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Histamine H_3 receptor antagonists: From target identification to drug leads

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ARTICLE INFO

Article history:
Received 15 August 2006
Accepted 31 October 2006

Keywords:
Histamine H3 receptor
Narcolepsy
Cognition
JNJ-5207852
JNJ-10181457

ABSTRACT

The successful cloning and functional expression of the histamine H_3 receptor in the late 1990s has greatly facilitated our efforts to identify small molecule, non-imidazole based compounds to permit the evaluation of H_3 antagonists in models of CNS disorders. High-throughput screening identified several series of lead compounds, including a series of imidazopyridines, which led to JNJ-6379490, a compound with high affinity for the human H_3 receptor. Analysis of structural features common to several series of non-imidazole H_3 receptor ligands resulted in a pharmacophore model. This model led to the design of JNJ-5207852, a diamine-based H_3 antagonist with good *in vitro* and *in vivo* efficacy but with an undesirable long half-life. However, further modifications of the template provided an understanding of the effect of structural modifications on pharmacokinetic properties, ultimately affording several additional series of compounds including JNJ-10181457, a compound with an improved pharmacokinetic profile. These compounds allowed *in vivo* pharmacological evaluation to show that H_3 antagonists promote wakefulness, but unlike modafinil and classical psychostimulants, they do not increase locomotor activity or produce any alteration of the EEG power spectral activity in rats. H_3 antagonists also increase extracellular acetylcholine and norepinephrine but not dopamine in rat frontal cortex and show efficacy in various models of learning-memory deficit. In addition, cFos immunoreactivity studies show H_3 antagonists activate neuronal cells in restricted rat brain regions in contrast to widespread activation after modafinil or amphetamine treatment. Therefore, H_3 antagonists are promising clinical candidates for the treatment of excessive day time sleepiness and/or cognitive disorders.

1. Introduction

Since its first pharmacological description as an endogenous substance [1], histamine has been found to exert tremendous influence over a variety of physiological processes. Most notable are its roles in the inflammatory "triple response" and in gastric acid secretion, which are mediated by H_1 [2] and H_2 [3] receptors, respectively. Antagonists of the histamine H_1 and H_2 receptors have been successful as "blockbuster" drugs for treating allergic conditions (allergic rhinitis) and gastric-acid-related disorders, respectively.

In the early 1970s, an understanding emerged that histamine was a neurotransmitter in the central nervous system [4,5]. Histamine synthesizing neurons are located in the tuberomammillary nucleus of the hypothalamus and project widely throughout the brain to regions that include the

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.10.031

cortex, the hippocampus, amygdala and striatum [6]. In 1983, a third subtype of histamine receptor, H_3, was pharmacologically identified as a presynaptic autoreceptor on histamine neurons in the brain controlling the stimulated release of histamine [7]. In 1987, the development of the agonist *R*-α-methylhistamine and the antagonist thioperamide validated the existence of the H_3 receptor [8]. The H_3 receptor was also shown to be a presynaptic heteroreceptor in non-histamine containing neurons in both the central and peripheral nervous systems [9]. Consequently, there are many potential therapeutic applications for histamine H_3 agonists and antagonists [10–13]. Since the histamine H_3 receptor is a presynaptic negative modulator of neurotransmitter release, it is rationalized that an H_3 receptor antagonist would enhance neurotransmitter release. By virtue of its unique CNS localization (striatum, thalamus, cortex) relative to other neurotransmitter receptors, it is hypothesized that H_3 receptor antagonists may produce a unique profile of CNS activation. In particular, activation of histaminergic neurotransmission leads to waking, improved cognition and suppression of food intake. By increasing the amount of histamine released from neurons, thereby promoting activation of H_1 receptor, H_3 antagonists increase waking [14]. H_3 antagonists are also thought to improve cognitive function possibly via an increase of acetycholine release [14,15]. The role of the H_3 receptor in the regulation of body weight is more controversial [11,16]. This paper describes the pharmacological characterization of several drug-like H_3 antagonists towards the identification of a suitable lead.

2. Cloning and functional expression of the human histamine H_3 receptor cDNA

Despite intensive efforts, the molecular identity of the H_3 receptor remained elusive for 15 years. In 1998, the successful cloning and functional expression of the histamine H_3 receptor by our group at J&JPRD greatly facilitated drug discovery efforts at this target [17,18]. As part of a directed effort to discover novel G protein-coupled receptors through homology searching of expressed sequence tag databases, a partial clone (GPCR97) that had significant homology to biogenic amine receptors was identified. The GPCR97 clone was used to probe a human thalamus library, which resulted in the isolation of a full-length clone encoding a putative G protein-coupled receptor. Subsequent analysis of GPCR97 revealed a pharmacological profile indistinguishable from that of the histamine H_3 receptor. The H_3 receptor cDNA contains an open reading frame of 445 amino acids and shows very low similarities with H_1 and H_2 receptors (22 and 20%, respectively), explaining why the H_3 receptor was not cloned by similarity screening with H_1 or H_2 receptor-related probes. *In situ* hybridization in rat brain revealed high levels of mRNA and confirmed the existence of the H_3 receptor in brain regions (cortex, striatum, thalamus, hippocampus, tuberomammillary nucleus, and locus coeruleus) consistent with its hypothesized function as a presynaptic release-controlling receptor that may regulate histamine (HA), norepinephrine (NE), serotonin (5-HT), dopamine (DA), gamma-aminobutyric acid (GABA), acetylcholine (ACh), and other neurotransmitters [19]. By cloning the H_3 receptor of other species, including rat [18], mouse [20] and guinea pig [21] major species differences were revealed. More specifically, many compounds have a lower affinity for the human receptor versus that of other species. For example, the antagonist thioperamide is a low affinity ligand for the human receptor whereas it is a high affinity ligand for the rat receptor (Table 1). In contrast, clobenpropit maintains high affinity for both the human and rat receptors (Table 1). GT-2331, an early clinical candidate from Gliatech [22], also displays more than 10-fold higher affinity for the rat receptor versus the human receptor (Table 1). These differences in pharmacology between rodents and humans are attributable to only two amino acid differences in the sequences [23]. More recently, the existence of various splice variants that differ in composition between species was revealed [19,24] and functional differences between human H_3 receptors isoforms have also been reported [25]. The significance of these findings is unclear, and our group and others have disputed the existence of the relevant human isoforms [26,27]. No isoforms have been reported in the mouse [20]. A number of studies have reported that the H_3 receptor is constitutively active in many experimental systems and probably also *in vivo* [28–31]. This has resulted in a large number of H_3 antagonists being reclassified from neutral

Table 1 – *In vitro* affinity and antagonist potency of reference compounds and investigational H_3 ligands (mean ± S.E.M.)

	hH_3 pK_i	rH_3 pK_i	hH_3 pA_2	rH_3 pA_2
Thioperamide	7.50 ± 0.10	8.40	7.20 ± 0.30[a]	8.70[a]
Clobenpropit	9.30 ± 0.10	9.0	9.1 ± 0.10[a]	9.30[a]
GT-2331	8.20	9.5	Agonist	Agonist
JNJ-280566	5.49	5.5	ND	ND
JNJ-6379490	8.57 ± 0.29	8.0	8.68 ± 0.07[a]	8.14 ± 0.09[a]
JNJ-132600	8.25 ± 0.12	7.8	8.74 ± 0.09[a]	8.47[a]
JNJ-10266386	6.54	ND	ND	ND
JNJ-5207852	9.24 ± 0.21	8.90 ± 0.17	9.84[a]	8.94[a]
JNJ-10181457	8.93 ± 0.16	8.15 ± 0.07	9.22[a]	8.33 ± 0.08[a]

The affinity of these ligands for the human and rat recombinant H_3 receptor stability expressed in SK-N-MC cells was determined by competitive radioligand binding using [^{3}H]-N-methylhistamine as the radioligand [18]. The functional antagonism was determined using a cAMP accumulation assay (SK-N-MC cells that expressed a reporter construct and either human or rat H_3 receptor) [18]. ND = not determined.
[a] Neutral antagonist.

antagonists to inverse agonists. However, classification of H_3 receptor ligands depends on the test system.

The initial development of H_3 receptor antagonists in the early 1980s focused on imidazole containing compounds (thioperamide, clobenpropit, ciproxifan, and proxyfan) [13]. Imidazole containing ligands are associated with inhibition of cytochrome P450 enzymes potentially leading to drug–drug interaction and these ligands are also subject to metabolism via histamine *N*-methyl transferase. The search for potent H_3 compounds structurally diverse from histamine was hampered by the limited throughput of the ''precloning'' H_3 screening assays. The successful cloning and functional expression of the histamine H_3 receptor by our group at J&JPRD prompted our efforts to identify small molecule, non-imidazole based compounds to permit the evaluation of H_3 antagonists in models of CNS disorders.

3. JNJ-6379490: a suitable tool for *in vivo* exploration of H_3 function

Several series of lead compounds were identified by high throughput screening (HTS) including imidazopyridines (JNJ-280566), *N*-methylimidazoles (JNJ-132600) and indolizidines (JNJ-10266386). Chemical structures are shown in Fig. 1, and corresponding *in vitro* binding and functional data for human and rat H_3 receptors are listed in Table 1. JNJ-280566, originally prepared for a calcium channel antagonist program [32], was found to have weak affinity for H_3 (Table 1). Subsequent medicinal chemistry efforts led to the optimized structure JNJ-6379490 containing a piperidine propyloxy moiety (Fig. 1 and Table 1).

JNJ-6379490 is a high affinity/potent H_3 neutral antagonist at the human and rat receptors (Table 1). It is devoid of activity at H_1, H_2 or H_4 receptors. The compound demonstrated good oral bioavailibility in rat and dog. JNJ-6379490 penetrated the brain following intraperitoneal (i.p.) administration as determined by *ex vivo* autoradiography (ED_{50} = 0.2 mg/kg i.p. in rat).

The best-known function of histamine in the brain is the regulation of wakefulness; therefore, a comparative study on the effects of the JNJ-6379490 and thioperamide on sleep–wake patterns was performed in rats (Fig. 2). JNJ-6379490 and thioperamide induced comparable dose-dependent modifications of the sleep–wake states. Both compounds produced a dose-related increase in wake duration associated with a decrease in the time spent in deep slow wave sleep (SWS2). Significant effects were observed from the dose of 0.63 mg/kg (s.c.) upwards with JNJ-6379490 (Fig. 2A) and from the dose of 2.5 mg/kg (s.c.) upwards with thioperamide (Fig. 2B). In addition, JNJ-6379490 produced a short-lasting (2 h) decrease in rapid eye movement (REM) sleep amounts from the dose of 2.5 mg/kg upwards. In contrast to methylphenidate and amphetamine, JNJ-6379490 and thioperamide did not produce any alteration of the EEG power spectral activity in awake free-moving rats and thereby preserved the qualitative aspect of vigilance (Fig. 3). JNJ-6379490 and thioperamide promote wakefulness, probably

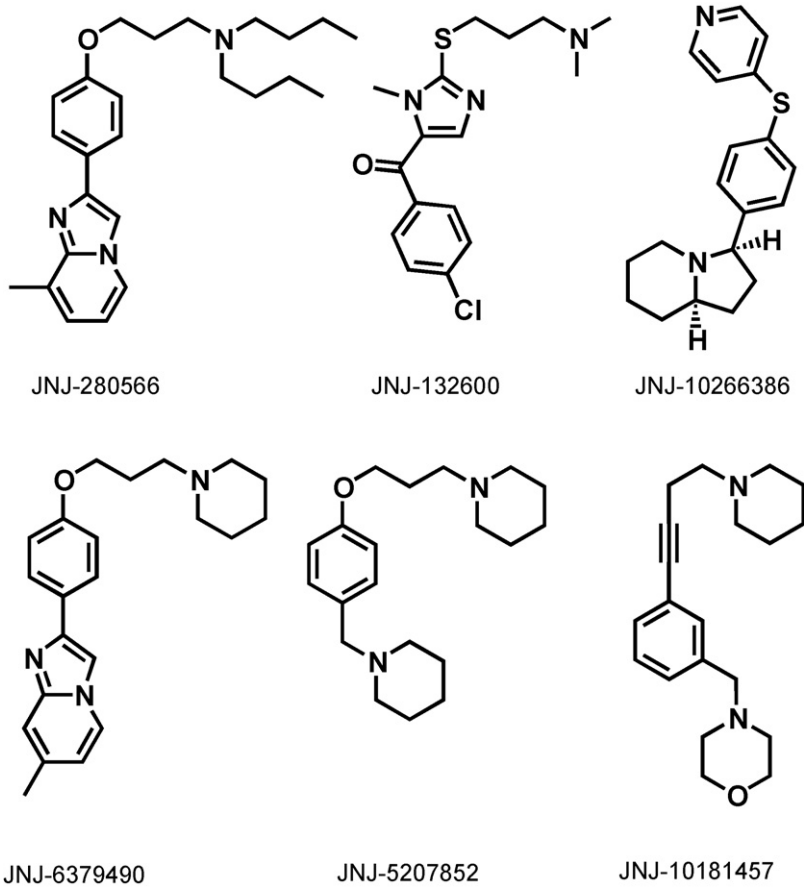

Fig. 1 – Chemical structures of investigational H_3 receptor ligands.

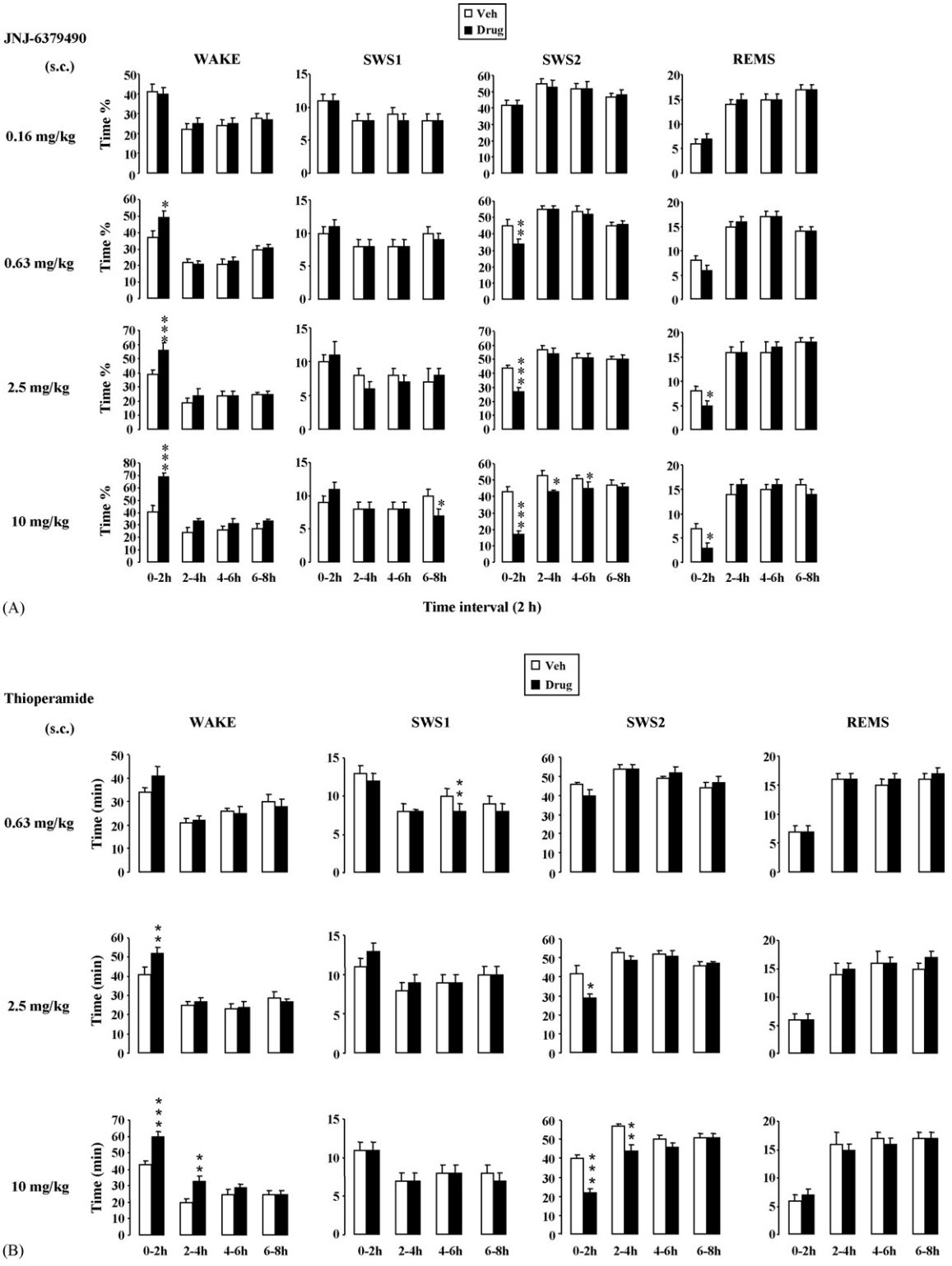

Fig. 2 – Effect of increasing doses of (A) JNJ-6379490 (0.16–10 mg/kg, s.c.) and (B) thioperamide (0.63–10 mg/kg, s.c.) on sleep–wake states in rats (male adult Wistar). The animals received the compounds or vehicle (saline) at the beginning of the light phase (8:00 a.m.). Polygraphic recordings were performed for 8 h following the treatment and were scored visually by 15 s epochs as either wake (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2) or rapid eye movement sleep (REMS), according to standard criteria. The sleep–wake parameters were analyzed per 2 h periods and were compared to control values, i.e., vehicle administration under the same recording conditions. Time spent in each vigilance state (W, SWS1, SWS2 and REMS) was expressed as percentage of recording time (mean ± S.E.M., $n = 8$ per dose). Statistical significance of the vehicle-drug comparisons was assessed by means of the paired two-tailed Student's *t*-test ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$).

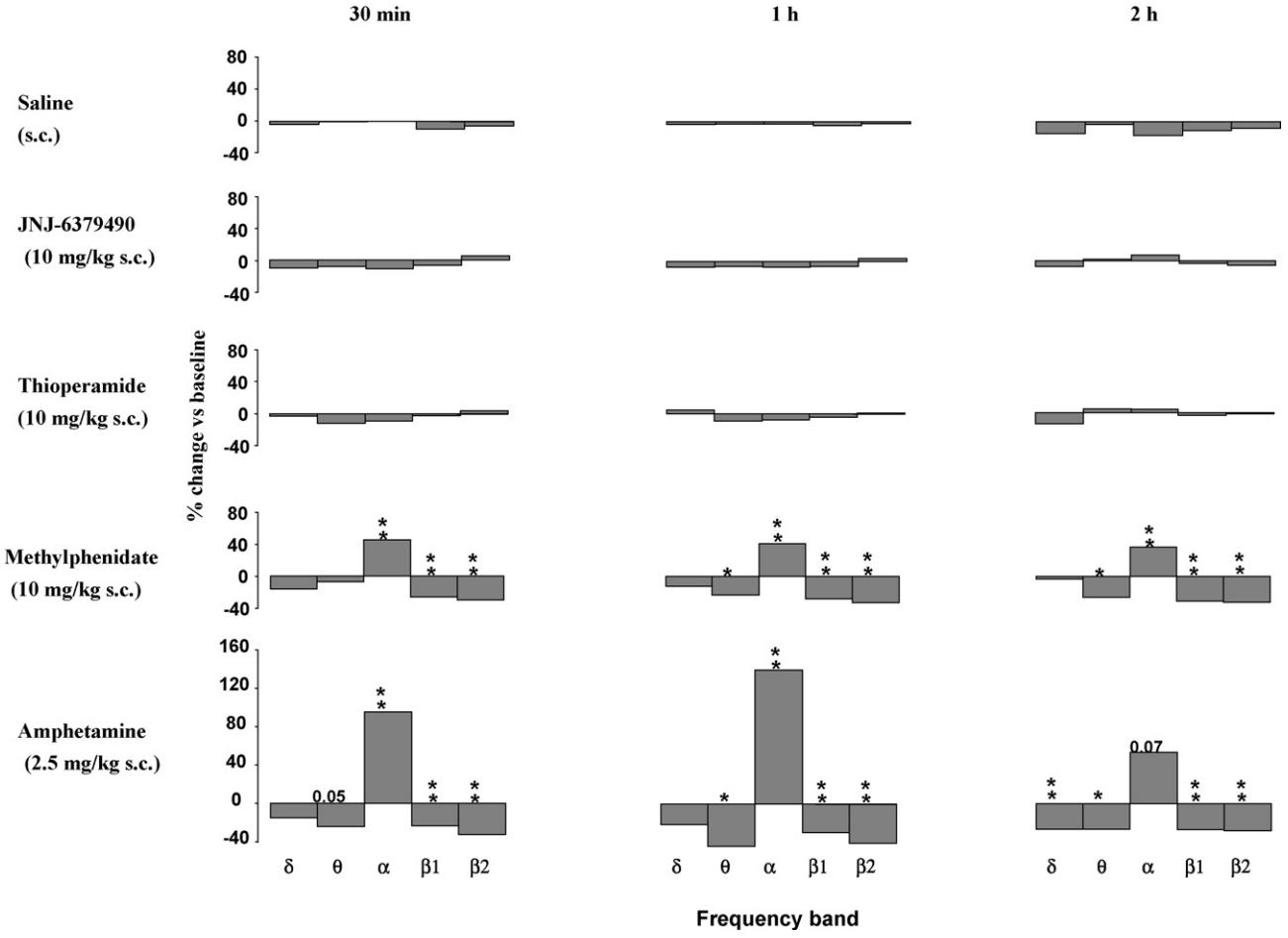

Fig. 3 – EEG power spectral activity in awake rats (adult Wistar) treated with JNJ-6379490 (10 mg/kg, s.c.) as compared to thioperamide (10 mg/kg, s.c.), methylphenidate (10 mg/kg, s.c.) and amphetamine (2.5 mg/kg, s.c.) treatments. The animals received the compounds or vehicle (saline) (n = 8–10 rats per group) at the beginning of the light phase (8:30 a.m.). Thirty minutes later, a series of 10 min recording sessions were performed 30 min, 1 h and 2 h after the treatment. A 10 min recording session was obtained immediately before the treatment to serve as baseline conditions. Spectral analysis of the EEG using the fast Fourier transform was performed on each of the 10-min EEG recording periods. The power densities in each of the five predetermined frequency bands (delta: 1.2–4 Hz, theta: 4.2–8 Hz, alpha: 8.2–12 Hz, beta-1: 12.2–20 Hz and beta-2: 20.2–30 Hz) of power spectra were averaged over all artefact-free epochs during the 10-min sampling and were expressed as percent of baseline values. Statistical significance of the baseline-vehicle and baseline-drug treatment comparisons was assessed by means of the Wilcoxon signed-rank test ($^{*}p < 0.05$ and $^{**}p < 0.01$).

because of their central histamine H_3 antagonistic properties, which would be expected to stimulate neuronal histamine release. In accordance with *ex vivo* receptor binding studies for central occupancy of H_3 receptors, JNJ-6379490 was effective at a four-fold lower dose than that of thioperamide.

JNJ-6379490 was also found to enhance short-term memory in the social interaction test in rat (unpublished results) supporting the hypothesis that H_3 antagonists may improve cognitive function, possibly via an increase of acetylcholine [14].

Due to idiosyncratic toxicity findings in dog, the development of this compound was stopped. In parallel to the evaluation of JNJ-6379490, a medicinal chemistry effort continued around the three series of HTS leads and these studies provided us with a working pharmacophore model [33] which distilled down to the simple bis-piperidine JNJ-5207852 (Fig. 1).

4. JNJ-5207852: a novel diamine-based H_3 antagonist

JNJ-5207852 exhibits high affinity for both human and rat H_3 receptors and behaves as a neutral antagonist (Table 1) [34]. This compound does not bind to H_1, H_2, or H_4 receptors and it retained its selectivity when tested in a CEREP panel containing approximately 50 G-protein coupled receptors, ion channels and other drug targets. [^{3}H]JNJ-5207852 failed to exhibit any appreciable binding in H_3 knockout mice, but exhibited patterns of binding in the cortex, hypothalamus and striatum of wild-type mice that were consistent with the distribution patterns of H_3 receptors in these brain areas. Autoradiography experiments revealed that full receptor occupancy is achieved in the striatum following the subcutaneous administration of a 1 mg/kg dose.

JNJ-5207852 produced a dose-dependent decrease in imetit-induced drinking confirming that JNJ-5207852 pharmacologically antagonizes the H_3 receptor *in vivo* in rats (Pudiak et al., in preparation). Imetit increased drinking in both rats and mice but not in H_3 knockout mice (unpublished data). After having demonstrated a central *in vivo* pharmacokinetic/pharmacodynamic (PK/PD) activity at the H_3 receptor, JNJ-5207852 was evaluated in more complex models to demonstrate the utility of this compound in fully integrated behavioral functions such as sleep–wake cycle, metabolism and cognitive tasks.

Consistent with the data obtained with JNJ-6379490 and thioperamide (Figs. 2 and 3), JNJ-5207852 promoted wakefulness [34]. In addition, JNJ-5207852 failed to have an effect on wakefulness in H_3 receptor knockout mice, providing strong evidence that the provigilant effect of JNJ-5207852 is due to an interaction with the H_3 receptor [34]. The wake-promoting effect of JNJ-5207852 was not associated with an increase in locomotor activity [34]. Methylphenidate and amphetamine induced a pronounced increase of the power density in alpha band (delta, theta, beta-1 and beta-2, Fig. 3). The increase in alpha power, which was particularly prominent after amphetamine treatment (Fig. 3), reflected the marked increase in locomotor activity probably due to the activation of the dopaminergic system. In contrast JNJ-6379490 and thioperamide did not produce any alteration of the EEG power spectral activity in the entire frequency range analyzed (up to 30 Hz, Fig. 3). In cats and mice, ciproxifan was shown to induce a marked increase in EEG fast rhythms (30–60 Hz) which has been interpreted to enhanced attention [35,36]. In our study (Fig. 3), EEG power densities were evaluated in rats (different species) up to 30 Hz frequency range.

The role of the H_3 receptor in the regulation of body weight is ambiguous [11,16]. Intracerebroventricular (i.c.v.) administration of histamine reduced food intake in two obese mouse models [37]. Intracerebroventricular or intraperitoneal administration of thioperamide reduced food intake in rat [38,39] presumably, by increasing histamine levels and subsequent stimulation of postsynaptic H_1 receptors. However, in a different study direct i.c.v. injection of thioperamide had no inhibitory effect on fasting-induced food and water intake, while higher doses increased food intake [40]. The H_3 knockout mouse produced by Takahashi et al. [41] displayed a mildly obese phenotype. In contrast, the growth curves for our in house H_3 knockout mice were parallel with the control animals [42]. Our H_3 receptor deficient mice were resistant to the effect of leptin on sleep probably due to their reduced histamine levels [42,43]. To shed light on these ambiguous data, the effect of JNJ-5207852 on body weight and food intake was assessed in normal and leptin deficient ob/ob mice. The ob/ob mice were included in that study because histamine has been proposed as a downstream target of leptin [44]. Chronic dosing with JNJ-5207852 did not have an effect on body weight in ob/ob mice or normal mice [34]. Similarly, in normal Beagles, there was no indication that appetite (food intake) was suppressed with JNJ-5207852 (1, 3, 10 mg/kg p.o. for 7 days) (unpublished data).

The effect of JNJ-5207852 on learning and memory deficits induced by pentylenetetrazol (PTZ) kindling in the brains of developing mice was investigated and compared to thioperamide [45]. JNJ-5207852 and thioperamide were found to ameliorate PTZ kindling induced learning and mnemonic deficits in social discrimination, acoustic fear conditioning and passive avoidance but not in the water maze test. These data suggest that an H_3 antagonist might be useful for the treatment of cognitive impairment in epilepsy.

Pharmacokinetic studies in rats and dogs revealed an undesirably long-half life for JNJ-5207852. The compound displayed a long brain residency time after single bolus administration as shown in Fig. 4. Full receptor occupancy was still observed after 24 h. In addition, JNJ-5207852 was found to induce phospholipidosis in rats.

However, further modifications of the template provided an understanding of the effect of structural modifications on pharmacokinetic properties, ultimately affording several additional series of compounds including JNJ-10181457, a compound with improved pharmacokinetic properties.

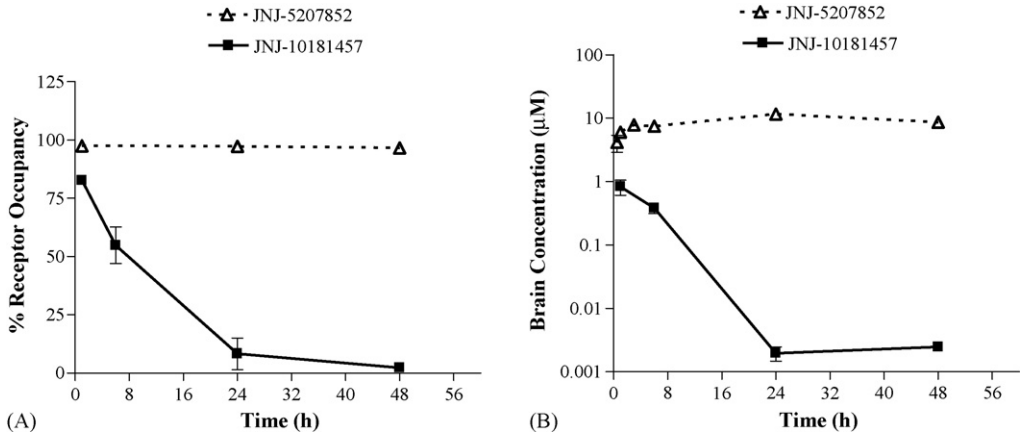

Fig. 4 – (A) *Ex vivo* occupancy of H_3 receptor by JNJ-5207852 and JNJ-10181457 (10 mg/kg p.o.) in rat striatum (coronal section): time dependency after oral administration. *Ex vivo* autoradiography was performed as previously described using [^{3}H]-*R*-α-methylhistamine [34]. (B) Blood–brain barrier penetration study with JNJ-5207852 and JNJ-10181457 (10 mg/kg, s.c.). Brain samples were analyzed for the content of JNJ-5207852 or JNJ-10181457 by LC-MS-MS.

5. JNJ-10181457: a short acting H_3 antagonist

JNJ-10181457 (or RWJ-662733 [46]) is a potent and selective H_3 neutral antagonist exhibiting a short residency in brain tissue. Its *in vitro* potency was ~10 times lower at the rodent H_3 receptor versus the human H_3 receptor. JNJ-10181457 showed rapid brain penetration and good receptor occupancy in striatum (Fig. 4). Maximal receptor occupancy (~85%) was achieved after 1 h following oral administration (10 mg/kg, Fig. 4A). The washout of JNJ-10181457 was much more rapid than that of JNJ-5207852 (Fig. 4B). After 24 h, there was no significant receptor occupancy (<10%) remaining with JNJ-10181457 in contrast to JNJ-5207852 which still had full receptor occupancy at 24 h (Fig. 4A). At 10 mg/kg i.p., JNJ-10181457 produced a decrease in imetit-induced drinking confirming that JNJ-10181457 pharmacologically antagonizes the H_3 receptor *in vivo* in rats (unpublished data).

The wake promoting effect of JNJ-10181457 was also demonstrated in mice [46] and rat [47]. The wake promoting effect of JNJ-10181457 was not associated with an increase in locomotor activity up to 30 mg/kg i.p. in rat [47]. Interestingly, orexin/ataxin-3 narcoleptic mice were more sensitive to JNJ-10181457 than their wild-type littermate as larger wake-promoting effects during the light period were observed in this animal model of narcolepsy [46]. JNJ-10181457 is likely to consolidate the wakefulness in these animals since the mean bout lengths of wake were longer than after vehicle administration. In addition, data obtained by Nishino at Stanford showed that JNJ-10181457 (1.25–10 mg/kg p.o.) significantly reduced cataplexy (number of cataplectic attacks and time spent in cataplexy) in familial narcoleptic Dobermans using the standard food elicited cataplexy test (Fig. 5A and B). The potency of the anticataplectic effects of JNJ-10181457 was comparable to that of desipramine (a tricyclic antidepressant currently used for the treatment of human cataplexy). Thioperamide (0.1–0.64 mg/kg i.v.) [48] and JNJ-5207852 (unpublished results) also potently reduced cataplexy in canine narcolepsy. There was no indication that appetite (food intake) was suppressed with JNJ-10181457 (1, 3, and 10 mg/kg p.o. for 7 days) in dogs (unpublished data) ruling out the compound is a false positive in the canine narcolepsy model. While the definitive mechanism of action of the anticataplectic effects of H_3 antagonists remains to be determined, it is relatively well established that enhancement of NE neurotransmission reduces cataplexy [49]. H_3 antagonists also increase terminal release of NE [50] and microdialysis in frontal cortex of freely moving rats showed that JNJ-10181457 increased extracellular NE levels (Fig. 6A and B).

The data obtained in narcoleptic mice and narcoleptic Dobermans suggest that H_3 antagonists may be a unique therapeutic choice to manage both excessive day time sleepiness and cataplexy attacks associated with narcolepsy.

Microdialysis studies in freely moving rats demonstrated that in addition to increasing NE levels, JNJ-10181457 (10 mg/kg, s.c.) also increased extracellular ACh levels in frontal cortex (Fig. 6A–D). In contrast, extracellular DA (Fig. 6E and F) and 5-HT levels (not shown) were not changed. The increase of ACh release induced by JNJ-10181457 may explain the significant improvement in acquisition memory observed in spontaneously hypertensive rat pups when treated with JNJ-10181457 (10 mg/kg, s.c.) and then subjected to a passive avoidance task (Fig. 7). Spontaneously hypertensive rat pups (SHR) are known to exhibit learning deficits compared to normotensive Wistar-Kyoto pups. This memory deficit can be corrected by the administration of methylphenidate, a compound clinically used to treat attention-deficit hyperactivity disorder (ADHD). A new behavioral method developed by Fox et al. [51] uses SHR pups tested in a five-trial repeated inhibitory passive avoidance task. SHR pups tested in this model appear to be more impulsive and exhibit a marked

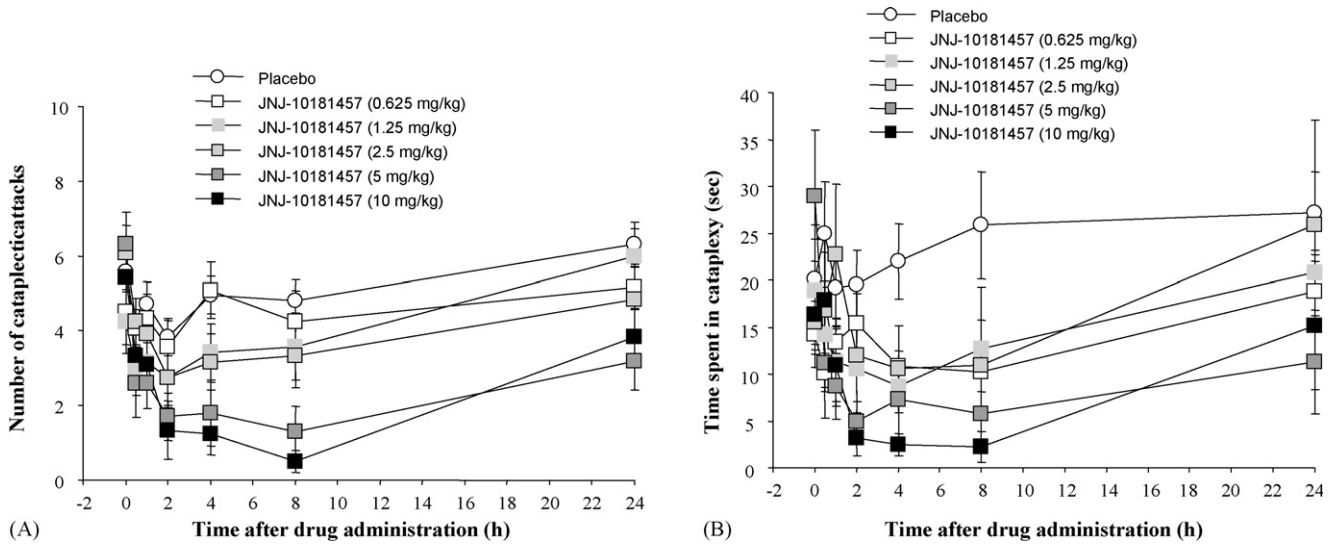

Fig. 5 – The effects of JNJ-10181457 on cataplexy were evaluated in six genetically narcoleptic Dobermans using the standard food elicited cataplexy test [48]. After the baseline cataplexy testing, JNJ-10181457 (0.625, 1.25, 2.5, 5 and 10 mg/kg) mixed with dog food (or placebo, food alone) was orally administered, and cataplexy testing was repeated six times over 24 h. JNJ-10181457 (1.25–10 mg/kg p.o.) significantly reduced cataplexy in the number of cataplectic attacks (A) and the time spent in cataplexy (B).

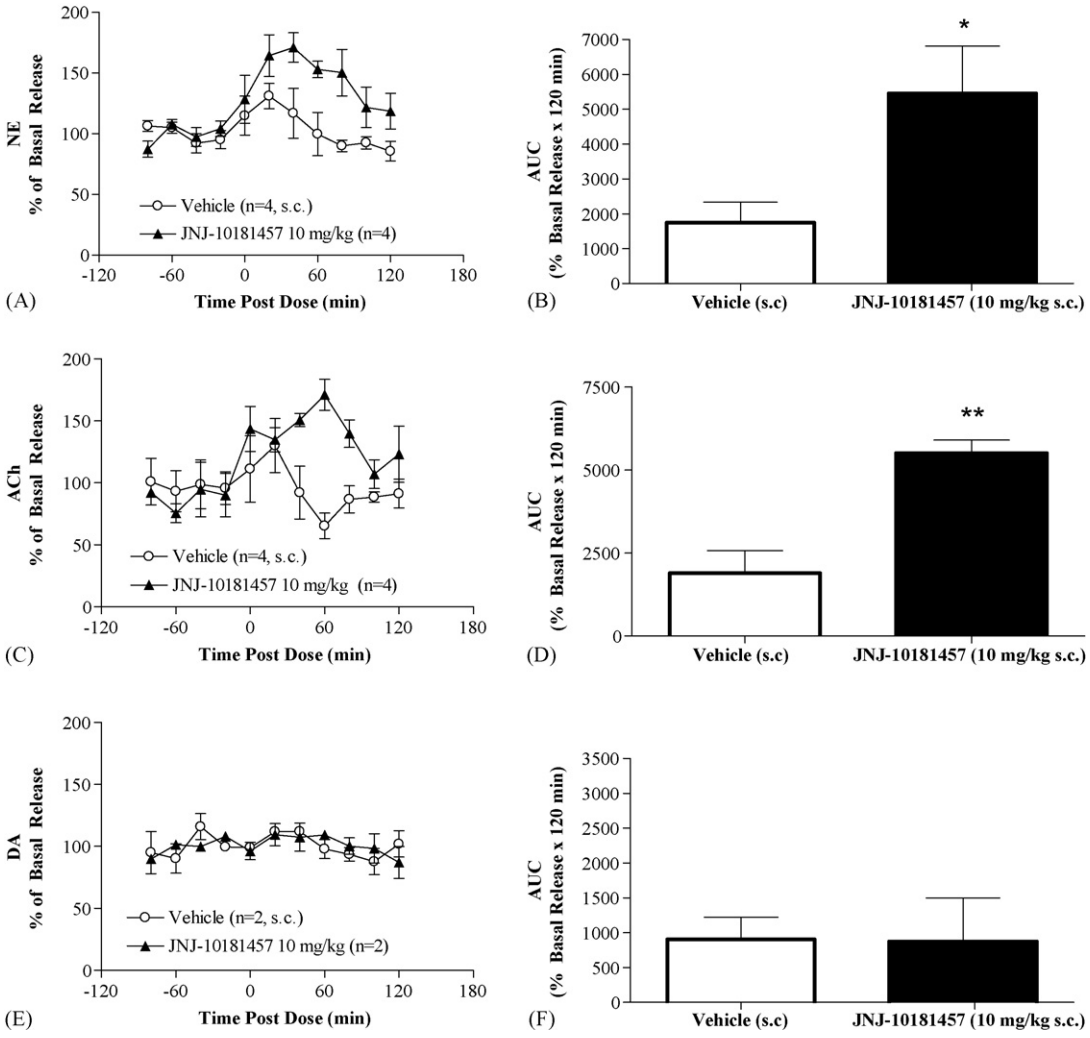

Fig. 6 – Effects of JNJ-10181457 (10 mg/kg, s.c.) on *in vivo* extracellular ACh (A and B), NE (C and D) and DA (E and F) levels in the rat prefrontal cortex (AP = −3.2 mm, ML = 0.8 mm, V = −5.0 mm from bregma and dura [66] of freely moving rats. All rats were administered the drug after measuring stable baseline. ACh levels were measured using LC-MS-MS. NE and DA were measured using HPLC/ECD. Mean basal dialysate levels of ACh, NE and DA were 0.5 ± 0.03, 0.23 ± 0.01, and 0.13 ± 0.01 pg/ml, respectively (n = 4–8). Data represents the means ± S.E.M., n = 2–5 animals. (B, D, and F) Area under the curve values (calculated from 0 to 120 min) of the data presented in panel A, C, and E. $^{**}p < 0.01$ by Student's t-test vs. vehicle treated rats.

impairment in vigilance and cognitive processes. Fig. 7A shows the effect of JNJ-10181457 (10 mg/kg, i.p.) and methylphenidate (2 mg/kg, i.p.) on acquisition memory in SHR pups during a 7-trial repeated acquisition passive avoidance task. SHR pups showed a significant improvement in acquisition memory. When no cue (i.e., shock) is present, treated animal's performed similarly to vehicle-treated animal (Fig. 7B) demonstrating that the effect of JNJ-10181457 in this model is specific to memory of the shock.

In order to gather more insight into the mechanism of action of an H_3 antagonist, we examined cFos activation patterns in mouse brain 2 h after acute treatment with JNJ-10181457 (10 mg/kg i.p.) (Fig. 8). cFos is an immediate early gene product which is transiently expressed after strong neuronal activation. The cFos activation pattern induced by JNJ-10181457 was compared to modafinil (300 mg/kg i.p.) and amphetamine (2.5 mg/kg i.p.). JNJ-10181457 activated neuronal cells in restricted mouse brain regions (hypothalamus and cortex) in contrast to a widespread activation after modafinil or amphetamine treatment (striatum, cingulated/motor cortex, and septum) (Fig. 8). Other H_3 antagonists (thioperamide and ciproxifan) have been reported to cause c-Fos labeling in the cat tuberomammillary histamine cell bodies [52,53]. The result from the present cFos study with modafinil is quite different from the findings in a previous study [54]. However, different doses and species might account for these differences. The dose used in this study (300 mg/kg i.p.) was significantly higher than the one used by Scammell et al. in rats (75 mg/kg i.p) [54] or by Lin et al. in cats (5 mg/kg p.o.) [55]. At high doses modafinil (300 mg/kg) increases locomotor activity [56]. It is possible that the cFos labeling observed in this study is related to the sustained wake state induced by modafinil rather than the drug itself as suggested in the literature [57]. However, even at this dosage modafinil induced a mild increase in locomotor activity relative to the large effect produced by metamphetamine (0.5–1 mg/kg) [56].

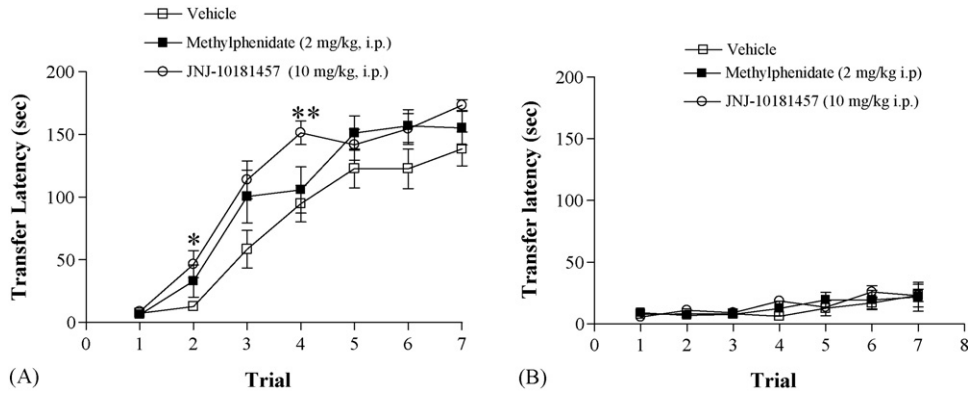

Fig. 7 – (A) Effect of JNJ-180181457 (10 mg/kg i.p.) on acquisition memory for SHR pups tested in a seven-trial repeated acquisition inhibitory passive avoidance task. Methylphenidate (2 mg/kg i.p.) was included for comparison. The model is a variant of the five-trial passive avoidance model described by Fox and colleagues [63]. Results show the mean ± S.E.M. transfer latency on trials 1–7 (n = 14–16 per group). $^{*}p < 0.05$ and $^{**}p < 0.01$; ANOVAs were performed across individual trials followed by a two-side Dunnet's test. (B) Effect of JNJ-180181457 (10 mg/kg i.p.) on acquisition memory when no shock is delivered during a seven-trial repeated acquisition inhibitory passive avoidance task. Results show the mean ± S.E.M. transfer latency on trials 1–7 (n = 10 per group).

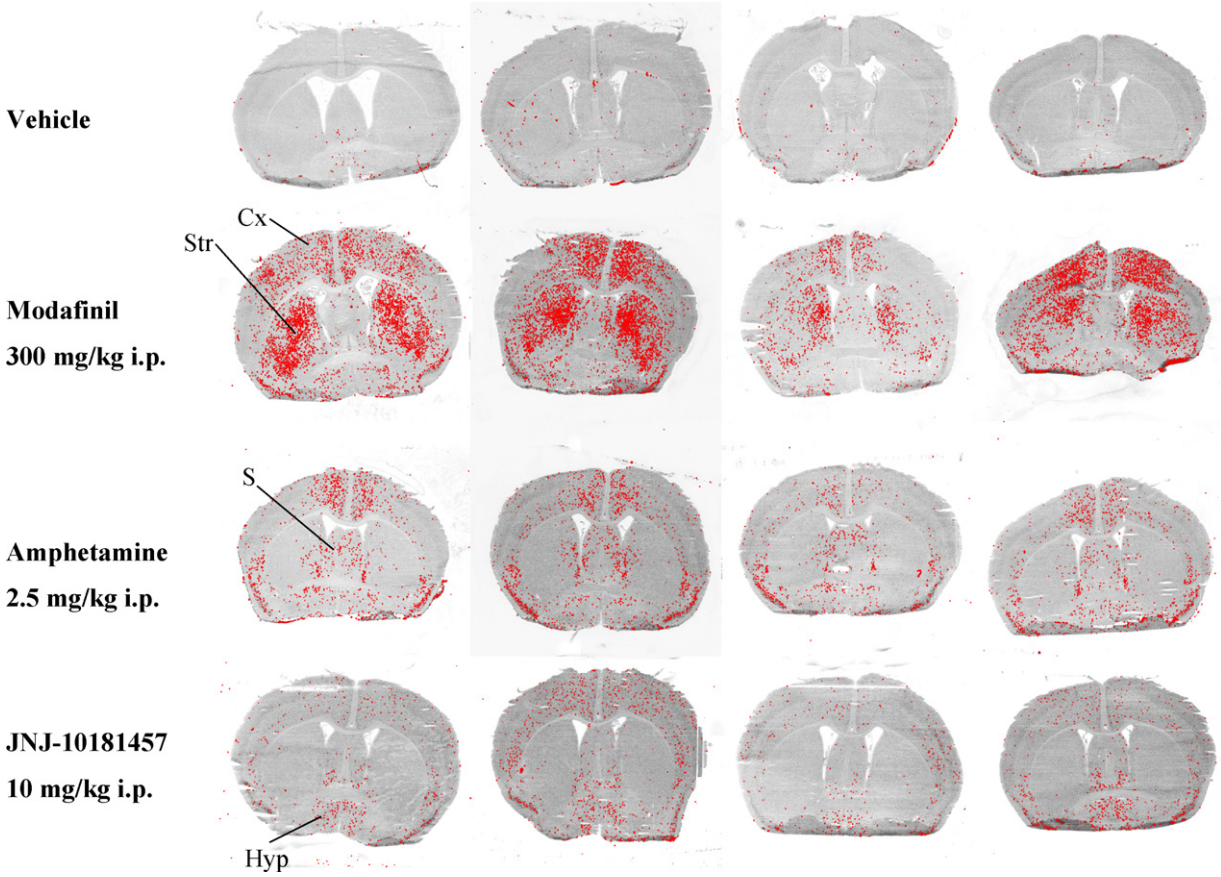

Fig. 8 – Representative images of cFos immunoreactivity in the striatum of adult C57BL/6 mice 2 h after acute treatment with modafinil (300 mg/kg, i.p.), amphetamine (2.5 mg/kg, i.p.) or JNJ-10181457 (10 mg/kg, i.p.). For each drug treatment, four different animals are shown. Cryosections were post-fixed in paraformaldehyde and stained for cFos using a goat polyclonal antibody (Santa Cruz Biotechnology) at 1:300 dilution. A secondary Cy5-labeled antibody (Jackson Immuno Research Lab, West Grove) was used for detection at a 1:500 dilution. Slides were scanned in a microarray scanner (Agilent, Foster City) at 5 mm resolution. Images were thresholded using Photoshop 6.0 (Adobe Systems, San Jose) and pseudocolored red to aid visualization. Cx, cortex; Hyp, hypothalamus; Str, striatum; S, septum.

6. Conclusions

Following the cloning of the histamine H_3 receptor cDNA, our group and others [11–13,19] have synthesized and preclinically tested numerous potent H_3 ligands. JNJ-6379490, JNJ-5207852 and JNJ-10181457 represent small molecule non-imidazoles that are potent and selective H_3 antagonists. These ''drug-like'' molecules have been profiled in various *in vivo* models (see Table 2 for a summary of the results) and several therapeutic opportunities have emerged from these preclinical data. The EEG, behavioral, neurochemistry and cFos activation profiles of these H_3 antagonists suggest a unique mechanism of action that contrasts with classical psychostimultants.

6.1. *H_3 receptor antagonists for the treatment of excessive day time sleepiness and narcolepsy*

Treatments that impair histamine-mediated neurotransmission, such as blockade of histamine synthesis or blockade of H_1 receptors all increase cortical slow waves and enhance sleep. Long-term abolition of histamine synthesis in a knockout mouse model deteriorates both sleep and waking quality, thus causing somnolence and behavioral deficits [36]. Mice that lack brain histamine are unable to remain awake when high vigilance is required. It was recently demonstrated by Huang et al. that an H_3 antagonist, ciproxifan did not increase wakefulness at all in H_1 knockout mice [58]. *In vivo* microdialysis revealed that ciproxifan was still able to increase histamine release in these H_1 knockout mice [58]. These results indicate that H_1 is involved in the regulation of behavioral state transitions from NREM sleep to wakefulness and that the arousal effect of the H_3 antagonist completely depends on the activation of histaminergic systems through H_1 receptor. Amphetamine-like stimulants increase locomotor activity and induce dependence whereas in rat JNJ-5207852 and JNJ-10181457 provide an increase in wakefulness without locomotor effect. These H_3 antagonists did not produce any alteration of the EEG power spectral activity in contrast to amphetamine and therefore preserve the qualitative aspect of vigilance. In addition, H_3 antagonists do not induce sensitization and do not cross-sensitize to stimulant effects of amphetamine and cocaine [59]. Therefore, H_3 antagonists should be devoid of addictive liability. The currently used 'waking' drugs (e.g. methylphenidate, and modafinil) have less clearly defined mechanisms of action. Modafinil does not increased locomotor activity in rats at wake-promoting doses [56]. Our cFos study clearly demonstrated that modafinil activated neuronal cells throughout the whole striatum and in contrast to this finding; JNJ-10181457 had a more restricted activation pattern. The more discrete cFos activation by JNJ-10181457 in hypothalamus suggests activation of histaminergic pathways. In addition, our microdialysis study in freely moving rats shows that JNJ-10181457 does not stimulate DA release in cortex. Because of this unique mechanism of action, H_3 antagonists are promising clinical candidates for the treatment of excessive day time sleepiness associated with different pathological conditions. In addition, the data showing that both JNJ-5207852 and JNJ-10181457 significantly reduced cataplexy in narcoleptic Dobermans suggest that H_3 antagonists may be a unique therapeutic choice to manage both cataplexy attacks and excessive day time sleepiness associated with narcolepsy. It is also noteworthy that decreased brain histamine content has been reported in narcoleptic dogs and decreased CSF histamine levels have been observed in narcoleptics [60].

Table 2 – Summary table, *in vivo* efficacy and potency of JNJ-6379490, JNJ-5207852 and JNJ-10181457 across *in vivo* models

Test model	JNJ-6379490	JNJ-5207852	JNJ-10181457
Ex *vivo* receptor occupancy (rats)	ED_{50} = 0.2 mg/kg i.p.	ED_{50} = 0.1 mg/kg, s.c. [34]	ED_{50} = 3 mg/kg, s.c.
Dipsogenia (rats)	ND	10 mg/kg i.p. (full blockade)	10 mg/kg i.p. (full blockade)
Free moving microdialysis ACh (rats)	ND	ND	↑ ACh 10 mg/kg, s.c.
Free moving microdialysis NE (rats)	ND	ND	↑ NE 10 mg/kg, s.c.
Free moving microdialysis DA (rats)	ND	ND	No change 10 mg/kg, s.c.
Free moving microdialysis 5-HT (rats)	ND	ND	No change 10 mg/kg, s.c.
Free moving EEG, wakefulness state (rats)	↑ Wake	↑ Wake [34]	↑ Wake [46]
Locomotor activity (rats)	ND	No change [34]	No change
Narcoleptic Doberman	ND	↓ Cataplexy	↓ Cataplexy
Passive avoidance (SHR pups)	ND	ND	↑ Acquisition
Social memory (rats)	↑ Short term memory	ND	ND
Water maze (PTZ-kindled mice)	ND	No effect [45]	ND
Social discrimination (PTZ-kindled mice)	ND	Ameliorate learning and memory deficits [45]	ND
Acoustic fear conditioning (PTZ-kindled mice)	ND	Ameliorate learning and memory deficits [45]	ND
Passive avoidance (PTZ-kindled mice)	ND	Ameliorate learning and memory deficits [45]	ND
Food intake (rats)	ND	No effect	No effect
Food intake (WT mice)	ND	No effect [34]	ND
Food intake (ob/ob mice)	ND	No effect [34]	NO
Food intake (dogs)	ND	No effect 10 mg/kg p.o.	No effect 10 mg/kg p.o.

ND: not determined, ↑: increase, ↓: decrease.

6.2. H_3 receptor antagonists for the treatment of cognitive disorders

It is difficult to separate the effect of H_3 receptor antagonists on waking from their effect on cognition. Promoting wakefulness also improves vigilance and cognitive responses. However, there is good evidence that H_3 receptors influence learning and memory by modulating the release of ACh. Decreased ACh mediated neurotransmission is believed to underlie cognitive deficits. Interestingly, H_3 receptor knockout mice are insensitive to the amnesic effect of scopolamine in a passive avoidance paradigm [42]. Microdialysis studies in freely moving rats demonstrated that JNJ-10181457 and other H_3 antagonists [14,61] increased extracellular ACh levels in frontal cortex (Fig. 6B and C). Several different H_3 antagonists have been shown to improve cognitive function in a variety of preclinical models (Table 2 and [14]). In contrast, imetit, an H_3 agonist, reduced cortical ACh release in freely moving rats and impaired rat performance in object recognition and passive avoidance tests [62]. H_3 antagonists may improve cognition either via promoting wakefulness or by improvement of cognitive processes via ACh or NA systems which are also regulated by H_3 receptors.

Various H_3 antagonists (Table 2 and [63]) have been shown to improve performance of relatively inattentive and impulsive SHR pups. This model fulfills several behavioral characteristics of ADHD [64]. The clinically used stimulant methylphenidate also improves performance in this model. In contrast to methylphenidate, H_3 antagonists are not associated with locomotor sensitization and are not associated with increased plasma level of ACTH or liabilities associated with dopamine increase [65].

Acknowledgment

The assistance of Dr. Kevin Sharp, Kenway Hoey, the vivarium staff and the bioanalytical group at J&JPRD, La Jolla is gratefully acknowledged.

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