugs presumably impact on inhibitory H_3-autoreceptors, as BLA H_3R receptor binding was strictly associated with the presence of histaminergic fibers [87]. Consistently, BLA perfusion with H_3R antagonists/inverse agonists increased endogenous histamine release [88], which, in turn, activated postsynaptic H_3Rs and inhibited ACh release [85]. The BLA receives the most abundant histaminergic innervation in the brain [89], and displays both high H_3R binding and its gene transcripts [90]. Crucial neural changes mediating emotional memory occur in the BLA [91,92]. Emotional memory may be assessed with contextual fear conditioning, in which experimental animals learn to associate a mild electrical shock to the foot with the environment where they receive the punishment. A critical event for aversive memory consolidation is the activation of muscarinic receptors within the BLA [85,86,93]. In this regard, it is relevant that BLA perfusion with H_3R antagonists/inverse agonists impaired [85], whereas with H_3R agonists ameliorated, expression of this form of associative memory [86]. These results contrast with the findings in the cortex. Nevertheless, since BLA is engaged in the development of mood disorders associated with extreme emotional traumas, the use of H_3R antagonists/inverse agonists that weaken traumatic memories may be proposed to alleviate disorders such as post-traumatic stress disorder (PTSD), panic attacks, specific phobias and generalized anxiety.

Brain histamine affects emotional memory, eliciting ERK2 phosphorylation in hippocampal CA3 pyramidal cells, an event that is crucial for the consolidation of contextual fear memory [94].

Arousal elicited by H_3R antagonists/inverse agonists & its contribution to procognitive effects

Cognition is a complex phenomenon involving the integration of multiple neurological activities, among which, arousal is crucial [95,96]. Histamine is, along with orexin, one of the major wake-promoting neurotransmitters in the CNS [97], as histidine decarboxylase-knockout mice that lack histamine are unable to remain awake when high vigilance is required [98]. In addition, narcoleptic dogs show histamine deficiency [99]. It is known that histaminergic neurons fire at higher frequency during wakefulness than during sleep [100]. Moreover, histamine is responsible for cortical EEG desynchronization [97], a salient sign of wakefulness [1,101]. Brain histamine elicits cortical activation both directly, through excitatory interactions with cholinergic corticopetal neurons originating from the substantia innominata [102] and the NBM [81], and indirectly through stimulation of cholinergic neurons in the mesopontine tegmentum, which activate thalamo- and hypothalamocortical circuitries [103]. H_3R blockade by local perfusion of thioperamide into the TMN increased the time spent in wakefulness, along with the release of TMN histamine in freely moving rats [82], thus suggesting that they, by increasing arousal, may enhance attention and improve cognitive performances. However, several H_3R antagonists/inverse agonists produced cognitive-enhancing effects at much lower doses than those required to elicit a robust wake enhancement [65,67]. For example, ABT-239 produced no detectable change in slow-wave EEG at 30 mg/kg, whereas it was effective in social recognition at 0.01 mg/kg [65]. Consistently, for ciproxifan, thioperamide or GSK189254, only a relatively low level of cumulative wake activity was linearly correlated with up to 80% of the receptor occupancy, and an abrupt break from linearity, along with a robust increase of waking activity, was observed at doses that produced greater than 80% occupancy [104]. High or low levels of H_3R occupancy may express activities mechanistically different, and H_3R antagonists/inverse agonists procognitive actions may not relate to increased arousal. Thus, lower dosage might be used to address H_3R antagonists/inverse agonists actions, especially towards cognition. This is an important issue, since nocturnal sleep should, ideally, not be disturbed by drug therapies. Nevertheless, at least at higher dosage, this class of drugs constitutes a novel effective treatment of narcolepsy and excessive daytime sleepiness (EDS), and this contention is supported by both preclinical and clinical data. Indeed, acute administration of GSK189254 reduced narcoleptic episodes in orexin-knockout mice [105]. Moreover, in a pilot single-blind clinical trial on 22 patients diagnosed with narcolepsy receiving a placebo for 1 week, followed by tiprolisant (BF2.649) for a second week, the Epworth sleepiness scale (ESS) score was reduced from a baseline value of 17.6 by 1.0 with the placebo ($p > 0.05$), and 5.9 with tiprolisant ($p < 0.001$) [106]. EDS, unaffected under placebo, was nearly suppressed on the last days

Table 1. Histamine H_3 receptor ligands in clinical trials for the treatment of CNS disorders.

Disorder	Compound	Condition	Phase
Alzheimer's disease	GSK-239512	Mild/moderate	II
	MK-0249		II
	PF-03654746	Mild/moderate	I
Narcolepsy	BF2.649 (tiprolisant/pitolisant)	Sleepiness	III
	BF2.649 (tiprolisant/pitolisant)	Cataplexy	III
	JNJ-17216498		II
	PF-03654746	Sleepiness	II
	MK-0249	Sleepiness in patients with OSA/HS	II
Attention-deficit hyperactivity disorder	MK-0249		II
	PF-03654746		II
Schizophrenia	BF2.649 (tiprolisant/pitolisant)	Cognitive impairment	II
	GSK-239512	Cognitive impairment	II
	MK-0249	Cognitive impairment	II
Parkinson's Disease	BF2.649 (tiprolisant/pitolisant)	Sleepiness	III
Epilepsy	BF2.649 (tiprolisant/pitolisant)	Photosensitive epilepsy	II

OSA/HS: Obstructive sleep apnea/hypopnea syndrome.

of tiprolisant dosing [106]. Very few human data on cognition and waking behavior are available (Table 1), hence preclinical studies can be taken as proof of concept. The cognitive-enhancing effects of the H_3R antagonist GSK239512 are being evaluated in patients with mild-to-moderate AD. The H_3R antagonist PF-03654746 is being evaluated for its effectiveness in the treatment of ADHD, and advanced to Phase II clinical trials for its efficacy in improving alertness and wakefulness in patients with EDS. BF2.649 also reduced EDS in narcoleptic patients, and currently running clinical trials are showing that it is a very promising alerting drug in Parkinson's disease [107].

H_3Rs & neuroprotection

Much of the recent interest in developing new ligands of the H_3R stems from the potential use of H_3R antagonists in controlling feeding behaviour, disorders of the sleep–wake cycle and cognitive impairments associated with AD or Parkinson's disease (reviewed in [108]). However, there are potential therapeutic applications for H_3R agonists as well. H_3R activation in the CNS results in lower hypothalamic histamine release, and an H_3R agonist may be used to treat insomnia [109]. In addition, Hough and coworkers revealed an antinociceptive role for spinal histamine H_3R [110]. In the past years, several studies have hinted at a role of the H_3R in neuroprotection. The first clear indication of how 'plastic' the brain histaminergic system is following injury, was provided by Panula and collaborators. They showed that H_3R mRNA is upregulated in the rat caudate and putamen following induction of transient global cerebral ischemia [111], or in the rat cortex following kainic acid-induced seizures [112], although with different time courses and recovery. A more recent paper published by the same group elegantly demonstrated that histamine protects hippocampal neurons from damage induced by kaininc acid in organotipic cocultures of hypothalamic and hippocampal tissue [113]. The hypothalamic histaminergic innervation of hippocampal neurons provides the neuroprotective effect and, presumably, the blockade of presynaptic autoinhibitory H_3R ameliorates the protective effect of histaminergic neurons. We recently showed that H_3R agonists activate antiapoptotic pathways, such as the PI3K/Akt/GSK-3β pathway [44]. The Akt pathway has been implicated in regulating several important cellular processes, including cell plasticity and survival, proliferation and metabolism. Akt promotes neuronal cell survival and opposes apoptosis by a variety of routes (e.g., modulating inhibitors of apoptosis, such as Bcl-2 and Bcl-x). Indeed, in our model H_3R agonists increased the expression of Bcl-2, and decreased the expression of pro-apoptotic elements, such as caspase-3, following neurotoxic insults in cultured murine cortical neurons [44]. Hence, stimulation of H_3R protects cortical neurons from NMDA-induced neurotoxic insults, and this observation may have relevance in the prevention of, for instance, ischemic neuronal damage or neurodegenerative diseases. As a matter of fact, schizophrenic patients display impaired Akt/GSK-3β signaling [114], and evidence points to a key role for GSK-3β in promoting neurodegeneration [115]. GSK3 is involved in a cascade of events, such as hyperphosphorylation of tau protein, increased production of β-amyloid and local cerebral inflammatory responses that may culminate in AD [116]. In this regard, binding studies showed that the expression of H_3R is spared in the brain of AD patients [84]. To fully understand the impact of H_3R-induced activation of antiapoptotic pathways in the CNS, *in vivo* experiments are necessary, even more so as H_3R antagonists are now viewed as potential therapeutics for schizophrenia [83] and AD [84].

Heterogeneity of histaminergic neurons

In comparable architecture of noradrenergic, dopaminergic and serotonergic systems [117,118], somata of histaminergic neurons are restricted to discrete cell clusters in the hypothalamic TMN, and send their axons to innervate nearly the entire CNS [7,8]. Cathecolaminergic and serotonergic nuclei are comprised of distinct compartments with respect to projection fields, as distinct sets of axons innervating separate brain regions originate from separate subgroups of noradrenergic (A1–A7), dopaminergic (A8–A17) and serotonergic (B1–B9) neurons [117,118]. This does not seem to be the case for the histaminergic system, as retrograde tracers injected into different CNS regions labeled histaminergic somata scattered throughout the TMN without a strict topographical pattern [9,12,119]. Noradrenergic, dopaminergic and serotonergic patterns imply independent functions of sets of neurons according to their origin and terminal projections. On the contrary, the morphological feature of the histaminergic system is consistent with the hypothesis of a single regulatory network for whole-brain activity, which modulates general states of metabolism and consciousness, rather than processing specific functions [16]. However, very recently, direct evidence demonstrated that

histaminergic neurons are also organized into functionally distinct circuits, impinging on different brain regions and displaying selective control mechanisms. Using the double-probe microdialysis technique in freely moving rats, it was observed that histaminergic neurons established distinct pathways related to independent functions according to their terminal projections, and to their sensitivity to H_3R antagonists/inverse agonists or $GABA_A$-receptor ($GABA_A$-R) antagonists [71,82]. $GABA_A$-R activation directly inhibits histaminergic cell firing rate [120,121], whereas $GABA_A$-R inhibition increases TMN histamine release significantly [122]. Depending on $GABA_A$-R-subunit expressions, histaminergic neurons displayed different sensitivities to GABA [123,124]. This may account for the functional heterogeneity of GABAergic responses displayed by histaminergic neurons following stimulation of the diagonal band of Broca, the antero–lateral hypothalamus, or the lateral preoptic area [120]. The finding that intrahypothalamic perfusion of bicuculline increased histamine release from the TMN, the nucleus accumbens and cortex, but not from the striatum [82], indicates that sensitivity to bicuculline relates to TMN neurons heterogeneity with respect to projection fields.

Application of imidazole or nonimidazole H_3R antagonists/inverse agonists locally into the TMN significantly increased histamine release from the TMN, the prefrontal cortex and the NBM, but not from the striatum or nucleus accumbens [71,82]. These findings indicate that histamine neurons projecting to the dorsal striatum and nucleus accumbens were insensitive to blockade of H_3Rs [71,82]. Spatial segregation caused by probe localization does not explain why histaminergic neurons projecting to the striatum or nucleus accumbens do not respond to H_3R antagonists/inverse agonists. Infact, bicuculline administered into the TMN significantly augmented histamine release from the nucleus accumbens [82], and TMN perfusion with cannabinoid 1-receptor agonists increased histamine release from the dorsal striatum [122], confirming the existence of histaminergic afferents to the striatum. Furthermore, retrograde tracing with dye injections into the striatum or prefrontal cortex labeled most histaminergic somata within the same area, the medial part of the ventral TMN [119]. This proximity suggests that the compounds administered through the microdialysis probe indiscriminately affected histaminergic cells projecting to the striatum and prefrontal cortex. Interestingly, previous studies showed that following GSK189254 administration, activation of c-fos occurred in cortical areas and the TMN, but not in striatum [84]. Moreover, local perfusion of the striatum with H_3R antagonists/inverse agonists did not alter spontaneous histamine release [71,82], suggesting that the entire somatodendritic domain of histaminergic neurons projecting to this region were insensitive to H_3R blockade. Since the magnitude of neuronal responses to extracellular signals might also depend on different receptor numbers at the membrane, it is important to underline that in the TMN, some HDC-positive cells displayed very low levels of H_3R immunoreactivity [82], although no evidence demonstrates that these cells are the ones innervating the nucleus accumbens or striatum. On the other hand, histamine increases in the prefrontal cortex and NBM were probably caused by discharge potentiation of histamine neurons, sending afferents to these regions, in analogy to TMN perfusion with prostaglandin E_2 [125], orexin-A [126] or endocannabinoids [122].

These observations suggest that the histaminergic system is organized into distinct circuits modulated by selective mechanisms. This could imply independent functions of subsets of histaminergic neurons according to their respective origin and terminal projections.

Conclusion

A wide variety of studies agree that the neuronal histaminergic system regulates some forms of cognition, and, inevitably, reports that pharmacological blockade of central H_3Rs exerts procognitive activity in several cognitive tasks have raised considerable interest. Advances in molecular pharmacology are uncovering the extraordinary complexity of the H_3R: it shows functional constitutive activity, polymorphisms in humans and rodents – with a differential distribution of splice variants in the CNS, and potential coupling to different intracellular signal-transduction mechanism. Thus, it will be a great challenge in the years to come to develop ever-more-selective agonists, inverse agonists, pure antagonists of the H_3R, as well as ligands for its various isoforms. All histaminergic neurons are believed to express H_3Rs, and responses to H_3R ligands are a criterion for their identification *in vitro*. Contrary to this general assumption, it has been recently reported that histamine neurons projecting to the striatum and nucleus accumbens are insensitive to thioperamide, an H_3R antagonist, thus suggesting that histamine neurons are more functionally heterogeneous than previously thought. Although further

Executive summary

Histamine is a neurotransmitter

- All histaminergic neurons are localized in the tuberomammillary nucleus (TMN) of the posterior hypothalamus and project to almost all regions of the CNS, mostly unmyelinated fibers that, with the exception of the mesencephalic trigeminal nucleus, do not form synaptic contacts, but present diffuse varicosities containing synaptic vesicles.
- This morphological feature, a compact cell group with widely distributed fibers, resembles that of other biogenic amines systems, such as norepinephrine or serotonin, thus suggesting that histaminergic neurons may also regulate several central functions.

Histamine receptors as therapeutic drug targets

- All four histaminergic receptor subtypes (H_1R, H_2R, H_3R and H_4R) belong to the rhodopsin-like family of G-protein-coupled receptors (GPCRs), and are functionally expressed on neurons in the mammalian CNS.
- The H_1R and H_2R are well-established drug targets, and antagonists of these receptors have been successfully used as blockbuster drugs for treating allergic conditions and gastric ulcers. H_4Rs are primarily distributed in immune cells where they mediate immune and inflammatory responses.
- The H_3R acts as a presynaptic autoreceptor that restricts histamine release as well as synthesis, and as a heteroreceptor, modulating the release of several neurotransmitters, including acetylcholine (ACh), dopamine, norepinephrine and serotonin.
- The regulatory role in the release of histamine and other neurotransmitters makes the H_3R an attractive target for therapies of CNS disorders, and H_3R ligands are good therapeutic candidates for their simultaneous exploitation of multiple neuronal systems.

Characteristics of the H_3R

- The H_3R is largely confined to the nervous system.
- Molecular pharmacology is uncovering the extraordinary complexity of the H_3R – it shows functional constitutive activity, polymorphisms in humans and rodents with a differential distribution of splice variants in the CNS, and potential coupling to different intracellular signal-transduction mechanisms.
- Constitutive activity of native H_3Rs appears to be one of the highest among the GPCRs in the brain.

Therapeutic potentials of H_3R antagonists/inverse agonists in cognitive & emotional disorders

- Considerable interest was raised by reports that pharmacological blockade of H_3Rs exerted procognitive effects in a variety of animal tasks analyzing different types of memory.
- Increase of ACh release in the prefrontal cortex exerted by H_3R antagonists/inverse agonists could account for the procognitive effects produced by these compounds. Neuronal alterations associated to cognitive deficits are not restricted to the cholinergic systems, as many neurotransmitter systems, including dopamine, contribute to specific aspects of cognition. Therefore, it is important to point out that systemic administration of H_3R antagonists/inverse agonists increases the release of cortical dopamine but fails to increase dopamine release from other regions, such as the striatum or the nucleus accumbens.
- These observations may provide the rational basis for clinical indication in disorders, such as Alzheimer's disease and other dementias, schizophrenia or attention-deficit hyperactivity disorder.
- The use of H_3R antagonists/inverse agonists that weaken traumatic memories may help to alleviate disorders such as post-traumatic stress syndrome, panic attacks, specific phobias and generalized anxiety.

Arousal elicited by H_3R antagonists/inverse agonists & its contribution to procognitive effects

- It is known that histaminergic neurons fire at a higher frequency during wakefulness than during sleep, and are responsible for cortical EEG desynchronization, a salient sign of wakefulness.
- This class of drugs constitutes a novel effective treatment of narcolepsy and excessive daytime sleepiness, and this contention is supported by both preclinical and clinical data.
- BF2.649 (tiprolisant/pitolisant) reduced excessive daytime sleepiness in narcoleptic patients, and current clinical trials are showing that it is a very promising alerting drug in Parkinson's disease.

H_3Rs & neuroprotection

- Several studies have hinted at a role of the H_3R in neuroprotection. H_3R mRNA is upregulated in the rat caudate and putamen following induction of transient global cerebral ischemia, or in the rat cortex following kainic acid-induced seizures.
- H_3R agonists activate antiapoptotic pathways, such as the PI3K/Akt/GSK-3β pathway.

Heterogeneity of histaminergic neurons

- Histamine neurons established distinct pathways related to independent functions according to their terminal projections, and to their sensitivity to H_3R antagonists/inverse agonists or $GABA_A$ receptor antagonists.
- This could imply independent functions of subsets of histaminergic neurons according to their respective origin and terminal projections.

studies are required to understand the full implications of such functional heterogeneity of histaminergic neurons, the possibility that H_3Rs control only some of those functions implies that H_3R-directed therapies may achieve selective effects with minimal side effects, and this may increase the interest for this class of drugs.

Future perspective

The H_3R plays a regulatory role in the release of histamine and other neurotransmitters, making it an attractive target for CNS indications, including cognitive disorders, narcolepsy, ADHD and pain. The interest in this receptor as a potential drug target has produced great advancement in novel compound series with different properties, providing a variety of preclinical tools, as well as advancing several candidates into clinical trials. As increasing numbers of H_3R antagonists/inverse agonists progress through the clinic for a number of potential indications, knowledge will be gained to define the profile of the ideal compound in terms of specificity, pharmacokinetic parameters, and both duration and magnitude of receptor occupancy. However, since recent evidence indicates that histaminergic neurons are heterogeneous and organized into functionally distinct circuits that influence different brain regions, and display selective control mechanisms, efforts will be focused towards the identification and pharmacological characterization of different compounds, each suitable for the treatment of specific disorders.

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▪ **Demonstrated that histamine neurons are involved in sleep regulation by orexin neurons.**