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REVIEW

The histamine H_3 receptor: an attractive target for the treatment of cognitive disorders

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The histamine H_3 receptor, first described in 1983 as a histamine autoreceptor and later shown to also function as a heteroreceptor that regulates the release of other neurotransmitters, has been the focus of research by numerous laboratories as it represents an attractive drug target for a number of indications including cognition. The purpose of this review is to acquaint the reader with the current understanding of H_3 receptor localization and function as a modulator of neurotransmitter release and its effects on cognitive processes, as well as to provide an update on selected H_3 antagonists in various states of preclinical and clinical advancement. Blockade of centrally localized H_3 receptors by selective H_3 receptor antagonists has been shown to enhance the release of neurotransmitters such as histamine, ACh, dopamine and norepinephrine, among others, which play important roles in cognitive processes. The cognitive-enhancing effects of H_3 antagonists across multiple cognitive domains in a wide number of preclinical cognition models also bolster confidence in this therapeutic approach for the treatment of attention deficit hyperactivity disorder, Alzheimer's disease and schizophrenia. However, although a number of clinical studies examining the efficacy of H_3 receptor antagonists for a variety of cognitive disorders are currently underway, no clinical proof of concept for an H_3 receptor antagonist has been reported to date. The discovery of effective H_3 antagonists as therapeutic agents for the novel treatment of cognitive disorders will only be accomplished through continued research efforts that further our insights into the functions of the H_3 receptor.
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Keywords: H_3 receptor; H_3 antagonist; histamine; cognition; neurotransmitter release; drug discovery

Abbreviations: 5-CSRTT, five-choice stimulus reaction time task; AD, Alzheimer's disease; ADHD, attention deficit hyperactivity disorder; RAMH, *R*-α-methylhistamine; RT, reverse transcription; SHR, spontaneously hypertensive rat

Introduction

There exist four distinct histamine receptor subtypes (H_1, H_2, H_3 and H_4) that mediate the many physiologic functions of endogenous histamine. Two of these, the H_1 and H_2 receptors, have been important drug targets with highly effective and clinically beneficial therapeutic agents designed to block effects mediated by these receptors. Classical antihistamines such as chlorpheniramine, fexofenadine and desloratidine have been developed that very effectively treat allergic responses mediated by histamine activation of H_1 receptors. The histamine H_2 receptor has also proven to be a therapeutically important drug target, and selective H_2 antagonists such as ranitidine and cimetidine have been developed that treat gastric ulcers through the blockade of gastric acid secretion. The histamine H_3 receptor represents yet another histamine receptor that is a very attractive CNS drug target and has generated intense research efforts in both academic and industrial laboratories in an effort to identify potent and selective H_3 receptor antagonists. Originally described as a presynaptic autoreceptor that inhibits histamine release in the brain (Arrang *et al.*, 1983), it was subsequently shown to also regulate the release of other important neurotransmitters via a parallel role as a heteroreceptor (Schlicker *et al.*, 1988, 1989, 1993; Clapham and Kilpatrick, 1992; Blandina *et al.*, 1996). To date, preclinical research with potent and selective H_3 antagonists suggests that this class of agents may offer a novel therapeutic approach for the treatment of a variety of cognitive disorders including attention deficit hyperactivity disorder (ADHD), Alzheimer's disease (AD) and schizophrenia. The aim of this paper is to review some of the important recent advances in understanding the molecular and functional aspects of the H_3 receptor with respect to the role of this receptor in cognition. In addition, the preclinical properties of some H_3 receptor antagonists that have recently advanced into human clinical studies for cognitive disorders will be highlighted.

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Histamine H_3 receptor: isoforms, localization, pharmacology and signalling

Like the other members of the histamine receptor family, the histamine H_3 receptor is a G-protein-coupled receptor (GPCR, the drug and histamine receptor nomenclature used in this review conforms with the *Br J Pharmacol* Guide to Receptors and Channels; Alexander *et al*., 2007). Much recent progress has been made in the basic understanding of the structure, localization, pharmacology and signalling properties of H_3 receptor isoforms since the original cloning and characterization of the histamine H_3 receptor in 1999 (Lovenberg *et al*., 1999). The H_3 receptor exhibits highest homology (~60% in the transmembrane domains) to the most recently cloned histamine H_4 receptor but much lower homology to other GPCRs including the H_1 and H_2 receptors (~20% homology) (Hancock *et al*., 2003; Leurs *et al*., 2005).

Isoforms and localization

Whereas the full-length H_3 receptor is described as consisting of 445 amino acids, alternative splicing of the receptor gene results in at least 20 possible human H_3 receptor mRNA isoforms identified by reverse transcription (RT)-PCR. These isoforms exhibit variable amino- and carboxyl-termini lengths, truncations of the third intracellular loop and deletions of transmembrane domains. To date, eight of these recombinant human H_3 receptor isoforms (H_3(445), H_3(453), H_3(415), H_3(413), H_3(409), H_3(373), H_3(365) and H_3(329)) have been shown to be functionally competent based upon either binding or signalling assays when expressed in heterologous cell expression systems (Table 1). The pharmacology and functionality of these isoforms will be further elaborated below. All eight functional H_3 receptor isoforms share the same transmembrane domains with differences arising from modifications of the amino and carboxyl termini and truncations of the third intracellular loop. The remaining 12 human H_3 receptor isoforms are either non-functional or their biological activity is yet to be determined. Many of these receptors have alterations within their transmembrane domains due to deletions or novel stop codons and therefore would not be expected to exhibit more typical H_3 receptor pharmacology and function; however, their relevance to physiological responses has not been fully probed and so remains to be determined.

The distribution of the histamine H_3 receptor has been characterized in the CNS largely on the basis of RNA *in situ* hybridization (Pillot *et al*., 2002; Rouleau *et al*., 2004) and radioligand-binding (Laitinen and Jokinen, 1998; Jansen *et al*., 2000) studies that have demonstrated expression throughout the brain. H_3 receptor expression is prominent in the basal ganglia, globus pallidus, hippocampus and cortex in humans (Martinez-Mir *et al*., 1990). Studies investigating the differential localization of human H_3 receptor isoforms using RT-PCR approaches suggest that the H_3(445) and H_3(365) isoforms predominate in many brain areas with approximately equivalent levels of expression (Bongers *et al*., 2007b). Brain areas in which they are more highly expressed include caudate nucleus, hippocampus, frontal cortex and hypothalamus, among others (Table 1). The levels of expression of the H_3(415), H_3(413) and H_3(329) isoforms are much lower but nevertheless can be detected in caudate nucleus and amygdala by RT-PCR (Table 1).

In rats, nine distinct recombinant H_3 receptor isoforms have been identified, with three of these (H_3(445), H_3(413) and H_3(397)) representing functional receptors (Drutel *et al*., 2001). The H_3(445), H_3(413) and H_3(397) isoforms as well as H_3(410) (Morisset *et al*., 2001) constitute four rat H_3 isoforms that differ by alterations in the third intracellular loop. Interestingly, there is no evidence for a rat H_3(365) receptor, a truncated isoform that is expressed in humans. Additionally, the H_3(397) isoform seen in rat is distinct from any seen in humans. Among the non-functional isoforms, H_3(497), H_3(465) and H_3(449) represent isoforms with transmembrane domain 7 truncations that interfere with the expression of H_3(445) but do not possess any H_3 receptor-binding activity themselves (Bakker *et al*., 2006). In general, the highest expression of the H_3 receptor in rodents is in the cerebral cortex, hippocampal formations, striatum and hypothalamus (Drutel *et al*., 2001). Studies examining the differential localization of the functional rat H_3 receptor isoforms using *in situ* hybridization approaches suggest that the H_3(445) and H_3(397) isoforms predominate in many brain areas (Drutel *et al*., 2001). Both are expressed in olfactory tubercle, but H_3(445) appears to be the major isoform in the nucleus accumbens, thalamus and caudate putamen, whereas H_3(397) is the predominate isoform in hippocampal and hypothalamic regions, locus coeruleus and cortical laminae. Conversely, H_3(413) is expressed in relatively lower abundance in striatum, thalamus and cortical regions.

Non-human primates also express multiple H_3 receptor isoforms including the functional isoforms H_3(445), H_3(413) and H_3(410) as well as an inactive H_3(335) isoform that has a truncated third intracellular loop and transmembrane 5 domain (Strakhova *et al*., 2007; Table 1). The monkey H_3(445) appears to be the predominant isoform, expressed in multiple brain regions such as the frontal cortex, hippocampus, caudate and hypothalamus. The monkey H_3(445), H_3(413) and H_3(410) isoforms display comparable pharmacology in both binding and functional assays (Strakhova *et al*., 2007).

Many human and rat brain areas that express H_3 receptor isoforms in relatively high abundance are those involved in cognition (that is, cortex and hippocampus, see below) or subcortical areas (that is, hypothalamus) that project neurons to these cognition-associated brain regions. Therefore, these receptors can function to regulate neuronal activity itself as is seen with histaminergic neurons arising from the hypothalamus or can regulate the release of neurotransmitters at the synaptic level in cognition-associated brain regions as will be elaborated further below. The observation of similar H_3 receptor expression patterns in humans and rats helps support the use of the rat as a preclinical model for testing the procognitive properties of H_3 antagonists. It should also be noted that the limited peripheral expression of the H_3 receptor is likely to reduce the potential for non-CNS side-effect liabilities that may be associated with the H_3 receptor. The potential impact of the

Table 1 Summary of known functional human and rat splice variants of H_3 receptors

Isoform	*Brain localization*[a]	*Binding*	*Signalling*	*References*
Human				
H_3(445)	Caudate, Cb, Th, Amg, Hipp, SN, FrCx, Hyp, cc, sp. cord	Yes	↑ GTPγS, ↓ cAMP, ↑ MAPK	Lovenberg *et al*. (1999); Coge *et al*. (2001); Tardivel-Lacombe *et al*. (2001);
H_3(453)	ND	Yes	↓ cAMP	Wellendorph *et al*. (2002); Baranowski
H_3(415)	Caudate, Cb, Th, Amg, Hipp, FrCx	Yes	↑ MAPK	*et al*. (2006); Bongers *et al*. (2007a, b)
H_3(413)	Caudate, Amg	Yes	↑ MAPK	
H_3(409)	Whole brain	Yes	ND	
H_3(373)	Th	ND	↑ R-SAT	
H_3(365)	Caudate, Cb, Th, Amg, Hipp, SN, FrCx, Hyp, cc, sp. cord	Yes	↑ GTPγS, ↓ cAMP, ↑ MAPK	
H_3(329)	Amg, SN, Cx, Hyp, Th, Cb, caudate, cc, Hipp	Yes	↑ MAPK	
Rat				
H_3(445)	AO, Tu, CPu, Acb, Th, GrCb, Hipp, Cx, Hyp, sp. cord	Yes	↓ cAMP, ↑ MAPK	Lovenberg *et al*. (2000); Drutel *et al*. (2001); Morisset *et al*. (2001)
H_3(413)	5 and 6b, CPu, Th, DR, VTM and VLTM neurons	Yes	↓ cAMP, ↑ MAPK	
H_3(397)	CPu, Tu, 5 and 6b, CA1 and CA2, DT, VMH, TMN, LC, Pk	Yes	↓ cAMP, ↑ MAPK	
Monkey				
H_3(445)	FrCx, Hipp, Amg, caudate, Th, Hyp, Cb	Yes	↑ calcium	Yao *et al*. (2003); Strakhova *et al*. (2007)
H_3(413)	Caudate	Yes	↑ calcium	
H_3(410)	Caudate	Yes	↑ calcium	

[a]Abbreviations: 5 and 6b, layers V and VIb of cortex; Acb, nucleus accumbens; Amg, amygdala; AO, anterior olfactory nucleus; CA1 and CA2, fields CA1 and CA2 of hippocampus; Cb, cerebellum; cc, corpus callosum; CPu, caudate putamen; Cx, cortex; DR, dorsal raphe; DT, dorsal thalamic nuclei; FrCx, frontal cortex; GrCb, granular cells of cerebellum; Hipp, hippocampus; Hyp, hypothalamus; LC, locus coerrulus; ND, not determined; Pk, Purkinje cell layer of cerebellum; SN, substantia nigra; sp. cord, spinal cord; Th, thalamus; TMN, tuberomammilary nucleus; Tu, olfactory tuberculum; VLTM, ventrolateral tuberomammilary nucleus; VMH, ventromedial hypothalamic nuclei; VTM, ventral tuberomammilary nucleus.
Signaling key: R-SAT, Receptor Selection and Amplification Technology; calcium levels were determined by FLIPR (Fluorescence Imaging Plate Reader) in HEK cells co-expressing chimeric $G\alpha_{q/i5}$.
The nomenclature system is based on amino-acid number (in parenthesis).

differential expression of H_3 receptor isoforms in the brain on the activity of H_3 receptor antagonists is difficult to determine at this time given the large number of isoforms and differences in isoform types across species. Thus, there is a need to increase our understanding of the role of the multiple isoforms on neuronal activity, including the modulation of neurotransmitter release and subsequent effects on behaviour.

Isoform pharmacology and function

The pharmacology of the H_3 receptor has been extensively reviewed (Hancock *et al*., 2003; Cowart *et al*., 2004; Celanire *et al*., 2005; Leurs *et al*., 2005) and the pharmacological properties of well-characterized H_3 antagonists developed by a number of H_3 receptor research groups are highlighted below. The pharmacology of H_3 receptor ligands at the various H_3 receptor isoforms other than H_3(445) is not well described but differential pharmacological profiles have been noted for the human isoforms, most especially for agonists (Wellendorph *et al*., 2002; Hancock *et al*., 2003; Esbenshade *et al*., 2006b; Bongers *et al*., 2007b). Comparison of the potencies of H_3 receptor agonists such as histamine, *R*-α-methylhistamine, imetit and others at the H_3(445) and H_3(365) receptors revealed approximately from 3- to 20-fold greater potencies of these agonists at the H_3(365) receptor than H_3(445) in binding and functional assays (Wellendorph *et al*., 2002; Bongers *et al*., 2007b). Interestingly, the increase in GTPγS binding induced by the agonists is greater at the H_3(445) than at the H_3(365) isoform (Bongers *et al*., 2007b). Both of these findings were attributed to the higher degree of constitutive activity demonstrated by the H_3(365) isoform. The agonist pharmacological profile of the H_3(415), H_3(413) and H_3(329) isoforms closely resembles that for the H_3(445) isoform (Esbenshade *et al*., 2006c), whereas there appears to be little difference in the pharmacological profile of H_3 antagonists across the human H_3 receptor isoforms.

The H_3 receptor is constitutively active and capable of signalling independently of agonist both *in vitro* and *in vivo* (Morisset *et al*., 2000; Wieland *et al*., 2001). In a similar manner to the isoform-dependent coupling to signalling pathways, the level of constitutive activity of the H_3 receptor also appears to be isoform dependent. Most notably, of the human isoforms, H_3(365) is the most constitutively active, exhibiting the highest relative degree of basal activity in recombinant systems and is the isoform that provides the largest reversal of basal activity in the presence of inverse agonists (Bongers *et al*., 2007b; Esbenshade *et al*., 2007). The potential impact on the differential coupling and constitutive activity of the multiple H_3 receptor isoforms on H_3 antagonist activity is not presently known. However, it has been demonstrated that H_3 inverse agonists can reverse constitutive H_3 receptor-mediated suppression of [^{3}H]histamine synthesis in rat brain cortical slices (Moreno-Delgado

et al., 2006) and [^{3}H]histamine release in mouse brain synaptosomes (Morisset *et al.*, 2000). Thus, although it may be important to design H_3 antagonists that can block the agonist activity of endogenous histamine as well as act as inverse agonists to decrease H_3 receptor constitutive activity at native H_3 receptors, no clinical data as yet have demonstrated whether H_3 receptor inverse agonists are superior to antagonists in blocking an agonist response.

Signalling pathways coupled to H_3(445) receptors have been identified using recombinantly expressed receptors where they have been shown to modulate multiple signal transduction pathways (Bongers *et al.*, 2007a). Activation of H_3 receptors can mediate $G\alpha_{i/o}$-protein-coupled inhibition of adenylate cyclase (Lovenberg *et al.*, 1999) and the Na^+/H^+ exchanger (Silver *et al.*, 2001) as well as stimulation of GTPγS binding (Morisset *et al.*, 2000; Wulff *et al.*, 2002), phospholipase A_2 (Morisset *et al.*, 2000), mitogen-activated protein kinase (MAPK) (Drutel *et al.*, 2001), GSK-3β and Akt (Bongers *et al.*, 2007a). It should be noted that isoform-dependent H_3 receptor differential activation of signalling pathways (MAPK and adenylate cyclase) has also been shown for both human and rat H_3 receptors (Drutel *et al.*, 2001; Esbenshade *et al.*, 2006c, 2007; Bongers *et al.*, 2007b). Interestingly, several of these signalling pathways have been associated with potential roles in various CNS processes including long-term plasticity (MAPK), neuronal cell death (PLA_2) and neuronal migration/neuroprotection (Akt/GSK-3β) (Bongers *et al.*, 2007a). It should also be noted that direct coupling of these signalling events to H_3 receptors has been demonstrated not only in recombinant systems but also in brain tissues expressing native H_3 receptors. Much remains to be determined concerning the role, whether directly through H_3 receptor activation or indirectly through the modulation of the release of multiple neurotransmitters (see below), of this important CNS receptor on these signalling pathways and their associated central functions.

It has been demonstrated that native H_3 receptors couple to $G\alpha_{i/o}$ proteins, activating GTPγS binding in brain tissues (Clark and Hill, 1996; Humbert *et al.*, 2007) and inhibiting adenylate cyclase in striatal slices (Sanchez-Lemus and Arias-Montano, 2004). Additionally, the native H_3 receptor modulates the synthesis and release of histamine (Arrang *et al.*, 1983; Gomez-Ramirez *et al.*, 1998, 2002) and the release of a variety of other neurotransmitters, including ACh, norepinephrine and others (Schlicker *et al.*, 1988, 1989, 1993; Clapham and Kilpatrick, 1992; Blandina *et al.*, 1996). The precise signalling events that contribute to this modulation in neurotransmitter release by H_3 receptors are not well defined; however, it has been demonstrated that histamine suppresses N- and P-type Ca^{2+} channels in dissociated rat tuberomammillary nucleus histaminergic neurons through an H_3 receptor-coupled pertussis toxin-sensitive G-protein-mediated mechanism (Takeshita *et al.*, 1998) Additionally, recent work examining norepinephrine release from cardiac synaptosomes suggests the involvement of protein kinase A and voltage-operated calcium channels (Seyedi *et al.*, 2005). Despite the limited understanding of the neuronal intracellular signalling associated with native H_3 receptors, the role of H_3 receptors in the modulation of neurotransmitter release and the ability of H_3 antagonists to enhance the release of multiple neurotransmitters is well established and is highlighted below.

H_3 receptor modulation of neurotransmitter release

It has been hypothesized that H_3 receptors are specifically located on axon terminals by neurons of multiple neurochemical phenotypes. Although originally described as a presynaptic autoreceptor controlling histamine release (Arrang *et al.*, 1983), the H_3 receptor is also thought to function as a postsynaptic heteroreceptor involving axoaxonic synapses that regulate the release of other neurotransmitters. Whereas their neuronal soma resides exclusively in the posterior hypothalamus, specifically the tuberomammillary nucleus, histaminergic fibres project throughout most regions of the brain, including cortex, striatum, thalamus, hippocampus, hypothalamus, locus coeruleus and spinal cord. By forming synapses with other axon terminals expressing H_3 receptors, release of histamine from these projections can modulate the release of neurotransmitters contained within the postsynaptic terminal. Consistent with autoreceptor inhibition, the release and interaction of histamine with $G\alpha_i$-protein-coupled H_3 heteroreceptors on axoaxonic postsynaptic terminals leads to inhibition of neurotransmitter release. Conversely, the inverse agonism associated with H_3 receptor antagonists has been shown to increase release of neurotransmitters that include ACh, dopamine, norepinephrine and serotonin, as supported by growing numbers of *in vitro* and/or *in vivo* neurotransmitter release studies (summarized in Table 2).

Histamine

Functioning as an excitatory neurotransmitter involving postsynaptic stimulation of H_1 and H_2 receptors throughout the CNS, histamine plays a key role in attention and vigilance (Passani *et al.*, 2000, 2004; Blandina and Passani, 2006). Activation of secondary pathways involved in attention may also be linked to histaminergic neurotransmission, in particular the noradrenergic reticular formation evolving from the locus coeruleus that receives histamine terminal projections from the tuberomammillary nucleus. In this regard, pharmacological-evoked histamine release may afford efficacy in attentional disorders such as ADHD. Whereas release of several different neurotransmitters can be mediated through the H_3 receptor, described below, histamine was first hypothesized to control its own release through the interaction with presynaptic H_3 autoreceptors. In initial studies, incubation with histamine or H_3 receptor agonists inhibited potassium-evoked release of [^{3}H]histamine from rat cortical slices, whereas H_3 receptor antagonists had a facilitatory effect on the stimulated release (Arrang *et al.*, 1983). Several subsequent studies have similarly demonstrated H_3 receptor regulation of histamine release *in vitro*, including the selective H_3 receptor antagonists A-304121, A-317920 and ABT-239 that competitively reversed histamine-mediated inhibition of [^{3}H]histamine

Table 2 Summary of reported *in vitro* and *in vivo* H_3 receptor ligand-mediated neurotransmitter release

Compounds	*ACh*	*Dopamine*	*Norepinephrine*	*Serotonin*	*Histamine*
Histamine		↓ stimulated *in vitro* (Schlicker *et al.*, 1993)		↓ stimulated *in vitro* (Schlicker *et al.*, 1988)	↓ stimulated *in vitro* (Arrang *et al.*, 1983)
RAMH	↓ stimulated *in vitro* (Clapham and Kilpatrick, 1992) ↓ stimulated *in vivo* (PFC) (Blandina *et al.*, 1996)	↓ stimulated *in vitro* (Schlicker *et al.*, 1993)	↓ stimulated *in vitro* (Schlicker *et al.*, 1989)		↓ stimulated *in vitro* (Arrang *et al.*, 1983)
Imetit	↓ stimulated *in vivo* (PFC) (Blandina *et al.*, 1996)				
Immepip	↓ stimulated *in vivo* (PFC) (Blandina *et al.*, 1996)				
Ciproxifan					↑ basal *in vivo* (PFC) (Horner *et al.*, 2007)
Thioperamide	↑ stimulated *in vitro* (Clapham and Kilpatrick, 1992)	∅ RAMH ↓ *in vitro* (Schlicker *et al.*, 1993) ↑ stimulated *in vivo* (NA) (Munzar *et al.*, 2004)	∅ RAMH ↓ *in vitro* (Schlicker *et al.*, 1989) ∅ RAMH ↓ *in vivo* (Di Carlo *et al.*, 2000)		
Clobenprobit		↑ stimulated *in vivo* (NA) (Munzar *et al.*, 2004)			
Burimamide				∅ histamine ↓ *in vitro* (Schlicker *et al.*, 1988)	
Impromidine				∅ histamine ↓ *in vitro* (Schlicker *et al.*, 1988)	↑ basal *in vivo* (ant hyp) (Mochizuki *et al.*, 1991) ↑ basal *in vivo* (amyg) (Cenni *et al.*, 2004)
A-304121					∅ histamine ↓ *in vitro* (Esbenshade *et al.*, 2003)
A-317920					∅ histamine ↓ *in vitro* (Esbenshade *et al.*, 2003)
ABT-239	↑ basal *in vivo* (PFC) (Fox *et al.*, 2005)	↑ basal *in vivo* (PFC) (Fox *et al.*, 2005)			∅ histamine ↓ *in vitro* (Esbenshade *et al.*, 2005)
BF2.649	↑ basal *in vivo* (PFC) (Ligneau *et al.*, 2007b)	↑ basal *in vivo* (PFC) (Ligneau *et al.*, 2007b)			
GSK189254	↑ basal *in vivo* (PFC) (Medhurst *et al.*, 2007a)	↑ basal *in vivo* (PFC) (Medhurst *et al.*, 2007a)	↑ basal *in vivo* (cing ctx) (Medhurst *et al.*, 2007a)		

Abbreviations: amyg, amygdala; ant hyp, anterior hypothalamus; cing ctx, cingulate cortex; NA, nucleus accumbens; RAMH, R-α-methylhistamine; PFC, prefrontal cortex.
Keys: ↑, increased NT release; ↓, decreased NT release; ∅, blocked pharmacological effect.

release from rat brain cortical slices (Esbenshade *et al.*, 2003, 2005).

The first report of histamine release in the whole animal was demonstrated in the hypothalamus of thioperamide-treated rats (Itoh *et al.*, 1991; Mochizuki *et al.*, 1991). H_3 receptor antagonism produced by systemic administration of GT-2016 was subsequently reported to increase histamine in the parietal cortex of awake, freely moving rats (Tedford *et al.*, 1995). Ciproxifan, along with the ADHD agents methylphenidate and atomoxetine, was shown to increase extracellular histamine levels in rat prefrontal cortex (Horner *et al.*, 2007). These later findings raised the intriguing possibility that efficacy associated with ADHD agents such as methylphenidate and atomoxetine may in part involve increased histaminergic tone, supporting the therapeutic potential of H_3 antagonists in the treatment of attention disorders. In studies using H_1 receptor knockout mice, ciproxifan increased wakefulness only in wild type, yet in both genotypes ciproxifan increased histamine release in the frontal cortex, supporting H_3 receptor antagonist-evoked histamine release and subsequent H_1 receptor-mediated vigilance (Huang *et al.*, 2006). Additionally, evoked histamine release has been demonstrated in the basolateral amygdala following local thioperamide administration (Cenni *et al.*, 2004). Taken together, these studies suggest that increased release of histamine by H_3 receptor antagonists may act as indirect H_1 and H_2 receptor agonists enhancing histaminergic neurotransmission within the brain with the potential to augment attention in cognitive disorders such as ADHD and AD.

Acetylcholine

Cholinergic transmission represents an essential neurophysiological component in cognitive functioning. One recognized therapeutic approach to improve cognitive

deficits associated with neurodegenerative disorders such as AD is the development of agents capable of increasing extracellular concentrations of ACh in brain regions associated with cognition (for example, hippocampus and prefrontal cortex). The clinical success of this approach is exemplified by acetylcholinesterase inhibitors such as donepezil (Aricept), widely used in the treatment of AD. Early *in vitro* evidence for H_3 receptor-mediated regulation of ACh neurotransmission was demonstrated in experiments examining potassium-stimulated tritium release from slices of entorhinal cortex preloaded with [^{3}H]choline (Clapham and Kilpatrick, 1992). Whereas the H_3 receptor agonist *R*-methylhistamine inhibited release, the H_3 receptor antagonist thioperamide augmented potassium-stimulated [^{3}H]ACh release. Blandina *et al*. (1996) later provided the first *in vivo* evidence for a role of histamine H_3 receptors in regulating ACh release in rat cortex, which receives cholinergic input originating primarily from the nucleus basalis. In a series of *in vivo* microdialysis experiments, it was demonstrated that histamine and the H_3 receptor agonists *R*-α-methylhistamine, imetit and immepip locally administered through the microdialysis probe inhibited potassium-evoked ACh release in the frontoparietal cortex (Blandina *et al*., 1996). The inhibition was prevented by the H_3 antagonist clobenpropit, but not by an H_1 antagonist (tripolidine) or H_2 antagonist (cimetidine). In addition, *R*-α-methylhistamine and imetit inhibited potassium-evoked ACh cortical release when administered systemically (i.p.) at doses shown to disrupt short-term memory performance, suggesting a potentially important role for the H_3 receptor as a target for neurodegenerative disorders associated with impaired cognitive function. H_3 receptors also regulate ACh release in other brain regions including the hippocampus where systemic administration of *R*-α-methylhistamine decreased electrically evoked ACh release, whereas thioperamide enhanced ACh release in the hippocampus (Mochizuki *et al*., 1994). Similarly, when administered locally into the medial septum diagonal band, *R*-α-methylhistamine decreased, whereas thioperamide augmented hippocampal ACh release (Bacciottini *et al*., 2002). Studies have also shown that in the basolateral amygdala, local administration of H_3 receptor agonists enhance ACh release from this brain region at doses corresponding with enhanced memory retention in a contextual fear-conditioning paradigm (Cangioli *et al*., 2002), whereas H_3 receptor antagonists reduce ACh release (Passani *et al*., 2001) with a dose-associated impairment in memory retention. Since these initial studies, there have been reports of novel histamine H_3 receptor antagonists increasing ACh release as demonstrated by *in vivo* microdialysis associated with procognitive efficacy in behavioural animal models. The selective histamine H_3 receptor antagonist ABT-239 increased ACh release in the frontal cortex and to a lesser extent in the hippocampus at doses (0.1–3 mg kg^{-1}) similar to those producing efficacy in rat cognition models (Fox *et al*., 2005), as described below. Similarly, the novel histamine H_3 receptor antagonists BF2.649 (Ligneau *et al*., 2007b) and GSK189254 (Medhurst *et al*., 2007a) increased ACh release in the frontal cortex and/or dorsal hippocampus.

Dopamine

Aberrant dopaminergic neurotransmission has long been recognized as a major aetiological component of schizophrenia psychopathology. The primary pharmacological approach to schizophrenia has employed the use of dopamine receptor antagonists for treating the hyperdopaminergic transmission associated with positive symptoms (hallucinations, delusions and thinking disturbances), In contrast, the negative symptoms (apathy, blunted affect and inattention) and cognitive deficits also observed in schizophrenia do not respond well to dopamine receptor antagonists, which in fact are considered to manifest through hypodopaminergic transmission, specifically in the prefrontal cortex. Pharmacological stimulation of dopamine release in the prefrontal cortex is being considered a viable approach in treating negative symptoms and cognitive impairment in schizophrenia, symptoms that are currently not well treated and thus currently representing a significant unmet medical need. There have been several reports indicating that histamine H_3 receptors can regulate dopamine release. The H_3 agonists histamine and *R*-α-methylhistamine were shown to inhibit preloaded [^{3}H]dopamine release from mouse striatal slices and this effect was blocked by the H_3 antagonist thioperamide, but not by H_1 or H_2 receptor antagonists (Schlicker *et al*., 1993). In the whole animal, H_3 receptor antagonism produced by systemic administration of either thioperamide or clobenprobit potentiated methamphetamine-induced dopamine release in the nucleus accumbens shell, but had no effect on extracellular dopamine when given alone (Munzar *et al*., 2004). Administration of the H_3 antagonist ABT-239 by itself increased extracellular dopamine concentrations of dopamine in rat prefrontal cortex, but not in the striatum (Fox *et al*., 2005). Enhanced dopamine release in rat prefrontal cortex has also been demonstrated with both BF2.649 (Ligneau *et al*., 2007b) and GSK189254 (Medhurst *et al*., 2007a). Taken together, these studies support the therapeutic potential of H_3 receptor antagonists for treating negative symptoms and cognitive deficits associated with schizophrenia as defined by hypodopaminergic function in prefrontal cortex.

Norepinephrine

Noradrenergic neurotransmission within the CNS plays an important role in attentional processing and affective behaviours, which is highly regulated through norepinephrine release in cortical and hippocampal regions from axon terminals of neurons located in the locus coeruleus. Several psychiatric therapeutics lead to enhanced noradrenergic transmission through various pharmacological means, including inhibition of synaptic reuptake or increased release of norepinephrine. Histamine H_3 receptors expressed on noradrenergic terminals innervating cortical and hippocampal regions may represent a potential target in modulating norepinephrine release. Support for this potential originates from studies demonstrating that *R*-α-methylhistamine inhibition of [^{3}H]norepinephrine release from rat cortical slices was prevented by the H_3 receptor antagonist thioperamide (Schlicker *et al*., 1989). Initial rat *in vivo* microdialysis studies involving both systemic and local administration of

thioperamide did not stimulate basal norepinephrine release in the hippocampus, but did prevent the reduction of norepinephrine that was produced by *R*-α-methylhistamine (Di Carlo *et al*., 2000). Although these results suggested that norepinephrine release mediated through histamine H_3 heteroreceptors located on noradrenergic terminals may only play a minor role in regulating hippocampal norepinephrine release, it was subsequently demonstrated that oral administration the novel H_3 receptor antagonist GSK189254 increased basal norepinephrine levels in the cingulate cortex of freely moving rats at doses improving cognitive performance (Medhurst *et al*., 2007a).

Serotonin

Similar to norepinephrine, pharmacological augmentation of extracellular brain serotonin represents a viable approach in the treatment of affective disorders, in particular unipolar depression, as evidenced by the clinical efficacy of selective serotonin reuptake inhibitors. Located in the midbrain nuclei, serotonergic neurons project axons throughout cortical and hippocampal forebrain regions where histamine H_3 receptors are located. Reports of H_3 receptor-mediated serotonin release have been primarily limited to *in vitro* studies. Inhibition of electrically evoked [^{3}H]serotonin from rat cortical slices by histamine was antagonized by the mixed H_2/H_3 receptor agonist/antagonists burimamide and impromidine, the later evoking release alone (Schlicker *et al*., 1988). Additionally, in studies utilizing rat midbrain slices, it has been shown that H_3 receptors regulate serotonin release in the substantia nigra pars reticulata where electrically evoked serotonin release was inhibited up to 60% by H_3 receptor agonists such as *R*-α-methylhistamine and immepip (Threlfell *et al*., 2004). Interestingly, this effect was reversed by the H_3 receptor antagonist thioperamide but not by antagonists of GABA or glutamate receptors, strongly suggesting a role for histamine and H_3 receptors in the function of the substantia nigra pars reticulata and a potential target for basal ganglia therapies (Threlfell *et al*., 2004). Whether *in vitro* demonstration of H_3 receptor-mediated serotonin release translates to significant *in vivo* effects remains to be determined. Whereas the selective H_3 receptor antagonist GSK189254 was shown to evoke ACh, dopamine and norepinephrine release in the rat cingulate cortex, there was no effect on serotonin (Medhurst *et al*., 2007a). On the other hand, there is substantial interest in the field for agents that combine H_3 receptor antagonism with serotonin uptake inhibition to increase neuronal serotonin levels (see below).

In summarizing the role of H_3 receptor-mediated neurotransmitter release, experimental results over the last 10 years support the hypothesis that the behavioural effects of H_3 receptor antagonists likely involves the release of various neurotransmitters in brain regions associated with cognitive function. Thus, functioning as 'indirect' agonists at multiple receptor classes within the CNS provides the potential for H_3 antagonists to treat psychiatric pathologies resulting from reduced neurotransmission. However, much remains unknown as to how the interaction and crosstalk between different neurotransmitters affected by H_3 receptor antagonism contribute to the potential efficacy afforded by these novel CNS agents. Sophisticated microdialysis studies capable of assessing several neurotransmitters at multiple sites simultaneously are warranted and may provide improved understanding of such mechanisms.

H_3 receptors and cognition

Histamine is a biogenic amine that exhibits high affinity for the H_3 receptor and has a demonstrated role in CNS activities including learning and memory. For example, the histaminergic system has been implicated in arousal and attention (influenced in ADHD), AD and schizophrenia. Increases in histamine levels in the brain of patients with AD have been reported in key brain areas such as the frontal cortex and hippocampus (Cacabelos *et al*., 1989), whereas others have reported a significant reduction in the content of histamine in the hippocampus and other areas (Mazurkiewicz-Kwilecki and Nsonwah, 1989; Panula *et al*., 1998). These differences between studies could reflect differences in the amount of neuronal damage or disease state (Fernandez-Novoa and Cacabelos, 2001), although no clear relationship between histamine levels and AD, for example, have been demonstrated. Interestingly, recent work has further supported a role for H_3 receptors in AD, demonstrating that H_3 receptor expression remains prevalent in the medial temporal cortex of patients diagnosed with AD, even in advanced stages of the disease (Medhurst *et al*., 2007a). A substantial body of evidence supports that increasing histaminergic tone can facilitate cognition (De Almeida and Izquierdo, 1986; Kamei *et al*., 1993; Miyazaki *et al*., 1995), supporting the utility of drugs that increase histaminergic activity in key brain regions, although there is also some evidence that indicates a decrease in histaminergic tone can increase cognition (Huston *et al*., 1997). These conflicting reports make it difficult to conclusively demonstrate that the beneficial effects of H_3 receptor antagonists in diverse cognition models are mediated solely by histamine or whether other neurotransmitter systems previously mentioned (for example, ACh, dopamine, etc.) play equally or more important roles.

The neuronal histamine system and specifically H_3 receptors have been suggested as modulators of the sleep–wake cycle and cognitive processes (Passani *et al*., 2004; Esbenshade *et al*., 2006a). In general, literature data indicate that the administration of H_3 receptor agonists can impair cognition (see Table 3 for a summary), although see also Rubio *et al*. (2002). These data, taken together with evidence from histamine H_3 receptor knockout animals that demonstrate enhanced spatial learning and memory in the Barnes maze (Rizk *et al*., 2004), support a role for H_3 receptors in cognition. As described above, histamine H_3 receptors are an attractive drug target as these receptors modulate neurotransmitter release and the localization and neurochemistry of H_3 receptors make this system uniquely poised to play a role in aspects of learning and memory. Given preclinical evidence suggesting that blockade of histamine H_3 receptors can decrease impulsivity, improve attention, and enhance learning and memory, research has focused on the ability of

Table 3 Summary of H_3 receptor-mediated cognitive effects across cognitive domains

Compounds	*Recognition memory*	*Spatial memory*	*Memory consolidation*	*Working memory*	*Executive function*	*Attention/ impulsivity*	*Representative references*
Histamine							
Histamine	↑ (SR)	↑ (scop. def. RM)	↑ (PA)				De Almeida and Izquierdo (1986); Prast *et al.* (1996); Chen (2000); Chen and Kamei (2000)
Histidine	↑ (SR)	↑ (scop. def. EPM)	↑ (AA, chronic)				Kamei *et al.* (1993); Miyazaki *et al.* (1995)
H_3 receptor agonists							
Immepip	↓ (SR)						Prast *et al.* (1996)
RAMH	↓ (OR)	↑ (facilitated SM)	↓ (PA)				Rubio *et al.* (2002)
Imetit	↓ (OR)		↓ (PA)				Blandina *et al.* (1996)
H_3 receptor antagonists							
Thioperamide	↑ (SR)	↑ (scop. def. WM; no effect BM)	↑ (PA senescence, SHR pup models)	↑(scop. def. y-maze)	↑ (scop. def. RM)	↑ (SHR 5-trial IA)	Meguro *et al.* (1995); Miyazaki *et al.* (1995); Prast *et al.* (1996); Chen (2000); Orsetti *et al.* (2002); Komater *et al.* (2003, 2005)
Ciproxifan	↑ (SR)	↑ (BM, scop. def. WM)				↑ (SHR 5-trial IA; 5-CSRTT)	Fox *et al.* (2002, 2005); Day *et al.* (2007)
GT-2331						↑ (SHR 5-trial IA)	Fox *et al.* (2002)
ABT-239	↑ (SR, adult/ aged)	↑ (scop. def. WM)	ND	↑ (y-maze)		↑ (SHR 5-trial IA)	Fox *et al.* (2005)
BF2.649	↑ (OR; naïve and scop. def.)						Ligneau *et al.* (2007b)
JNJ-10181457						↑ (SHR 7-trial IA)	Bonaventure *et al.* (2007)
GSK189254	↑ (OR)	↑ (aged WM)	↑ (PA)		↑ (executive set shifting)		Medhurst *et al.* (2007a)

Abbreviations: 5-CSRTT, five-choice serial reaction time test; AA, active avoidance; BM, Barne's maze; EPM, elevated plus maze; IA, inhibitory avoidance; OR, object recognition; PA, passive avoidance; RAMH, *R*-α-methylhistamine; RM, radial maze; scop. def., scopolamine deficit; SM, spatial memory; SR, social recognition; WM, water maze.
Keys: ↑, improvement; ↓, impairment.

H_3 receptor antagonists to potentially treat cognitive disorders. These cognitive disorders can be further subdivided into different cognitive domains and much of the preclinical research in these areas has focused on cognition assays that measure the different learning and memory domains thought to be most affected in diseases such as AD. Other domains, such as attention and impulsivity, are likely to be of importance in AD and especially in other patient populations, especially those with ADHD (see Table 3). Broad efficacy has been demonstrated across these multiple domains with H_3 receptor antagonists, even if not all compounds have been tested in each assay or not every H_3 receptor antagonist tested was demonstrated active in all tasks.

Attention/impulsivity

Whereas attentional deficits span multiple disease states including AD, schizophrenia and ADHD, of particular relevance to ADHD are deficits in impulsivity. Tests that measure aspects of attention and impulsivity that have been used to evaluate efficacy of H_3 receptor antagonists are the five-trial (or seven-trial) inhibitory avoidance paradigm in spontaneously hypertensive rat (SHR) pups, as well as the five-choice stimulus reaction time test (5-CSRTT). As detailed in Table 3 as well as in multiple publications (Fox *et al*., 2002; Hancock and Fox, 2004; Esbenshade *et al*., 2006a), a number of H_3 receptor antagonists, including thioperamide, ciproxifan, ABT-239 and GT-2331, are efficacious in the five-trial inhibitory avoidance in SHR pups (with a recent report also describing the efficacy of JNJ-10181457 in a seven-trial inhibitory avoidance version of the model (Bonaventure *et al*., 2007)). SHRs exhibit many behaviours commonly observed in patients with ADHD, and as such are often used as a model of ADHD (Davids *et al*., 2003; Russell, 2007). SHR pups are normotensive at the age of testing in the five-trial and seven-trial inhibitory avoidance assay and thus cognitive deficits are independent of hypertension. Deficits in the SHR may be linked to a reduction in nicotinic-ACh receptors observed in a number of brain regions including cortex, hippocampus, thalamus and striatum (Gattu *et al*., 1997; Terry *et al*., 2000). It is also possible that behavioural abnormalities in SHRs may be due to an impaired release of dopamine from nerve terminals in the prefrontal cortex (Davids *et al*., 2003). As H_3 receptors have been shown to regulate the release of both ACh and dopamine, blockade of H_3 receptors with antagonists would be expected to improve function in either or both cases. The terms attention and impulsivity are quite general, referring to multiple processes. As an example, attention covers selective and divided attention, vigilance and distractability. Impulsivity is typically defined as the inability to withhold a response.

Another way of modelling aspects of attention and impulsivity is using the 5-CSRTT in which individual measures of attention, impulsivity, motivation and motor function can be quantified (Robbins, 2002). The 5-CSRTT is analogous to a test used to assess humans (the Continuous Performance Test), thus data from 5-CSRTT may serve as a useful translational assay for efficacy in these behavioural domains. The 5-CSRTT uses visual cues to predict a food reward; a reward that is presented only when the animal correctly responds to the appropriate stimuli. By measuring the percent correct or incorrect choices or the number of missed responses, attention can be measured. Impulsivity can be measured by assessing the number of responses in between trials (when it is inappropriate to respond). Motor function can be assessed by measuring variables such as latency to respond. Previous studies have demonstrated some conflicting results with H_3 receptor antagonists in this task, with one report indicating efficacy (Ligneau *et al*., 1998), whereas another study found thioperamide did not reverse a scopolamine-induced deficit (Kirkby and Higgins, 1998). A recent publication found that ciproxifan is efficacious on measures of impulsivity, with some efficacy on measures of attention (Day *et al*., 2007).

Recognition memory

One of the early domains studied with H_3 receptor antagonists was social memory (a form of short-term recognition memory). Social recognition is frequently impaired in patients with AD, and as such efficacy in this domain may be relevant for domains impaired in patients with AD. Social recognition relies on the retention of memory in rats, in which an adult animal uses olfactory cues to recall a social interaction with a conspecific juvenile. Evidence supporting a role for the histaminergic system in social memory came from an early study demonstrating that i.c.v. administration of histamine facilitated social memory; an effect that was also observed with thioperamide (Prast *et al*., 1996). Further, recognition recall can be blocked by the inhibition of histamine synthesis (Prast *et al*., 1996). Another form of short-term recognition memory, object recognition, has been used to evaluate short-term recognition memory. In this task, rodents are assessed for their ability to remember a familiar vs an unfamiliar test object (that is, this type of recognition is less likely to involve the possibility of social performance). As detailed in Table 3, several H_3 receptor antagonists have demonstrated efficacy in the recognition memory domain.

Recognition memory involves multiple brain regions and neuromodulatory systems. Evidence has implicated the hippocampus (Kogan *et al*., 2000) and entorhinal cortex (Bannerman *et al*., 2002) in recognition memory, but research suggests that the parahippocampal region (including the entorhinal, perirhinal, parahippocampal/postrhinal cortices) is important in this cognitive domain (Mishkin, 1978). ACh (Winslow and Camacho, 1995) and histamine (Prast *et al*., 1996) are important neurotransmitters involved in social memory. Taken together, it is likely that the observed improvement of recognition memory with H_3 receptor antagonists in the current studies reflects enhanced ACh and histamine release in these key brain regions and it is possible that this will translate into improved social memory and cognition in patients.

Spatial memory
Spatial memory is another aspect of cognition that is impaired in patients with AD. There are a number of assays that have been used to measure spatial memory, including the Barnes maze or variants of the water maze or radial arm maze. All of these tasks used for evaluating spatial memory have the shared feature of requiring the animal to use local information in navigating through their environment. In addition to the results with H_3 receptor antagonists listed in Table 3, in the Barnes maze, mice lacking H_3 receptors learn spatial cues more readily than do their wild-type counterparts (Rizk *et al*., 2004). Taken together, these data indicate that blockade of H_3 receptors enhances spatial memory.

There are many different types of spatial memory tasks that have been used to evaluate H_3 receptor antagonists, but in general these assays rely on the hippocampus and the septal–hippocampal pathway. It is likely that the improvements in spatial memory observed with H_3 receptor antagonists, therefore, are due to effects on ACh.

Memory consolidation

One important behavioural assay that has been commonly used to assess cognitive function, especially components of longer term memory consolidation, is the inhibitory avoidance assay. Thioperamide improves memory in this assay in a model of senescence (Meguro *et al*., 1995) as well as in SHRs (Hancock and Fox, 2004). Further, thioperamide can prevent the deficit induced by scopolamine in this assay (Giovannini *et al*., 1999), and mice lacking the H_3 receptor are known to be insensitive to the cognitive-impairing effects of scopolamine in this test (Toyota *et al*., 2002). Therefore, the observation of efficacy of H_3 receptor antagonists in this task implies that they may be acting via modulation of cholinergic function in this cognitive domain. One point of interest is that in this particular assay H_3 receptor antagonists generally have efficacy when the administration is given before training, rather than post-training (Giovannini *et al*., 1999), suggesting a role for H_3 receptors specifically in memory acquisition, rather than memory recall. Another rodent model where H_3 receptors appear to play a role in memory consolidation is contextual fear conditioning. Local post-training administration of H_3 receptor agonists and antagonists into the basolateral amygdala augment and reduce ACh release, respectively, with corresponding enhancement and impairment of memory retention in this model (Passani *et al*., 2001; Cangioli *et al*., 2002), suggesting a role for a H_3 receptor-regulated cholinergic component in this cognitive domain.

Working memory
Working memory is a cognitive domain that is impaired in schizophrenia as well as AD and ADHD. Working memory generally refers to a memory system that is used to temporarily store information required to successfully complete complex cognitive tasks. H_3 receptor antagonists have demonstrated efficacy in this domain (Table 3), and examples of frequently used assays include the y-maze, variations of the water maze, radial arm maze and tests of delayed match-to-sample memory. Increased error rates (radial arm maze) have been associated with declining histamine content in key brain regions, and these deficits can be reversed by either i.c.v. histamine or thioperamide (Chen *et al*., 1999). As another example, thioperamide can reverse scopolamine-induced deficits in the y-maze task (Orsetti *et al*., 2002).

Executive function
A recent report by Medhurst *et al*. (2007a) is the first support for efficacy of H_3 receptor antagonists in the cognitive domain of executive function, a domain impaired in patients with schizophrenia. In attentional set-shifting assays, or models of cognitive flexibility, rats are required to learn that a previously rewarded rule no longer is the correct strategy and they must now use a new rule to obtain a reward. The H_3 receptor antagonist GSK189254 was found efficacious; improving both reversal learning and attentional set-shifting (Medhurst *et al*., 2007a). The demonstration of efficacy with H_3 receptor antagonists in a model of cognitive flexibility and executive function could have implications for the treatment of cognitive deficits of schizophrenia.

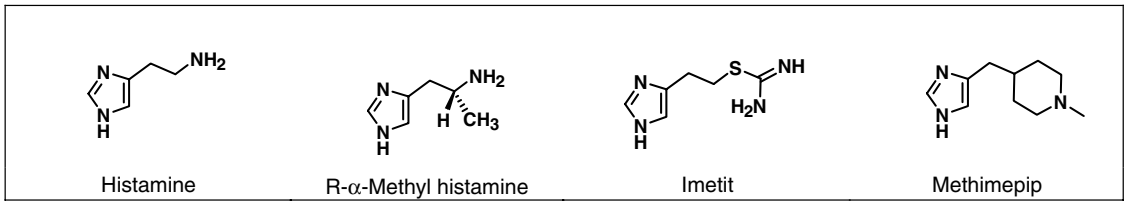
H_3 receptor antagonists have demonstrated efficacy across a number of cognitive domains relevant to disorders such as AD, cognitive deficits associated with schizophrenia and ADHD. Many groups in the H_3 field are currently evaluating the safety and efficacy of H_3 receptor antagonists in the clinic, and the results of these studies will provide more specific evidence of the role of H_3 receptors, and potentially the broader role of histamine in cognitive functioning. A selection of prominent H_3 antagonists (Table 4), which have been used preclinically as tool compounds to elucidate the effects of H_3 receptor blockade on cognition have been identified in patents of companies with extensive activity in the H_3 antagonist arena, or those H_3 antagonists identified as currently being in Phase I/Phase II trials are highlighted in the following section.

Properties of H_3 receptor antagonists

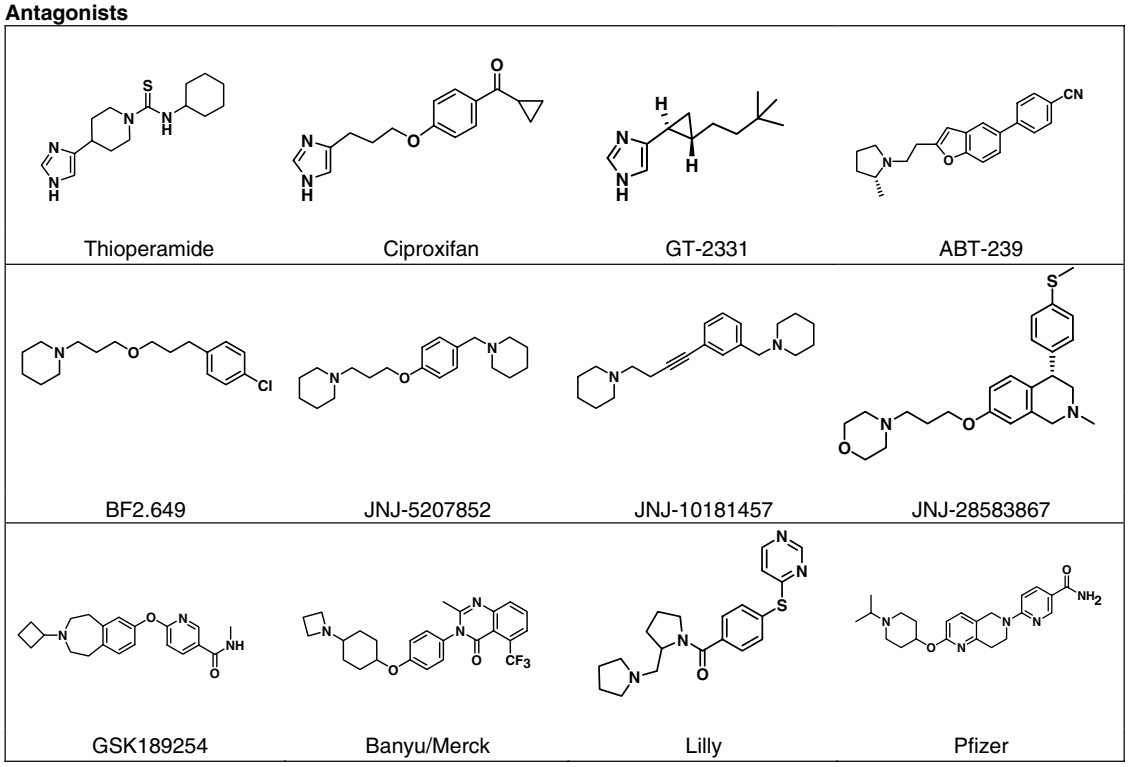
ABT-239
ABT-239 (Table 4) is a 2-ethylaminobenzofuran that has high potency in rat ($K_i = 1.3$ nM) and at all other H_3 receptor species (Cowart *et al*., 2005). The compound is selective for H_3 receptors vs over 80 other CNS receptor sites tested, exhibits good pharmacokinetic properties in several animal species and is broadly effective in diverse behavioural models (Esbenshade *et al*., 2005; Fox *et al*., 2005). ABT-239 improves short-term recognition memory in adult rats (0.01 mg kg^{-1} i.p.) and aged rats (0.3 mg kg^{-1}), and it ameliorates attention deficits in the five-trial inhibitory avoidance model of ADHD in SHR rat pups (0.1 mg kg^{-1}). At higher doses, ABT-239 partially reversed scopolamine-induced deficits in spatial memory in rats in the two-platform water maze (3 mg kg^{-1}) and enhanced N40 gating in DBA2 mice (1 and 3 mg kg^{-1}).

Table 4 Chemical structures of histamine H_3 receptor agonists and antagonists described in the scientific and patent literatures

Agonists



Antagonists



The structures shown for Banyu/Merck, Lilly and Pfizer are representative of the series described in the patent literature.

Whereas the specific neurotransmitters mediating the behavioural effects of ABT-239 cannot yet be stated with certainty, *in vivo* microdialysis studies show that ABT-239 increases cortical and hippocampal ACh at doses (0.1–3.0 mg kg^{-1}) and time courses (30–120 min) that parallel the behavioural efficacy in cognitive models. Significantly, both the *in vivo* ACh release and behavioural efficacy are retained upon chronic (5 day) dosing in rats. ABT-239 increases the release of histamine *in vitro* from rat brain synaptosomes, indicating that the release of either or both ACh and histamine could modulate the procognitive effects of ABT-239 *in vivo*. On the basis of drug exposures (14–507 nM), rat binding potency and protein binding, ABT-239 is effective in the ADHD model at calculated H_3 receptor occupancies from 37 to 96%, values consistent with other close analogues (Cowart *et al*., 2007). ABT-239 exhibited an unfavourable compound-related cardiovascular profile associated with QT prolongation in monkeys that precluded further clinical development (Hancock, 2006). However, due to its potency and selectivity for the H_3 receptor, it is an excellent tool compound to investigate the role of H_3 receptors in the CNS.

BF2.649

BF2.649 (Table 4) is a piperidinylpropoxyalkylphenyl H_3 antagonist that exhibits potent binding to the rat ($K_i = 2.7$ nM) and mouse ($K_i = 14$ nM) histamine H_3 receptors (Ligneau *et al*., 2007a, b). It also binds to the human cortical H_3 receptor with $IC_{50} = 5.3$ nM. Systemic injections of BF2.649 increased ACh and dopamine release in the rat cortex at 10 mg kg^{-1} i.p., and in the object recognition model BF2.649 (15 mg kg^{-1}, i.p.) reversed the cognitive deficits induced by scopolamine, consistent with the ability to release ACh in the rat brain. Behavioural studies also demonstrated that BF2.649 (5 mg kg^{-1}, i.p.) attained a 63% attenuation of methamphetamine-induced hyperlocomotion, but only at lower but not higher doses of methamphetamine

(Ligneau *et al*., 2007a). BF2.649 induced a modest attenuation of MK-801 hyperactivity, had limited efficacy against apomorphine-induced prepulse inhibition deficits and it did not affect apomorphine-induced climbing. Despite the claim that BF2.649 exhibits antipsychotic potential (Ligneau *et al*., 2007a), based on these preclinical data, the overall effects of BF2.649 in these models were minimal.

Pharmacokinetic parameters measured by a radioreceptor assay indicated that BF2.649 exhibits 84% oral bioavailability in mice. However, these data were not replicated in our lab (with 30, 5 and 2% bioavailability in mice, rat and dog). One recent report indicated a 2-h half-life and good brain penetration in mice using a superior analytical assay but no detailed pharmacokinetic parameters were provided (Ligneau *et al*., 2007a). This limited oral bioavailability questions the data related to the ability of BF2.649 to increased histamine brain levels after oral administration in mice and the EEG studies conducted in cats. Further studies are needed to demonstrate the role of BF2.649 or its metabolites in preclinical models, with detailed analysis of the plasma levels of parent as well as the main metabolites in these species. BF2.649 is presently under clinical investigation in several Phase II trials for the treatment of schizophrenia, ADHD, dementia and Parkinson's disease. (www.stanleyresearch.org/programs/trialgrants.htm). From the development point of view, our laboratory findings suggest that CYP2D6 inhibition, potent hERG binding and the potential for phospholipidosis would likely be important hurdles for this novel compound.

JNJ compounds

Several novel series of H_3 antagonists have been reported by Johnson & Johnson (Table 4). JNJ-5207852 is a potent dibasic amine antagonist that binds potently to rat H_3 receptors ($K_i = 1.2$ nM), and has good brain penetration. In *ex vivo* binding studies in mice, the compound had an ED_{50} of $0.13\,mg\,kg^{-1}$, subcutaneously (Barbier *et al*., 2004). It promotes wakefulness in rodents at $10\,mg\,kg^{-1}$ s.c. but not at $1\,mg\,kg^{-1}$, and significantly, this effect was absent in H_3 receptor KO mice. This compound appears to have not advanced to the clinic, possibly due to a long brain residency and/or induction of phospholipidosis. JNJ-10181457 is also a dibasic amine antagonist that exhibits high-affinity binding for the rat H_3 receptor ($K_i = 7.1$ nM), promoting wakefulness in rodents and reducing cataplectic attacks in narcoleptic dogs (Bonaventure *et al*., 2007). JNJ-10181457 improved cognitive performance in SHR pups at $10\,mg\,kg^{-1}$ s.c., consistent with data obtained with JNJ-5207852 that reversed pentylenetetrazol-induced memory deficits in several learning and memory tests (Jia *et al*., 2006).

The data obtained with these potent H_3 receptor antagonists demonstrate that they can promote wakefulness and improve cognition in preclinical animal models. Some of these aforementioned agents did not advance to the clinic (due to different reasons), but JNJ-17216498 is reportedly in Phase II studies in patients with narcolepsy (www.clinicaltrials.gov). A recent publication described the pharmacology of a new class of compounds exemplified by JNJ-28583867, a combined H_3 antagonist and serotonin reuptake inhibitor that increases serotonin, norepinephrine and dopamine release in rat brain (Barbier *et al*., 2007). This compound showed antidepressant-like activity in mice and promoted wakefulness in rats. In view of these combined behavioural effects in animals, the authors proposed that JNJ-28583867 might be useful for the treatment of several symptoms in depressed patients.

GSK189254

The benzo[*d*]azepine H_3 receptor antagonist GSK189254 (Table 4) binds with high affinity to the rat and human histamine H_3 receptor ($K_i = 3$ and 0.2 nM, respectively) and increases the release of ACh, norepinephrine and dopamine in rat cortex after oral administration of $1–3\,mg\,kg^{-1}$ (Medhurst *et al*., 2007a). It reversed scopolamine-induced amnesia in the inhibitory avoidance assay at the same dose range and it was also efficacious in other cognitive models (i.e., water maze and object recognition test). Interestingly, despite the high affinity of this compound for the rat H_3 receptor and *ex vivo* binding studies showing that the ED_{50} for cortical H_3 receptor occupancy is $0.17\,mg\,kg^{-1}$ (oral), efficacy in animal models of cognition is reportedly achieved only at 10-fold higher doses. The published preclinical data are consistent with the ability of H_3 antagonists to improve cognition. However, available clinical information indicates that GSK189254 is presently under clinical evaluation in patients suffering narcolepsy and in an electrical hyperalgesia model in healthy volunteers as a translational model of neuropathic pain (www.clinicaltrials.gov).

Preclinical data on pain models have not been disclosed for GSK189254 but a recent paper described the effects of GSK207040 and GSK334429 in animal models of cognition and pain (Medhurst *et al*., 2007b). These compounds are potent antagonists at the rat H_3 receptor ($K_i = 1$ and 0.8, respectively) that reversed scopolamine-induced amnesia in the inhibitory avoidance test and significantly reversed capsaicin-induced reduction in the paw withdrawal threshold, indicating that these H_3 antagonists can reduce tactile allodynia. Duloxetine (Cymbalta) has recently been approved for the treatment of neuropathic pain and it has been suggested that its efficacy may be related to its ability to increase serotonin and norepinephrine levels in the brain. As H_3 antagonists can increase neurotransmitter release in the brain, H_3 antagonists may increase these or other relevant neurotransmitters, and be useful for the treatment of neuropathic pain in humans. Despite this initial finding in the capsaicin model, evidence for efficacy in other models of neuropathic pain such as the Chung and Bennett models is needed to support to this notion. Other advanced H_3 antagonists from GSK include GSK239512 in a brain imaging study (www.clinicaltrials.gov), GSK357868 and GSK678103.

MK-0249

Merck is conducting three Phase II clinical trials to determine the efficacy of the H_3 antagonist MK-0249 in AD, ADHD and cognitive deficits of schizophrenia (www.clinicaltrials.gov). The chemical structure of this compound has not been disclosed but several series have been disclosed

in patents applied for by Banyu/Merck (Nagase *et al.*, 2005, 2006). A representative compound of the quinazolinone series filed by Banyu is shown in Table 4.

Lilly
Lilly has filed a number of patent applications. One recent application names a compound (Table 4) with a K_i of 11.7 nM for antagonism of *R*-α-methylhistamine-stimulated [^{35}S]GTPγS binding to the human H_3 receptor (Beavers *et al.*, 2007). The presence and preference for the (*S*)-2-pyrrolidin-1-ylmethyl-pyrrolidine moiety is reminiscent of another H_3 antagonist chemical series from Novo-Nordisk, including NNC 0038-0000-1202 (Peschke *et al.*, 2006).

Pfizer
Pfizer has more than two dozen published patent applications on diverse genera with claimed therapeutic targets that include CNS diseases. At least one application focused on only a single compound (Table 4) that also names treatment of inflammation and respiratory diseases and combinations with anti-inflammatory agents (Lunn, 2007). The compound had a K_i of 2.6 nM in blocking imetit-dependent inhibition of forskolin-stimulated cAMP synthesis in HEK-293 cells transfected with the human H_3 receptor.

Concluding remarks

There has been considerable progress made in our understanding of the complex biology and properties of the H_3 receptor that has correspondingly led to an increased interest in developing H_3 antagonists to treat cognitive disorders. Although there is indeed great complexity associated with the H_3 receptor including the heterogeneity of isoforms as well as their corresponding differential localization, pharmacological and signalling properties that can complicate drug discovery efforts, considerable efforts have been expended by academic and industrial laboratories to identify potent and selective H_3 antagonists for the treatment of cognitive disorders. Much of the interest in the therapeutic potential of H_3 antagonists arises from the ability of H_3 antagonists to enhance the release of key neurotransmitters such as histamine, ACh, norepinephrine and dopamine that play critical roles in cognitive processing. Additionally, the cognitive-enhancing effects of H_3 antagonists across multiple cognitive domains in a wide variety of preclinical cognition models also bolster confidence in this therapeutic approach for the treatment of ADHD, AD and schizophrenia. Despite these many advances, to date no clinical proof of concept for an H_3 receptor antagonist has been reported. However, a number of clinical studies examining the efficacy of H_3 receptor antagonists for a variety of cognitive disorders are currently underway, so the first reports of the efficacy of these compounds may be reported soon. In the mean time, research efforts are sure to continue to gain further insights into the functions of the H_3 receptor in the quest to discover selective therapeutic H_3 antagonists for the novel treatment of cognitive disorders.

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Conflict of interest

We are all employees of Abbott Laboratories.

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