Discovery of histamine H₃ antagonists for the treatment of cognitive disorders
and Alzheimer's disease

Jorge D. Brioni, Tim A. Esbenshade, Tiffany Runyan Garrison,
Scott R. Bitner and Marlon D. Cowart

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Corresponding Author:

Jorge D. Brioni
Neuroscience Research (Dept. 4MN)
Global Pharmaceutical Research and Development
Abbott Laboratories
100 Abbott Park Road
Abbott Park, IL 60064

Phone: 847-937-9079
e-mail: jorge.brioni@abbott.com

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Key Abbreviations:

AD, Alzheimer’s disease; ADHD, attention deficit hyperactivity disorder; CDS, cognitive deficits of schizophrenia; SHR, Spontaneously Hypertensive Rat;
Abstract

H₃ antagonists increase the release of brain histamine, ACh, NE and DA, neurotransmitters that are known to modulate cognitive processes. The ability to release brain histamine support the effect on attention and vigilance, but histamine also modulates other cognitive domains like short-term and long-term memory. A number of H₃ antagonists including BF2.649, PF-03654746, GSK189254, MK-0249, JNJ-17216498 and ABT-288 have advanced to the clinical area for the potential treatment of human cognitive disorders. H₃ antagonists exhibited wake promoting effects in humans and efficacy in narcoleptic patients indicating target engagement but some of them were not efficacious in ADHD and schizophrenic patients. Preclinical studies have also shown that H₃ antagonists activate intracellular signaling pathways that may improve cognitive efficacy as well as disease modifying effects in Alzheimer’s. Ongoing clinical studies will be able to determine the utility of H₃ antagonists for the treatment of cognitive disorders in humans.
Introduction

Histamine is an important biogenic amine that modulates many physiological responses in humans. Its biological actions are mediated via four histamine receptors named H₁, H₂, H₃ and H₄, a classification based on their sequence, their link to differential intracellular signaling mechanisms and their unique pharmacological profile (Haas and Panula, 2003; Leurs et al., 2005; Esbenshade et al., 2008). The H₁ and H₂ receptors are druggable targets as indicated by the efficacy of these antagonists in the treatment of allergy and ulcers, respectively; the role of the H₄ receptors is unclear at the present time although preclinical evidence suggests a potential role in inflammation and pain processes.

Extensive preclinical data with histamine H₃ receptor antagonists support their potential utility for the treatment of human cognitive disorders. The discovery of potent and selective H₃ antagonists have overcome many of the liabilities of earlier antagonists, have confirmed the preclinical data obtained with early agents, and have significantly expanded our knowledge in this area. In this article we reviewed the latest preclinical and clinical data on histamine H₃ antagonists as BF2.649, PF-03654746, GSK189254, GSK239512, MK-0249, MK-3134, JNJ-17216498 and ABT-288 have advanced to Phase 1 and Phase 2 stages in the clinical area for the potential treatment of sleep disorders, attention-deficit hyperactivity disorder (ADHD), the cognitive deficits in schizophrenia (CDS) and Alzheimer’s disease (AD).
Histamine H₃ Receptor Pharmacology

The histamine H₃ receptor was first described in 1983 as an autoreceptor that regulated histamine release and 16 years later the DNA sequence was elucidated, structurally confirming it as a member of the G-protein coupled receptor (GPCR) family (Arrang et al., 1983; Lovenberg et al., 1999). This receptor exhibits highest homology (~60% in the transmembrane domains) to the H₄ receptor but much lower homology (~20%) to the H₁ and H₂ receptors. In the time since its cloning, there has been considerable advancement in our knowledge about H₃ receptor molecular properties that have been described in detail in recent reviews (Esbenshade et al., 2008; Leurs et al., 2005).

The full length human and rat H₃ receptor is composed of 445 amino acids; however, at least 20 human and 9 rat H₃ receptor mRNA isoforms resulting from alternative splicing of the receptor gene have been identified. Truncations of the third intracellular loop, variations in the amino and carboxyl termini, and deletions of transmembrane domains account for the number and diversity of H₃ receptor isoforms. At least 8 human and 3 rat isoforms are functionally active, demonstrating binding and/or signaling activity when expressed in recombinant cell systems (Bongers et al., 2007; Esbenshade et al., 2008).

The distribution of H₃ receptors was examined in postmortem human brain tissue and autoradiographic studies indicate high levels in the globus pallidus, caudate, putamen, hippocampus, limbic and frontal cortical regions. Recent studies with a novel ¹¹C-PET ligand in humans confirmed a high expression of H₃ receptors in the caudate and putamen, intermediate expression in the cortex and low levels in the cerebellum.
(Ashworth et al., 2010). A comparable pattern of $H_3$ receptor expression is observed in rats, with high expression in the cortex, hippocampus, striatum and hypothalamus. Of particular interest is the differential expression of three rat isoforms in the hippocampus, locus coeruleus and raphe nucleus that could lead to a unique regulation of acetylcholine (ACh), noradrenaline (NE) and serotonin (5-HT) (Drutel et al., 2001). On the other hand, low expression of $H_3$ receptors has been detected in heart, placenta, lung, liver, as well as other peripheral tissues (Lovenberg et al., 1999). The high levels of expression in the brain in comparison to the periphery makes the $H_3$ receptor an attractive drug target as the possibility of mechanistic-based peripheral side effects is low.

The $H_3$ receptor plays a modulatory role as an autoreceptor in regulating the release of histamine and as an heteroreceptor regulating the release of ACh, NE and dopamine (DA). While the precise signaling events associated with this function at the synaptic level are not well understood, mechanistic studies on neurotransmitter release suggest a role for protein kinase A and voltage gated calcium channels. These signaling events are downstream from $H_3$ receptor activation of $G_{\alpha i/o}$-proteins that results in increased GTP$\gamma$S binding and inhibition of adenylate cyclase in brain tissues. A wide range of other $H_3$ receptor/$G_{\alpha i/o}$ mediated signal transduction pathways have also been identified in recombinant cell systems that include activation of MAPK, GSK-3$\beta$, Akt, and phospholipase A2, as well as inhibition of adenylate cyclase and the Na+/H+ exchanger (Bongers et al., 2007).
There are differences in the binding affinities of H₃ receptor antagonists across species that are attributable to differences in two amino acids in transmembrane 3. While early generation H₃ receptor antagonists including imidazole and non-imidazole-based structures were generally more potent at rodent than human receptors, more recent non-imidazole based H₃ receptor antagonists are up to 10-fold more potent at human than rat receptors. These differences are important to be able to identify compounds with both human and rat potency as well as from a clinical perspective in order to translate exposure from rodents to humans related to cognition or CNS signs of target engagement (wakefulness).

An interesting characteristic of the H₃ receptor is its ability to transduce signaling in the absence of agonist activation thus demonstrating inherent constitutive activity (Arrang et al., 2007). By definition, all H₃ antagonists block the activity of endogenous histamine. Additionally, the vast majority also acts as inverse agonists by reversing its constitutive activity. It is unclear at the present time the pharmacological relevance of inverse agonism versus antagonism in the in vivo situation, thus, for the purposes of this review these compounds will be referred to as H₃ receptor antagonists.

**H₃ Receptor Modulation of Neurotransmitter Release**

While histaminergic neuronal soma reside exclusively in the posterior hypothalamus, specifically the tuberomammillary nucleus (TMN), histaminergic fibers project throughout most regions of the brain including the cortex, striatum, thalamus, hippocampus, hypothalamus, locus coeruleus and spinal cord. Although originally
described as a presynaptic autoreceptor controlling histamine release (Arrang et al., 1983), the H₃ receptor also functions as a heteroreceptor regulating the release of other neurotransmitters. Similar to autoreceptor inhibition, the release and interaction of histamine with Gi-protein coupled H₃ heteroreceptors on axoaxonic postsynaptic terminals leads to inhibition of neurotransmitter release (ACh, etc). Histaminergic neurons were initially characterized as a homogenous cell population by anatomical studies, however, recent data have revealed that these neurons are organized into distinct circuits enabling H₃ receptors to selectively influence signaling in different brain regions.

The initial characterization of H₃ autoreceptors utilized histamine release from brain slices and the first report of H₃ antagonist-evoked histamine release in the whole animal was demonstrated in the hypothalamus of thioperamide-treated rats (Itoh et al., 1991; Mochizuki et al., 1991). H₃ receptor antagonism produced by systemic administration of GT-2016 increased histamine in the parietal cortex of awake, freely moving rats (Tedford et al., 1995). Additionally, several selective H₃ antagonist have been shown to increase extracellular histamine levels in the rat prefrontal cortex, in the TMN and the basolateral amygdala following systemic thioperamide administration (Cenni et al., 2004). Together, these studies demonstrate that systemic administration of an H₃ antagonist enhances histaminergic neurotransmission in the CNS.

A more recent awareness of the heterogeneity of H₃-autoreceptor regulation of histaminergic neurons has developed from studies utilizing local application of
compounds and dual-probe microdialysis (Giannoni et al., 2009; Giannoni et al., 2010). Local TMN application of the H₃ antagonists thioperamide and GSK189254 increased histamine release in the TMN, prefrontal cortex and nucleus basalis magnocellularis (NBM). Conversely, despite an increase in histamine in the TMN upon local administration, no change was observed in histamine levels in the striatum or nucleus accumbens (NAcc). Direct application of thioperamide into the prefrontal cortex and NBM increased the local concentration of histamine, however, direct application into the striatum and NAcc does not. The presence of histaminergic projections to these brain areas was confirmed as TMN-application of a GABA-A receptor antagonist or a CB1 receptor agonist increases histamine levels in the NAcc or striatum, respectively. These results demonstrate that histaminergic neurons differentially regulate neurotransmitter release in a region-specific manner in the brain.

Blandina and colleagues provided the first in vivo evidence for H₃ heteroreceptors regulating ACh release in rat cortex, which receives cholinergic input originating primarily from the nucleus basalis (Blandina et al., 1996). A series of microdialysis experiments demonstrated that histamine and the H₃ receptor agonists R-α-methyl histamine, imetit and immepip locally administered through the microdialysis probe inhibited potassium-evoked ACh release in the frontoparietal cortex. The inhibition was prevented by the H₃ antagonist clobenprobit, but not by the H₁ antagonist tripolidine or the H₂ antagonist cimetidine. After these studies were published there have been several reports of H₃ receptor antagonists increasing ACh release as demonstrated by in vivo microdialysis. The H₃ 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) similar to those producing efficacy in rat cognition models (Fox et al., 2005). Similarly, BF2.649 (Ligneau et al., 2007) and GSK189254 (Medhurst et al., 2007a) increased ACh release in the frontal cortex and/or dorsal hippocampus.

H3 heteroreceptor regulation of neurotransmission is not limited to ACh as microdialysis studies have demonstrated that H3 receptors can also regulate DA and NE release. Enhanced DA release in rat prefrontal cortex has been demonstrated with ABT-239, BF2.649 and GSK189254. The initial microdialysis studies examining NE release reported that both systemic and local administration of thioperamide did not stimulate basal NE release in the hippocampus. Despite this lack of effect when administered alone, thioperamide prevented the reduction of NE produced by R-α-methylhistamine (Di Carlo et al., 2000). More recently, oral administration the novel H3 receptor antagonist GSK189254 increased basal NE levels in the cingulate cortex of freely moving rats at doses improving cognitive performance (Medhurst et al., 2007a). The effect on NE release has also been associated to a potential analgesic effect in animals. However, the increase of DA and NE is modest in comparison to the magnitude of the effect of H3 antagonists on histamine or ACh release in the prefrontal cortex.

Similar to the heterogeneity demonstrated by H3 autoreceptors, inhibition of H3 heteroreceptors can also produce functionally distinct effects. Systemic administration of ABT-239 increases the release of ACh from the prefrontal cortex and hippocampus, as well as DA in the prefrontal cortex. However, ABT-239 does not increase DA levels
in the striatum (Fox et al., 2005). Similarly, the local application of GSK189254 in the TMN produces heterogenous modulation of neurotransmitter release (Giannoni et al., 2010). While GSK189254 increases histamine in the TMN, no concurrent increase in DA was observed in the NAcc. Conversely, GSK189254 induced increases in histamine release in the TMN and increased ACh release in the prefrontal cortex.

In summary, the neurochemical effects of H₃ antagonists have been confirmed with the use of novel and selective agents. The preclinical data demonstrate robust increases in histamine release from the TMN, cortex and hippocampus, brain regions associated with cognitive processing. H₃ antagonists also induce a robust release of ACh in the cortex and hippocampus. Results on NE release have been variable, perhaps due to the small magnitude of the effect. The magnitude of H₃-modulation of DA is also modest. Interestingly, differential effects on DA release have been observed with increases detected in the mesocortical but not the nigrostriatal or mesolimbic regions. Histamine and ACh are significant contributors to arousal, attention and memory, as well as other cognitive domains, and H₃ antagonist mediated increases in these neurotransmitters supports their utility in human cognitive disorders.

Histamine H₃ Receptors and Cognition

Cognitive processes in humans include several domains including attention, short-term memory, working memory, and long-term memory. In view of the neurochemical effects of H₃ antagonists enhancing the release of brain histamine, it is important to review the
role of histamine on attention and wake. Wakefulness during the day is maintained by the actions of several neurotransmitters systems including histamine, NE, glutamate, ACh, orexin, GABA, playing a unique role in the initiation and maintenance of wake. Wakefulness is characterized by cortical activation and behavioral arousal that can be detected by EEG and EMG techniques (Jones, 2005; Schwartz and Roth, 2008).

Histamine plays a physiological role in the light-dark cycle as histamine release increases during the light phase while it decreases to baseline levels during the dark phase. The widespread projections from the TMN histaminergic neurons to different brain areas is one of the outputs of the circadian rhythm set by the hypothalamus leading to activation of brain arousal mechanisms. These neurons induce activation of the cortex as they are active during the wake period while they eventually cease firing during the REM sleep phase. Agents that increase histaminergic activity (histamine, H₁ agonists or inhibitors of histamine metabolism) increase wake in animals; in contrast, agents that decrease histaminergic activity (inhibitors of histidine decarboxylase like α-FMH, or the H₁ antagonists mepyramine and diphenhydramine) promote sedation and sleep (Monti et al., 1986; Barbier and Bradbury, 2007). Genetic manipulation of the histamine pathway also supports its key role in vigilance. Histidine decarboxylase-KO mice show permanent changes in the sleep-wake cycle with increased somnolence and these KO mice are unable to be awake when vigilance is required; similarly, H₁-KO mice exhibit disruption of the circadian rhythm with decreased activity during the active phase (Inoue et al., 1996; Barbier and Bradbury, 2007).
H₃ antagonists increase histamine release in rats, and thioperamide, ciproxifan and BF2.649 increase wake in cats; similarly, JNJ-5207852, JNJ-10181457, GSK189254 and ABT-239 increase wake in rodents. As ciproxifan does not promote wakefulness in H₁-KO mice, these data indicate that the wake-promoting effect of H₃ antagonists is mediated by increased histamine release stimulating post-synaptic H₁ receptors (Lin et al., 1990; Barbier et al., 2004; Bonaventure et al., 2007).

Preclinical studies have shown that CNS-penetrant H₁ antagonists like diphenhydramine (DPH) increase total sleep, an effect that is not observed with the second generation H₁ antagonists that do not readily cross the blood-brain barrier. The effects of DPH on sleep in healthy volunteers are not robust, but in humans suffering insomnia DPH provides a significant decrease in the severity of insomnia. These data indicate that DPH is more effective in states of higher arousal in comparison to normal individuals. DPH increases subjective sleepiness in humans and impairs performance in attentional/working memory tests (Gevins et al., 2002). H₁ antagonists also exhibit affinity for muscarinic receptors and although the contribution of the muscarinic effect is difficult to resolve, considering the totality of the data, the effects of DPH and H₁ blockers on sleep are consistent with the histaminergic hypothesis and most likely H₁-related in human.

The importance of the histaminergic system in vigilance is also demonstrated by clinical studies in which H₃ antagonists increase wake in humans. During Phase 1 studies with PF-03654746, the clinical team observed sleep disturbances after multiple dosing
(Soares et al., 2009) and estimated that it occurred at high receptor occupancy levels in human (>70%). Furthermore, the relationship between H₃ receptor occupancy and sleep in humans was determined with MK-0249 by the Merck team; MK-0249 (2.5 – 50 mg) reached high occupancy in the striatum (93% at 50 mg), with patients reporting difficulty getting to sleep starting at the 5 mg dose that reached 72% occupancy (Iannone et al., 2009). These observations are consistent with the preclinical evidence demonstrating wake-promoting effects of H₃ antagonists in animals.

H₃ antagonists as a class exhibit alerting effects due to brain H₃ receptor occupancy, and if occupancy is high at night a common side-effect is the difficulty getting to sleep. If the desired occupancy is achieved during the day, H₃ antagonists may thus be useful for the treatment of excessive sleepiness and narcolepsy. H₃ antagonists are effective in two animal models of narcolepsy; GSK189254 is a potent H₃ antagonist that exhibits pro-cognitive effects in several animal models and it also increases wake in wild-type mice and orexin-KO mice. Similarly, JNJ-5207852 and JNJ-10181457 are potent H₃ antagonists with pro-cognitive and wake-promoting effects in mice and rats. JNJ-10181457 also reduced the number of cataplectic attacks in narcoleptic dogs (Barbier et al., 2004; Bonaventure et al., 2007).

A study in narcoleptic patients demonstrated that the H₃ antagonist BF2.649 (Pitolisant, 40 mg qd during 7 days) produced a significant reduction in the number of diurnal sleep episodes, with efficacy equal in magnitude to the approved agent modafinil (Lin et al., 2008). BF2.649 also reduced the duration of the sleep episodes in narcoleptic patients.
After five days of treatment BF2.649 was effective in both measures at 100 ng/ml plasma levels. BF2.649 also decreased excessive sleepiness in patients with Parkinson’s disease and Phase 3 trials are ongoing (5-40 mg doses).

These clinical data indicate that H₃ antagonists can have therapeutic properties in patients suffering excessive sleepiness and that the mechanism is likely due to the increased release of brain histamine. Taken together, a large body of research demonstrates that the histaminergic system plays a key role in waking and attention. Several H₃ antagonists promote wake in preclinical models of narcolepsy as we previously discussed, and BF2.649 is effective in humans suffering narcolepsy.

Preclinical and clinical data indicate that H₃ antagonists increase vigilance and wake which impact other cognitive domains. The exogenous administration of histamine facilitated long-term retention in the inhibitory avoidance test in mice after icv administration (Almeida and Izquierdo, 1986) and improved short-term memory in the social recognition test. A similar effect was induced by histidine administration, while inhibition of histamine synthesis by α-FMH impaired short-term memory (Prast et al., 1996). Intrahippocampal injections of histamine also ameliorate spatial memory deficits induced by MK-801 (Xu et al., 2005). These studies indicate that brain histamine plays a role in short-term memory, long-term memory as well as spatial memory.

The icv administration of the H₃ antagonist thioperamide improved short-term memory in the social recognition test (Prast et al., 1996), and when injected systemically it
improved performance in an attention model in SHR pups as well as other cognitive domains as shown in Table 1 (see also Hancock and Fox, 2004). In contrast, systemic injections of the H₃ agonists imetit and RAMH impaired performance in the object recognition test that measures working memory and in the 24 h inhibitory avoidance test measuring long term memory (Blandina et al., 1996).

ACh plays an important role in attention and memory processes, supported by the clinical efficacy of donepezil in AD. The ability of H₃ antagonists to increase ACh release in the brain makes this an attractive target for the treatment of cognitive disorders. Interestingly, the effect of H₃ agonists impairing memory have been correlated with the inhibitory effect on ACh release indicating the H₃ antagonists may regulate memory via the central cholinergic system (Blandina et al., 1996).

With regards to the effects of novel H₃ antagonists, ABT-239 binds with 2 nM potency to rat H₃ receptors and increases the release of histamine, ACh and DA in the rat prefrontal cortex (Esbenshade et al., 2005). ABT-239 improved performance in the 5-trial inhibitory avoidance test in SHR pups, in the social recognition test, in the 24 h inhibitory avoidance test, and in spatial memory tasks (Fox et al., 2005). GSK189254 is a potent H₃ antagonist (human Ki= 0.2 nM; rat Ki=1 nM) that increases the release of ACh, NE and DA in the rat cingulate cortex. At the behavioral level, GSK189254 improved performance in the rat object recognition task after 24-48 h delays, improved attentional set shifting and spatial learning in aged rats (Medhurst et al., 2007a).
BF2.649 binds with potent affinity to human (Ki = 2.7 nM) and rat (Ki = 17 nM) H₃ receptors, and it increases the release of ACh and DA from rat cortex. In behavioral studies BF2.649 improved retention of memory in the object recognition test that measures working memory (Ligneau et al., 2007). Similar data have been reported for JNJ-10181457; this compound has been described as a potent H₃ antagonist (Ki = ~1 nM) that increases the in vivo release of ACh and NE without effects on DA release in the rat prefrontal cortex. JNJ-10181457 facilitated acquisition of the inhibitory avoidance response in SHR pups and improved performance in the delayed non-matching to position (DNMTP) task in scopolamine-treated rats (Leurs et al., 2005; Bonaventure et al., 2007; Galici et al., 2007).

In addition to the facilitatory effects on different cognitive domains in rodents (Table 1), H₃ antagonists including ABT-239, GSK189254, GSK207040, GSK334429, JNJ-10181457 and BF2.649 attenuated scopolamine-induced deficits in cognitive tests in rodents (Fox et al., 2005; Ligneau et al., 2007; Medhurst et al., 2007a; Medhurst et al., 2007b). Thus, H₃ antagonists may be beneficial in CNS disorders like Alzheimer’s that exhibit cholinergic deficits related to the cognitive symptoms in these patients.

**Potential for Disease-progression in Alzheimer’s Disease**

Cholinergic transmission is well recognized as a major modulator of cognitive processing (Bartus, 2000). Acetylcholinesterase (AChE) inhibitors that increase synaptic ACh by inhibiting the enzymatic degradation of ACh provide a modest symptomatic
relief that declines with later stage AD progression. The progressive cholinergic cell loss associated with AD likely limits the therapeutic effectiveness of these agents dependent on endogenous ACh synthesis. Nonetheless, AChE inhibitors like donepezil currently represent the primary therapeutic approach for AD. Thus, there exists a significant unmet need for the development of superior drugs that in addition to symptomatic alleviation may slow pathological progression, i.e. disease modifying efficacy.

H₃ antagonists elevate ACh levels in cortex and hippocampus as well as enhance memory in preclinical models. Functioning as indirect agonists at a variety of postsynaptic receptors through evoked release of different neurotransmitters, the mechanism and efficacy of H₃ antagonists as AD therapeutics may involve activation of multiple biochemical pathways. Moreover, the multi-signaling potential of H₃ antagonists may afford therapeutic benefits beyond symptomatic alleviation. Drug discovery efforts towards developing therapeutics that have disease modifying effects have focused on the two proteins involved in AD pathology, viz., β-amyloid (Aβ), a product of aberrant amyloid precursor protein (APP) leading to production of extracellular Aβ plaques, and tau, a microtubule-associated protein that when hyperphosphorylated results in the formation of intracellular neurofibrillary tangles (NFTs) (Giacobini and Becker, 2007). In the latter case, pharmacological activation of cellular pathways that inhibit kinase signaling and subsequent tau hyperphosphorylation may represent a viable approach for targeting AD pathology.
It may be hypothesized that H₃ antagonist-evoked neurotransmitter release leads to activation of postsynaptic-receptor pathways such as phosphorylation of CREB (cyclic AMP response element binding protein), a transcription factor germane to cognitive function; and the inhibitory residue Ser-9 of glycogen synthase kinase3β (GSK3β), a primary tau kinase in AD responsible for tau hyperphosphorylation (Grimes and Jope, 2001; Hooper et al., 2008). With respect to GSK3β, it is constitutively active and a substrate to other kinases capable of phospho-regulating its activity through both inhibition and activation (Grimes and Jope, 2001). In the case of deactivation, signaling through phosphoinositide 3-kinase (PI3k) and subsequent activation of the serine-threonine kinase Akt inhibits GSK3β activity via Ser-9 phosphorylation, a cellular cascade known to be associated with neuroprotection. Administration of the H₃ antagonist ABT-239 in normal mice increase cortical CREB and S⁹-GSK3β phosphorylation at doses producing cognitive efficacy (Markosyan et al., 2009). It was also demonstrated that donepezil at doses associated with clinical exposures induced CREB phosphorylation consistent with a procognitive action, but in contrast to ABT-239, did not have effects on S⁹-GSK3β phosphorylation. Together, these findings suggest that increased S⁹-GSK3β phosphorylation induced by ABT-239 is not dependent on increased ACh release.

Both CREB and S⁹-GSK3β phosphorylation have been shown to be down-regulated in the Tg2576 (APP/Aβ) transgenic mouse model of AD (Bitner et al., 2009). However, a 2-week infusion of ABT-239 in Tg2576 mice normalized cortical CREB and hippocampal pS⁹-GSK3β phosphorylation. In similar studies conducted in TAPP mice, an AD
transgenic line containing both APP and tau transgenes, ABT-239 infusion reversed tau hyperphosphorylation in the spinal cord and hippocampus. Mechanistically, ABT-239 produced signaling changes (pS9-GSK3β) in α7 nicotinic ACh receptor (nAChR) knockout mice that do not exhibit α7 nAChR agonist-induced phosphorylation, suggesting that H₃ antagonist-mediated signaling is not dependent on ACh-stimulated α7 nAChR activation.

In contrast to the in vivo findings, studies conducted in cortical cell cultures have demonstrated that H₃ receptor agonism induces phosphorylation of the Akt/GSK3β pathway and protects against neurotoxic insults, which are blocked by the H₃ antagonist thioperamide (Mariottini et al., 2009). These results indicate that H₃ receptor agonist activation in vitro leads to signaling changes similar to those observed with H₃ antagonism in the whole animal. In this regard, H₃ antagonist-evoked neurotransmitter release and subsequent postsynaptic receptor stimulation, not present in an in vitro system, may indeed produce a signaling phenotype distinct from H₃ receptor-mediated biochemical signaling when examined in vitro. In summary, these in vivo signaling studies raise the intriguing possibility that H₃ antagonists activate signaling pathways that may translate into improved efficacy in AD patients, with symptomatic alleviation and disease modifying effects.

**Development Status of H₃ Receptor Antagonists**

Several H₃ antagonists have advanced to the clinical stage including BF2.649, PF-03654746, GSK189254, GSK239512, MK-0249, MK-3134, JNJ-17216498 and ABT-
288. These compounds have completed Phase 1 studies in human volunteers to
determine the pharmacokinetics and tolerability after single-dose and multiple-dose
administration; some of these drugs have also advanced to the Phase 2 stage. Although
limited clinical data have been released at this time, analysis of the available data can
enable researchers to determine which are the effects common to all agents versus
those effects that are unique to each pharmacophore.

More than 20 industrial and academic groups have worked on the development of H₃
antagonists. The earliest compounds have significant shortcomings as clinical drugs
(CYP inhibition, low brain penetration) but have become pharmacological tools,
particularly ciprofanz, thioperamide and GT-2331. The first cited agent into clinical trials
was GT-2331 but clinical development did not advance perhaps due to its imidazole-
associated liabilities. The problems of these early structures drove efforts toward non-
imidazoles, wherein the imidazole moiety is replaced by a tertiary basic amine,
addressing CYP inhibition, and improving CNS penetration and H₃ selectivity. Most
modern H₃ antagonists share these improved drug-like properties.

An early compound into the clinic was BF2.649 (Ligneau et al., 2007) and it has been
reported in several Phase 2 clinical trials on daytime sleepiness in Parkinson’s disease,
sleep apnea syndrome, and cognition enhancement in schizophrenic patients
(clinicaltrials.gov). BF2.649 showed efficacy in a 22 patient narcolepsy trial of single-
blind design: a 40 mg daily dose reduced sleepiness versus placebo, with efficacy in the
Epworth Sleepiness Scale (ESS) equivalent to modafinil (Lin et al., 2008). Most patients
with Parkinson’s disease have nighttime insomnia and daytime sleepiness, and BF2.649 was reported as effective in patients given 5-40 mg QD, as assessed in the ESS scale. The optimal dose was 20 mg, which is being targeted in a Phase 3 trial. No changes in nighttime sleep or Parkinson’s symptoms were noted.

Pfizer has reported preclinical as well as Phase 1 studies on PF-03654746. It is a potent H₃ antagonist (human H₃ Ki=3.2 nM, rat Ki=37 nM) and active in the object recognition model. It exhibits a long human t½ (9-18 hs), with insomnia noted as the main adverse event at the 3 mg dose that reached 15 ng/ml (Soares et al., 2009). PF-03654746 recently completed a Phase 2 trial in adult ADHD patients and no efficacy was observed in 2 drug groups versus placebo; however, a Phase 2 trial for daytime sleepiness in narcolepsy, a receptor occupancy PET study at 0.5-4 mg doses, and clinical trials in AD and narcolepsy are ongoing.

GSK189254 is a potent H₃ antagonist (human H₃ Ki=0.2 nM) with broad spectrum efficacy in a number of rodent models of cognition and narcolepsy (Medhurst et al., 2007a). GSK189254 increased ACh, NE, and DA as measured by microdialysis. This compound has been listed as in early trials targeting Alzheimer’s, pain and narcolepsy, but is no longer under clinical development. A ¹¹C-labeled analog has been reported as a radiotracer tool to probe the receptor occupancy of GSK239512 (clinicaltrials.gov). The structure and properties of GSK239512 have not been described, but GSK is recruiting patients for Phase 2 trials in schizophrenia and AD.
Merck has published the SAR of several chemical series, with one especially potent compound (human H₃ Ki=1.7 nM) named as selected for clinical development for various CNS dysfunctions. The structure and properties of MK-0249 have not been specifically disclosed, but it has completed three Phase 2 trials: in adult ADHD patients, AD and schizophrenia. A Phase 2 trial for the treatment of daytime sleepiness in patients with sleep apnea was terminated. Both MK-0249 and MK-3134, were evaluated in a clinical PET study to determine dose versus receptor occupancy. MK-0249 dosed at 2.5-50 mg and MK-3134 at 0.5-25 mg achieved up to 93% and 96% H₃ receptor occupancy, respectively. Data suggest that H₃ antagonist-induced alerting effects were observed at 67% occupancy, increasing with higher levels of H₃ occupancy (Iannone et al., 2009). However, MK-0249 failed to improve cognition in schizophrenic patients dosed for 4 weeks at 10 mg (Egan et al., 2009), a dose that achieved >85% H₃ receptor occupancy. MK-3134 has been described as completing Phase 1 testing in a biomarker study using BOLD fMRI at 1, 5 and 25 mg doses.

Johnson and Johnson was an early leader in industrial H₃ research with JNJ-5207852 and other discontinued agents. JNJ-17216498 was the first candidate named as having completed human testing, with a Phase 2 trial in 2007 in a small (65 patients) group of narcoleptic subjects. It was dosed at 10 and 50 mg with modafinil as comparator but results have not been disclosed. A later compound, JNJ-31001074 is likely to be an H₃ antagonist. It has been probed extensively, completing PK and a ketoconazole interaction studies, and a Phase 2 ADHD trial in adults dosed at 10 and 30 mg. Several
additional trials are recruiting patients, including a Phase 1 in pediatric and teen ADHD groups, and a Phase 2 multidose adult ADHD trial with atomoxetine and methylphenidate as active comparator. The structures of the most advanced candidates are undisclosed.

The Abbott group has developed a number of chemical series and an early compound was ABT-239 with Ki=0.45 nM affinity to human H₃ (Esbenshade et al., 2005). ABT-239 had robust activity in rodent models of cognition and has been used as a reference standard to probe H₃ effects in vivo (Fox et al., 2005). A recent study demonstrated that ABT-239 ameliorated ethanol-induced deficits on hippocampal LTP indicating that H₃ antagonists can affect changes in synaptic plasticity related to cognitive processes (Varaschin et al., 2010). However, it also potently binds to the cardiac hERG channel in vitro. This problem has been solved in later series by systematic changes of structure. Abbott recently disclosed preclinical data on ABT-288, a potent and selective H₃ antagonist that binds to rat and human H₃ receptors with Ki= 8.1 nM and 1.9 nM, respectively (Esbenshade et al., 2009). ABT-288 increased the release of histamine and ACh from the rat cortex and facilitates performance in attention, short-term memory and long-term memory tests. The compound penetrated the CNS efficiently, and effectively occupied rat H₃ receptors with ED₅₀= 3.2 ng/ml. Studies are presently ongoing to determine its efficacy in patients suffering AD and schizophrenia.

In addition to the companies mentioned above, other companies are active in the field including Schering (SCH-497079), Servier (S-41150), Arena (APD916), Wyeth/Pfizer
(WAY-361046), Cephalon (CEP-26401), Sanofi-Aventis (SAR-110894), Roche, UCB, Lilly and Ligand. Table 2 shows clinical data on the most advanced antagonists in the clinic today. It is important to note that all the H₃ antagonists on Table 2 are more potent to bind the human versus the rat H₃ receptor and the proper adjustment should be made to extrapolate the efficacious plasma levels.

Conclusions

There are significant needs for an effective treatment of the cognitive disorders in patients suffering ADHD, schizophrenia and AD, and although a common feature in these patients is that they suffer cognitive deficits, the particular domains that are affected in each disease must be individually considered.

Inattention, impulsivity and hyperactivity are the three clinical symptoms domains most affected in ADHD, deficits that are hypothesized to be caused by a combination of abnormalities in alertness and executive functions. This disorder affects 3-9% of school-age children but it is now recognized that it persists in adults as indicated by the decreased level of education, limited employment success and increased incidence of drug addiction in adults previously diagnosed with ADHD (Wilens et al., 2004). However, clinical data with H₃ antagonists provided mixed results as PF-03654746 was ineffective in adult ADHD patients, while a positive effect has been reported with the JNJ compounds in these patients. It has also been reported that H₃ antagonists increase attention in humans as BF2.649 improved performance in the Flicker-Fusion test in normal volunteers, but it is clear that a positive effect on attentional tasks may not
be sufficient to improve a pathological condition like ADHD. Studies that were previously conducted (data not disclosed yet) and those presently ongoing with BF2.649 and MK-0249 will provide useful information in the near future with regards to efficacy in this population of patients.

Schizophrenia is a chronic disorder that is no longer considered as a unitary disease. In schizophrenia there are three major clusters of symptoms: positive symptoms like delusions, hallucinations; negative symptoms like anhedonia and social withdrawal; and cognitive deficits in attention, memory, speed of processing and executive functions. Present pharmacological treatments are effective against the positive symptoms but they show limited efficacy against negative symptoms and do not improve the cognitive deficits in schizophrenic patients (Lublin et al., 2005). Most important, indices of cognitive function are better predictor of functional improvement than indices of the other domains and there is consensus in the scientific community that the cognitive domains of relevance are attention, vigilance, speed of processing, working memory and social cognition. Agents that improve cognitive deficits may represent a major breakthrough in the treatment of schizophrenia.

The efficacy of MK-0249 was evaluated in schizophrenia and it was not effective in patients receiving standard anti-psychotic medication. Patients received 4 weeks of treatment with a 10 mg dose of MK-0249, a dose that had previously shown high brain H₃ receptor occupancy in normal volunteers. These negative findings may indicate that the increased brain histamine was not sufficient to exhibit a therapeutic effect on
cognitive domains of importance in schizophrenic patients. On the other hand, the $H_1$ antagonist properties of the antipsychotic drugs could have interfered in this trial (risperidone $H_1$ Ki=15 nM; olanzapine $H_1$ Ki=2 nM) and further studies may be needed to demonstrate occupancy in the presence of antipsychotic medication. Studies with BF2.649 and ABT-288 are ongoing to treat CDS in this patient population.

AD is a progressive neurodegenerative disease and patients exhibit impairments in cognition and activities of daily living. Our knowledge of the pathological process in AD have significantly increased and evolved since the first description of AD in 1910, as patients in the advanced stage (severe) suffer a global cognitive decline, while selective deficits are observed in the early (mild-to-moderate) stages of AD (Hodges, 2006). Other neuropsychiatric deficiencies are also observed including lack of motivation, depression, delusions, agitation and aggression. Major impairments in the domain of anterograde episodic memory are observed in AD, as indicated by the inability to learn/retain new information, and impairments of semantic memory or inability to store new facts. Attentional and executive deficits are present in AD as these patients perform poorly in tests of selective, sustained and divided attention.

Acetylcholine inhibitors (donepezil, rivastigmine, galantamine) and NMDA antagonists (memantine) are the approved therapies for AD. These agents have modest efficacy and their symptomatic benefits are short-lived. Furthermore, the GI-related side effects of the AChE inhibitors (nausea, vomiting) lead to non-compliance issues in the AD population. Thus, in view of the limitations of the available therapies there is a need for
drugs with increased cognitive efficacy and, potentially, therapies that can slow AD progression. The ability of H₃ antagonists to improve attention, vigilance, and wake (as previously discussed) may differentiate these agents from other approaches presently under evaluation in AD patients, although it will be important to avoid high plasma levels late in the day to avoid inducing sleep disturbances in these patients. Clinical data on the effect of H₃ antagonists in AD have not been disclosed despite that several studies were initiated in this area. Compounds from GSK, Pfizer, Merck and Abbott are under evaluation in this patient population.

The ability of H₃ antagonists to improve attention and wake is unique to these agents in view of their ability to increase extracellular levels of histamine. The sleep disturbances noted in the Phase 1 studies suggest that H₃ antagonists are able to increase histamine in humans, consistent with the preclinical data. H₃ antagonists can also increase extracellular levels of ACh, a neurotransmitter that has been effective to improve cognition in the early phases of AD. H₃ antagonists may also bring another exciting biochemical effect by increasing the phosphorylation of key intracellular proteins that play a role in the neurodegenerative process. The different clinical studies presently ongoing to test the efficacy of H₃ antagonists in these human conditions may be able to provide an answer to these hypotheses and determine the place for H₃ antagonists in therapeutics.
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Statement of Conflict of Interest: The authors are all employees of Abbott Laboratories.
References


Table 1: Cognitive domains modulated by histamine and H3 antagonists in the different preclinical models that have been extensively used to detect the pro-cognitive effect of novel agents. (+) Indicates that efficacy in this model was demonstrated. (a) Evaluated in the 5-trial inhibitory avoidance test in SHR pups or the 5-choice serial reaction time test; (b) Short-term memory evaluated in the social recognition rat test; (c) Working memory in the radial maze, Y-maze, object recognition or DNMTP test; (d) Long-term memory or consolidation in the inhibitory avoidance test with retention measured 24 hs after 1-trial learning; (e) Spatial memory evaluated in the water maze or Barnes maze.

<table>
<thead>
<tr>
<th>Cognitive Domains</th>
<th>Attention - Impulsivity (a)</th>
<th>Short-term memory (b)</th>
<th>Working memory (c)</th>
<th>Long-term memory (d)</th>
<th>Spatial memory (e)</th>
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<td>Histamine</td>
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<td>Thioperamide</td>
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<tr>
<td>BF2.649 (pitolisant)</td>
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<td>ABT-239</td>
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Table 2: Summary of key preclinical and clinical data on histamine H3 antagonists that have been reported to advance to the clinical area. NA: data are not available.

<table>
<thead>
<tr>
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<th>Rat</th>
<th>Human</th>
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<tbody>
<tr>
<td></td>
<td>$H_3$ Ki nM</td>
<td>$H_3$ Ki nM</td>
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</tbody>
</table>
| BF2.649 (pitolisant) | 17 | 2.7 | 10 | - Efficacy reported in narcoleptic patients and Parkinson's disease.  
- Sleep disturbances, insomnia reported in humans  
- Phase 2 trial in schizophrenia ongoing, and Phase 3 trial in Parkinson's planned. |
| PF-03654746    | 37 | 3.2 | 9-18 | - Sleep disturbances, insomnia in Phase 1.  
- No efficacy in adult ADHD patients.  
- Phase 2 trials in AD and narcolepsy ongoing. |
| GSK189254      | 1  | 0.2 | >24  | - Sleep disturbances, insomnia in humans.  
- Discontinued from development in AD. |
| GSK239512      | NA | NA | NA   | - Advanced to Phase 1. Phase 2 trials in AD and schizophrenia ongoing |
| MK-0249        | NA | NA | NA   | - Sleep disturbances, insomnia in Phase 1.  
- Alerting effects noted at 67% receptor occupancy.  
- No efficacy in schizophrenic patients. |
| MK-3134        | NA | NA | NA   | - Advanced to Phase 1. |
| ABT-288        | 8  | 1.9 | NA   | - Phase 2 trials in AD and schizophrenia ongoing. |
| JNJ-17216498   | NA | NA | NA   | - Advanced to Phase 1. Evaluated in narcolepsy and ADHD patients. |