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JPET Miniseries: H_3 Receptors

Discovery of Histamine H_3 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

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, Illinois

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ABSTRACT

H_3 antagonists increase the release of brain histamine, acetylcholine, noradrenaline, and dopamine, neurotransmitters that are known to modulate cognitive processes. The ability to release brain histamine supports the effect on attention and vigilance, but histamine also modulates other cognitive domains such as short-term and long-term memory. A number of H_3 antagonists, including 1-{3-[3-(4-chlorophenyl)propoxy]propyl}piperidine hydrochloride (BF2.649), (1*R*,3*R*)-*N*-ethyl-3-fluoro-3-[3-fluoro-4-(pyrrolidin-1-ylmethyl)phenyl]cyclobutane-1-carboxamide (PF-03654746), 6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-3-pyridinecarboxamide hydrochloride (GSK189254), MK-0249 (structure not yet disclosed), JNJ-17216498 (structure not yet disclosed), and ABT-288 (structure not yet disclosed), have advanced to the clinical area for the potential treatment of human cognitive disorders. H_3 antagonists exhibited wake-promoting effects in humans and efficacy in narcoleptic patients, indicating target engagement, but some of them were not efficacious in patients suffering from attention-deficit hyperactivity disorder and schizophrenic patients. Preclinical studies have also shown that H_3 antagonists activate intracellular signaling pathways that may improve cognitive efficacy and disease-modifying effects in Alzheimer's disease. Ongoing clinical studies will be able to determine the utility of H_3 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 H_1, H_2, H_3, and H_4, 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 H_1 and H_2 receptors are druggable targets as indicated by the efficacy of these antagonists in the treatment of allergy and ulcers, respectively; the role of the H_4 receptors is unclear at the present time, although preclinical evidence suggests a potential role in inflammation and pain processes.

Extensive preclinical data with histamine H_3 receptor antagonists support their potential utility for the treatment of human cognitive disorders. The discovery of potent and selective H_3 antagonists have overcome many of the liabilities of earlier antagonists, confirmed the preclinical data obtained with early agents, and significantly expanded our knowledge in this area. In this article, we review the latest preclinical and clinical

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ABBREVIATIONS: ADHD, attention-deficit hyperactivity disorder; AD, Alzheimer's disease; SHR, spontaneously hypertensive rat; ACh, acetylcholine; NE, noradrenaline; DA, dopamine; CNS, central nervous system; TMN, tuberomammillary nucleus; NAcc, nucleus accumbens; GSK3β, glycogen synthase kinase 3β; KO, knockout; DPH, diphenhydramine; AChE, acetylcholinesterase; nAChR, nicotinic ACh receptor; PET, positron emission tomography; Aβ, β-amyloid; APP, amyloid precursor protein; CREB, cAMP response element binding protein; BF2.649, 1-{3-[3-(4-chlorophenyl)propoxy]propyl}piperidine hydrochloride; PF-03654746, (1*R*,3*R*)-*N*-ethyl-3-fluoro-3-[3-fluoro-4-(pyrrolidin-1-ylmethyl)phenyl]cyclobutane-1-carboxamide; GSK189254, 6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-3-pyridinecarboxamide hydrochloride; ABT-239, 4-(2-{2-[(2*R*)-2-methylpyrrolidin-1-yl]ethyl}-benzofuran-5-yl)benzonitrile; GT-2016, 5-cyclohexyl-1-(4-imidazol-4-ylpiperidyl)pentan-1-one; JNJ-5207852, 1-{3-[4-(piperidin-1-ylmethyl)phenoxy]propyl}piperidine; JNJ-10181457, 4-(3-(4-piperidin-1-ylbut-1-ynyl)benzyl)morpholine; MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate; GT-2331, (1*S*,2*S*)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl)imidazole.

data on histamine H_3 antagonists, because 1-{3-[3-(4-chlorophenyl)propoxy]propyl}piperidine hydrochloride (BF2.649), (1*R*,3*R*)-*N*-ethyl-3-fluoro-3-[3-fluoro-4-(pyrrolidin-1-ylmethyl)phenyl]cyclobutane-1-carboxamide (PF-03654746), 6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-3-pyridinecarboxamide hydrochloride (GSK189254), GSK239512, MK-0249 (structure not yet disclosed), MK-3134 (structure not yet disclosed), 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), cognitive deficits in schizophrenia, and Alzheimer's disease (AD).

Histamine H_3 Receptor Pharmacology

The histamine H_3 receptor was first described in 1983 as an autoreceptor that regulated histamine release (Arrang et al., 1983), and 16 years later the DNA sequence was elucidated, structurally confirming it as a member of the G protein-coupled receptor family (Lovenberg et al., 1999). This receptor exhibits highest homology (~60% in the transmembrane domains) to the H_4 receptor but much lower homology (~20%) to the H_1 and H_2 receptors. In the time since its cloning there has been considerable advancement in our knowledge about H_3 receptor molecular properties that have been described in detail previously (Leurs et al., 2005; Esbenshade et al., 2008).

The full-length human and rat H_3 receptor is composed of 445 amino acids; however, at least 20 human and nine rat H_3 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 H_3 receptor isoforms. At least eight human and three 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 H_3 receptors was examined in postmortem human brain tissue, and autoradiographic studies indicate high levels in the globus pallidus, caudate, putamen, hippocampus, and limbic and frontal cortical regions. Recent studies with a novel ^{11}C-PET ligand in humans confirmed a high expression of H_3 receptors in the caudate and putamen and 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 (Drutel et al., 2001). On the other hand, low expression of H_3 receptors has been detected in heart, placenta, lung, liver, and other peripheral tissues (Lovenberg et al., 1999). The high levels of expression in the brain in comparison with the periphery makes the H_3 receptor an attractive drug target, because 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). Although 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 Gαi/o proteins that results in increased guanosine 5′-3-*O*-(thio)triphosphate binding and inhibition of adenylate cyclase in brain tissues. A wide range of other H_3 receptor/Gαi/o-mediated signal transduction pathways have also been identified in recombinant cell systems that include activation of mitogen-activated protein kinase, glycogen synthase kinase 3β (GSK3β), 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 H_3 receptor antagonists across species that are attributable to differences in two amino acids in transmembrane 3. Whereas early-generation H_3 receptor antagonists, including imidazole- and nonimidazole-based structures, were generally more potent at rodent than human receptors, more recent nonimidazole-based H_3 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 to translate exposure from rodents to humans related to cognition or CNS signs of target engagement (wakefulness).

An interesting characteristic of the H_3 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 H_3 antagonists block the activity of endogenous histamine. In addition, the vast majority act as inverse agonists by reversing its constitutive activity. It is unclear at the present time what is 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 H_3 receptor antagonists.

H_3 Receptor Modulation of Neurotransmitter Release

Whereas 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 H_3 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 H_3 heteroreceptors on axoaxonic postsynaptic terminals leads to the 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 H_3 receptors to selectively influence signaling in different brain regions.

The initial characterization of H_3 autoreceptors used histamine release from brain slices, and the first report of H_3 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). H_3

receptor antagonism produced by systemic administration of 5-cyclohexyl-1-(4-imidazol-4-ylpiperidyl)pentan-1-one (GT-2016) increased histamine in the parietal cortex of awake, freely moving rats (Tedford et al., 1995). In addition, several selective H_3 antagonist have been shown to increase extracellular histamine levels in the rat prefrontal cortex, the TMN, and the basolateral amygdala after systemic thioperamide administration (Cenni et al., 2004). Together, these studies demonstrate that systemic administration of an H_3 antagonist enhances histaminergic neurotransmission in the CNS.

A more recent awareness of the heterogeneity of H_3 autoreceptor regulation of histaminergic neurons has developed from studies using local application of compounds and dual-probe microdialysis (Giannoni et al., 2009, 2010). Local TMN application of the H_3 antagonists thioperamide and GSK189254 increased histamine release in the TMN, prefrontal cortex, and nucleus basalis magnocellularis. 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 nucleus basalis magnocellularis 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, because 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 et al. (1996) provided the first in vivo evidence for H_3 heteroreceptors regulating ACh release in rat cortex, which receives cholinergic input originating primarily from the nucleus basalis. A series of microdialysis experiments demonstrated that histamine and the H_3 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 H_3 antagonist clobenprobit, but not by the H_1 antagonist tripolidine or the H_2 antagonist cimetidine. Since those studies were published there have been several reports of H_3 receptor antagonists increasing ACh release as demonstrated by in vivo microdialysis. The H_3 antagonist 4-(2-{2-[(2*R*)-2-methylpyrrolidin-1-yl]ethyl}-benzofuran-5-yl)benzonitrile (ABT-239) increased ACh release in the frontal cortex and to a lesser extent in the hippocampus at doses (0.1 to 3 mg/kg) similar to those producing efficacy in rat cognition models (Fox et al., 2005). Likewise, BF2.649 (Ligneau et al., 2007) and GSK189254 (Medhurst et al., 2007a) increased ACh release in the frontal cortex and/or dorsal hippocampus.

H_3 heteroreceptor regulation of neurotransmission is not limited to Ach, because microdialysis studies have demonstrated that H_3 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 of the novel H_3 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 with the magnitude of the effect of H_3 antagonists on histamine or ACh release in the prefrontal cortex.

Similar to the heterogeneity demonstrated by H_3 autoreceptors, inhibition of H_3 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). Likewise, the local application of GSK189254 in the TMN produces heterogenous modulation of neurotransmitter release (Giannoni et al., 2010). Whereas 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 H_3 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. H_3 antagonists also induce a robust release of ACh in the cortex and hippocampus. Results on NE release have been variable, perhaps because of the small magnitude of the effect. The magnitude of H_3 modulation of DA is also modest. It is noteworthy that 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 H_3 antagonist-mediated increases in these neurotransmitters supports their utility in human cognitive disorders.

Histamine H_3 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 H_3 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, and 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 electroencephalogram and electromyography techniques (Jones, 2005; Schwartz and Roth, 2008).

Histamine plays a physiological role in the light/dark cycle, because histamine release increases during the light phase whereas it decreases to baseline levels during the dark phase. The widespread projection 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 because they are active during the wake period and eventually cease firing during the rapid

eye movement sleep phase. Agents that increase histaminergic activity (histamine, H_1 agonists, or inhibitors of histamine metabolism) increase wake in animals; in contrast, agents that decrease histaminergic activity [inhibitors of histidine decarboxylase such as α-fluoromethylhistidine, or the H_1 antagonists mepyramine and diphenhydramine (DPH)] 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, H_1-KO mice exhibit disruption of the circadian rhythm with decreased activity during the active phase (Inoue et al., 1996; Barbier and Bradbury, 2007).

H_3 antagonists increase histamine release in rats, and thioperamide, ciproxifan, and BF2.649 increase wake in cats; similarly, 1-{3-[4-(piperidin-1-ylmethyl)phenoxy]propyl}piperidine (JNJ-5207852), 4-(3-(4-piperidin-1-ylbut-1-ynyl)benzyl)morpholine (JNJ-10181457), GSK189254, and ABT-239 increase wake in rodents. Because ciproxifan does not promote wakefulness in H_1-KO mice, these data indicate that the wake-promoting effect of H_3 antagonists is mediated by increased histamine release stimulating postsynaptic H_1 receptors (Lin et al., 1990; Barbier et al., 2004; Bonaventure et al., 2007).

Preclinical studies have shown that CNS-penetrant H_1 antagonists such as DPH increase total sleep, an effect that is not observed with the second-generation H_1 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 with normal individuals. DPH increases subjective sleepiness in humans and impairs performance in attentional/working memory tests (Gevins et al., 2002). H_1 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 H_1 blockers on sleep are consistent with the histaminergic hypothesis and most likely H_1-related in human.

The importance of the histaminergic system in vigilance is also demonstrated by clinical studies in which H_3 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 humans (>70%). Furthermore, the relationship between H_3 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 H_3 antagonists in animals.

H_3 antagonists as a class exhibit alerting effects caused by brain H_3 receptor occupancy, and if occupancy is high at night a common side effect is difficulty getting to sleep. If the desired occupancy is achieved during the day, H_3 antagonists may thus be useful for the treatment of excessive sleepiness and narcolepsy. H_3 antagonists are effective in two animal models of narcolepsy; GSK189254 is a potent H_3 antagonist that exhibits procognitive effects in several animal models and also increases wake in wild-type mice and orexin-KO mice. Likewise, JNJ-5207852 and JNJ-10181457 are potent H_3 antagonists with procognitive 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 H_3 antagonist BF2.649 (pitolisant, 40 mg every day for 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 5 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- to 40-mg doses).

These clinical data indicate that H_3 antagonists can have therapeutic properties in patients suffering excessive sleepiness and that the mechanism is probably caused by 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 H_3 antagonists promote wake in preclinical models of narcolepsy as we discussed previously, and BF2.649 is effective in humans suffering narcolepsy.

Preclinical and clinical data indicate that H_3 antagonists increase vigilance and wake, which affect other cognitive domains. The exogenous administration of histamine facilitated long-term retention in the inhibitory avoidance test in mice after intracerebroventricular administration (de Almeida and Izquierdo, 1986) and improved short-term memory in the social recognition test. A similar effect was induced by histidine administration, whereas inhibition of histamine synthesis by α-fluoromethylhistidine impaired short-term memory (Prast et al., 1996). Intrahippocampal injections of histamine also ameliorate spatial memory deficits induced by (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK-801) (Xu et al., 2005). These studies indicate that brain histamine plays a role in short-term, long-term, and spatial memory.

The intracerebroventricular administration of the H_3 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 H_3 agonists imetit and (*R*)-α-methylhistamine 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, which is supported by the clinical efficacy of donepezil in AD. The ability of H_3 antagonists to increase ACh release in the brain makes this an attractive target for the treatment of cognitive disorders. It is noteworthy that the effect of H_3 agonists impairing memory have been correlated with the inhibitory effect on ACh release, indicating the H_3 antagonists may regulate memory via the central cholinergic system (Blandina et al., 1996).

TABLE 1
Cognitive domains modulated by histamine and H_3 antagonists in the different preclinical models that have been extensively used to detect the procognitive effect of novel agents

	Cognitive Domains				
	Attention–Impulsivity[a]	Short-Term Memory[b]	Working Memory[c]	Long-Term Memory[d]	Spatial Memory[e]
Histamine		+		+	+
Thioperamide	+	+	+	+	+
BF2.649 (pitolisant)			+		
ABT-239	+	+	+	+	+
ABT-288	+	+		+	+
GSK189254	+			+	+
JNJ-10181457	+		+		
PF-03654746				+	

+ indicates that efficacy in this model was demonstrated.
[a] Evaluated in the five-trial inhibitory avoidance test in SHR pups or the five-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 delayed nonmatching to position test.
[d] Long-term memory or consolidation in the inhibitory avoidance test with retention measured 24 h after one-trial learning.
[e] Spatial memory evaluated in the water maze or Barnes maze.

With regard to the effects of novel H_3 antagonists, ABT-239 binds with 2 nM potency to rat H_3 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 five-trial inhibitory avoidance test in SHR pups, the social recognition test, the 24-h inhibitory avoidance test, and spatial memory tasks (Fox et al., 2005). GSK189254 is a potent H_3 antagonist (human K_i = 0.2 nM; rat K_i = 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- to 48-h delays and improved attentional set shifting and spatial learning in aged rats (Medhurst et al., 2007a).

BF2.649 binds with potent affinity to human (K_i = 2.7 nM) and rat (K_i = 17 nM) H_3 receptors, and it increases the release of ACh and DA from rat cortex. In behavioral studies BF2.649 improved the 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 H_3 antagonist (K_i = ~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 nonmatching to position task in scopolamine-treated rats (Leurs et al., 2005; Bonaventure et al., 2007; Galici et al., 2009).

In addition to the facilitatory effects on different cognitive domains in rodents (Table 1), H_3 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,b). Thus, H_3 antagonists may be beneficial in CNS disorders such as Alzheimer's disease 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 probably limits the therapeutic effectiveness of these agents dependent on endogenous ACh synthesis. Nonetheless, AChE inhibitors such as 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.

H_3 antagonists elevate ACh levels in cortex and hippocampus and 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 H_3 antagonists as AD therapeutics may involve activation of multiple biochemical pathways. Moreover, the multisignaling potential of H_3 antagonists may afford therapeutic benefits beyond symptomatic alleviation. Drug discovery efforts toward developing therapeutics that have disease-modifying effects have focused on the two proteins involved in AD pathology, namely 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 (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 H_3 antagonist-evoked neurotransmitter release leads to activation of postsynaptic receptor pathways such as phosphorylation of cAMP response element binding protein (CREB), a transcription factor germane to cognitive function, and the inhibitory residue Ser9 of 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 and subsequent activation of the serine–threonine kinase Akt inhibits GSK3β activity via Ser9 phosphorylation, a cellular cascade known to be associated with neuroprotection. Administration of the H_3 antagonist ABT-239 in normal mice increase cortical CREB and

S^9-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 S^9-GSK3β phosphorylation. Together, these findings suggest that increased S^9-GSK3β phosphorylation induced by ABT-239 does not depend on increased ACh release.

Both CREB and S^9-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 pS^9-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 (pS^9-GSK3β) in α7 nicotinic ACh receptor (nAChR) knockout mice that do not exhibit α7 nAChR agonist-induced phosphorylation, suggesting that H_3 antagonist-mediated signaling does not depend on ACh-stimulated α7 nAChR activation.

In contrast to the in vivo findings, studies conducted in cortical cell cultures have demonstrated that H_3 receptor agonism induces phosphorylation of the Akt/GSK3β pathway and protects against neurotoxic insults, which are blocked by the H_3 antagonist thioperamide (Mariottini et al., 2009). These results indicate that H_3 receptor agonist activation in vitro leads to signaling changes similar to those observed with H_3 antagonism in the whole animal. In this regard, H_3 antagonist-evoked neurotransmitter release and subsequent postsynaptic receptor stimulation, not present in an in vitro system, may indeed produce a signaling phenotype distinct from H_3 receptor-mediated biochemical signaling when examined in vitro. In summary, these in vivo signaling studies raise the intriguing possibility that H_3 antagonists activate signaling pathways that may translate into improved efficacy in patients with AD, with symptomatic alleviation and disease-modifying effects.

Development Status of H_3 Receptor Antagonists

Several H_3 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- 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 H_3 antagonists. The earliest compounds have significant shortcomings as clinical drugs (cytochrome P450 inhibition, low brain penetration) but have become pharmacological tools, particularly ciprofan, thioperamide, and (1*S*,2*S*)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl) imidazole (GT-2331). The first cited agent into clinical trials was GT-2331, but clinical development did not advance perhaps because of its imidazole-associated liabilities. The problems of these early structures drove efforts toward nonimidazoles, wherein the imidazole moiety is replaced by a tertiary basic amine, addressing cytochrome P450 inhibition and improving CNS penetration and H_3 selectivity. Most modern H_3 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 (http://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 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 to 40 mg every day as assessed in the Epworth Sleepiness 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 and phase 1 studies on PF-03654746. It is a potent H_3 antagonist (human H_3 K_i = 3.2 nM; rat K_i = 37 nM) and active in the object recognition model. It exhibits a long human $t_{½}$ (9–18 h), 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 two drug groups versus placebo; however, a phase 2 trial for daytime sleepiness in narcolepsy, a receptor occupancy PET study at 0.5- to 4-mg doses, and clinical trials in AD and narcolepsy are ongoing.

GSK189254 is a potent H_3 antagonist (human H_3 K_i = 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 in early trials as targeting Alzheimer's, pain, and narcolepsy, but is no longer under clinical development. A ^{11}C-labeled analog has been reported as a radiotracer tool to probe the receptor occupancy of GSK239512 (http://clinicaltrials.gov). The structure and properties of GSK239512 have not been described, but GlaxoSmithKline is recruiting patients for phase 2 trials in schizophrenia and AD.

Merck has published the structure–activity relationship of several chemical series, with one especially potent compound (human H_3 K_i = 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 to 50 mg and MK-3134 at 0.5 to 25 mg achieved up to 93 and 96% H_3 receptor occupancy, respectively. Data suggest that H_3 antagonist-induced alerting effects were observed at 67% occupancy, increasing with higher levels of H_3 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 blood oxygen level-dependent functional magnetic resonance imaging at doses of 1, 5, and 25 mg.

Johnson and Johnson was an early leader in industrial H_3 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 H_3 antagonist. It has been probed extensively, in pharmacokinetic and 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 K_i = 0.45 nM affinity to human H_3 (Esbenshade et al., 2005). ABT-239 had robust activity in rodent models of cognition and has been used as a reference standard to probe H_3 effects in vivo (Fox et al., 2005). A recent study demonstrated that ABT-239 ameliorated ethanol-induced deficits on hippocampal long-term potentiation, indicating that H_3 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 H_3 antagonist that binds to rat and human H_3 receptors with K_i = 8.1 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 H_3 receptors with ED_{50} = 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, Servier, Arena, Wyeth/Pfizer, Cephalon, Sanofi-Aventis, 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 H_3 antagonists in Table 2 are more potent for binding the human versus the rat H_3 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 to 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 H_3 antagonists provided mixed results because PF-03654746 was ineffective in adult ADHD patients, whereas a positive effect has been reported with the JNJ compounds in these patients. It has also been reported that H_3 antagonists increase attention in humans because 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 such as ADHD. Studies that were previously conducted (data not disclosed yet) and those presently ongoing with BF2.649 and MK-0249 will provide useful informa-

TABLE 2
Summary of key preclinical and clinical data on histamine H_3 antagonists that have been reported to advance to the clinical area

	Rat H_3	Human		
		H_3	$t_{1/2}$	Clinical Observations and Status
	nM		*h*	
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	N.A.	N.A.	N.A.	Advanced to phase 1. Phase 2 trials in AD and schizophrenia ongoing
MK-0249	N.A.	N.A.	N.A.	Sleep disturbances, insomnia in phase 1. Alerting effects noted at 67% receptor occupancy. No efficacy in schizophrenic patients.
MK-3134	N.A.	N.A.	N.A.	Advanced to phase 1.
ABT-288	8	1.9	N.A.	Phase 2 trials in AD and schizophrenia ongoing.
JNJ-17216498	N.A.	N.A.	N.A.	Advanced to phase 1. Evaluated in patients with narcolepsy and ADHD.

N.A., data are not available.

tion in the near future with regard 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 such as delusions and hallucinations; negative symptoms such as 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 predictors 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 antipsychotic medication. Patients received 4 weeks of treatment with a 10-mg dose of MK-0249, a dose that had previously shown high brain H_3 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 K_i = 15 nM; olanzapine H_1 K_i = 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 cognitive deficits of schizophrenia 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. Patients in the advanced stage (severe) suffer a global cognitive decline, whereas 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, impairments of semantic memory, or inability to store new facts. Attentional and executive deficits are present in AD because these patients perform poorly in tests of selective, sustained, and divided attention.

Acetylcholine inhibitors (donepezil, rivastigmine, galantamine) and *N*-methyl-D-aspartate antagonists (memantine) are the approved therapies for AD. These agents have modest efficacy and their symptomatic benefits are short-lived. Furthermore, the gastrointestinal-related side effects of the AChE inhibitors (nausea, vomiting) lead to noncompliance 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 H_3 antagonists to improve attention, vigilance, and wake (as discussed previously) may differentiate these agents from other approaches presently under evaluation in patients with AD, 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 H_3 antagonists in AD have not been disclosed, and several studies have been initiated in this area. Compounds from GSK, Pfizer, Merck, and Abbott are under evaluation in this patient population.

The ability of H_3 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 H_3 antagonists are able to increase histamine in humans, consistent with the preclinical data. H_3 antagonists can also increase extracellular levels of ACh, a neurotransmitter that has been effective to improve cognition in the early phases of AD. H_3 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 H_3 antagonists in these human conditions may be able to provide an answer to these hypotheses and determine the place for H_3 antagonists in therapeutics.

Authorship Contributions

Participated in research design: Brioni, Esbenshade, Garrison, Cowart, and Bitner.

Conducted experiments: Brioni, Esbenshade, Garrison, and Bitner.

Performed data analysis: Cowart.

Wrote or contributed to the writing of the manuscript: Brioni, Esbenshade, Garrison, Cowart, and Bitner.

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Address correspondence to: Jorge D. Brioni, Neuroscience Research (Dept. 4MN), Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064. E-mail: jorge.brioni@abbott.com