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Acute wake-promoting actions of JNJ-5207852, a novel, diamine-based H_3 antagonist

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1 1-[4-(3-piperidin-1-yl-propoxy)-benzyl]-piperidine (JNJ-5207852) is a novel, non-imidazole histamine H_3 receptor antagonist, with high affinity at the rat ($pK_i = 8.9$) and human ($pK_i = 9.24$) H_3 receptor. JNJ-5207852 is selective for the H_3 receptor, with negligible binding to other receptors, transporters and ion channels at 1 μM.

2 JNJ-5207852 readily penetrates the brain tissue after subcutaneous (s.c.) administration, as determined by *ex vivo* autoradiography (ED_{50} of 0.13 mg kg^{-1} in mice). *In vitro* autoradiography with ^{3}H-JNJ-5207852 in mouse brain slices shows a binding pattern identical to that of ^{3}H-R-α-methylhistamine, with high specific binding in the cortex, striatum and hypothalamus. No specific binding of ^{3}H-JNJ-5207852 was observed in brains of H_3 receptor knockout mice.

3 In mice and rats, JNJ-5207852 (1–10 mg kg^{-1} s.c.) increases time spent awake and decreases REM sleep and slow-wave sleep, but fails to have an effect on wakefulness or sleep in H_3 receptor knockout mice. No rebound hypersomnolence, as measured by slow-wave delta power, is observed. The wake-promoting effects of this H_3 receptor antagonist are not associated with hypermotility.

4 A 4-week daily treatment of mice with JNJ-5207852 (10 mg kg^{-1} i.p.) did not lead to a change in body weight, possibly due to the compound being a neutral antagonist at the H_3 receptor.

5 JNJ-5207852 is extensively absorbed after oral administration and reaches high brain levels.

6 The data indicate that JNJ-5207852 is a novel, potent and selective H_3 antagonist with good *in vitro* and *in vivo* efficacy, and confirm the wake-promoting effects of H_3 receptor antagonists.
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Keywords: Histamine H_3 receptor; sleep; REM sleep; arousal; food intake

Abbreviations: ADHD, attention deficit/hyperactivity disorder; EEG, electroencephalogram; EMG, electromyogram; H_3 receptor, histamine H_3 receptor; i.c.v., intracerebroventricular; REM, rapid eye movement; SWS, slow-wave sleep

Introduction

The histamine receptor family currently consists of four members, all of which are G-protein-coupled receptors mediating important physiological functions. The H_1 receptor mediates the typical allergic symptoms induced by histamine release from mast cells (vasodilation, extravasation, pruritus). Histamine H_1 receptor antagonists represent the mainstay of the treatment of seasonal rhinitis and other allergies. The H_2 receptor is expressed in the immune system, brain and on the parietal cells of the gastric epithelium, where it is involved in the regulation of gastric acid secretion. H_2 receptor antagonists are used to treat gastric and duodenal ulcers and gastro-esophageal reflux disease. Although there is evidence for the presence of functional H_3 receptors on peripheral nerve terminals, for example, in the heart (Silver *et al*., 2002), the highest density of H_3 receptors is found in the central nervous system. Autoradiographic studies show a strong presence of the H_3 receptor in the striatum, substantia nigra and cortex (Pillot *et al*., 2002). The recently identified H_4 receptor is mainly found in the bone marrow and on eosinophils, and plays a role in chemotaxis (Hofstra *et al*., 2003).

At the molecular level, the H_3 receptor is a G_i-coupled pre-synaptic auto- and hetero-receptor whose activation leads to a decreased release of neurotransmitter. It may also be constitutively active in certain systems (Morisset *et al*., 2000). The H_3 receptor has been shown to play a role in the regulation of the release of histamine, glutamate, norepinephrine and acetylcholine, among others (reviewed in Brown *et al*., 2001). H_3 receptor antagonists increase the release of these neurotransmitters and are thus expected to offer therapeutic benefits in diseases characterized by disturbances of neurotransmission, such as depression, Alzheimer's disease and schizophrenia (Stark *et al*., 1996). Although H_3 receptor antagonists are reported to be in clinical trials for attention-deficit disorder (Tedford *et al*., 2000), their therapeutic potential remains to be confirmed.

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As a neurotransmitter, the best-defined physiological role of histamine in the brain is the regulation of arousal and sleep–waking states. Histaminergic neurons display a higher firing rate during waking than during sleep (reviewed in Brown *et al*., 2001) and injection of histamine into the ventricles or brain causes an increase in waking and a decrease in slow-wave sleep (SWS; Lin *et al*., 1996). Depletion of neuronal histamine by injection of α-fluoromethylhistamine, an inhibitor of histidine decarboxylase, the enzyme responsible for the synthesis of histamine, leads to a reduction in the time spent awake (Monti *et al*., 1988). Similarly, impaired histamine synthesis in histidine decarboxylase knockout mice leads to reductions in wake time (Parmentier *et al*., 2002). The wake-promoting effect of neuronal histamine is mediated mainly *via* the H_1 receptor. Brain-penetrating H_1 antagonists are sedative in humans and laboratory animals, while H_2 antagonists do not consistently affect the sleep–wake cycle (Monti *et al*., 1986). Since activation of the H_3 receptor leads to inhibition of the histaminergic neurons and decreased histamine synthesis (Arrang *et al*., 1983; 1987), it is expected that H_3 agonists should increase sleep, while H_3 antagonists should increase waking. In a recent study, the firing rates of specific 'wake-on' neurons in the tuberomammillary region of the posterior hypothalamus were attenuated by H_3 receptor agonists and enhanced by an antagonist (Vanni-Mercier *et al*., 2003). Consistent with this hypothesis, H_3 agonists have been demonstrated to increase the behavioral and electroencephalogram (EEG) indices of sedation (Monti *et al*., 1996; McLeod *et al*., 1998). Conversely, the H_3 antagonists (carboperamide, thioperamide) increase waking and decrease SWS and REM sleep in rats (Monti *et al*., 1991; 1996) and cats (Lin *et al*., 1990). Ciproxifan, an H_3 receptor antagonist, increased wakefulness at the expense of SWS and REM in wild-type mice, but failed to have an effect in histidine decarboxylase knockout mice (Parmentier *et al*., 2002). In a previous study, we have shown that the provigilant effect of thioperamide is abolished in $H_3^{-/-}$ mice, thus confirming the involvement of the H_3 receptor in the wake-promoting actions of this H_3 antagonist (Toyota *et al*., 2002).

The development of novel, non-imidazole ligands is an area of active research. Several ligand families (Walczynski *et al*., 1999; Linney *et al*., 2000; Apelt *et al*., 2002; Faghih *et al*., 2002; Shah *et al*., 2002) have been described. We set out to identify a novel H_3 antagonist that had suitable properties to evaluate the therapeutic potential of this target in humans. A result of this work was 1-[4-(3-piperidin-1-yl-propoxy)-benzyl]-piperidine (JNJ-5207852). The synthesis of JNJ-5207852 and structure–activity relationships of its analogs have been described in an earlier work (Apodaca *et al*., 2003). The present study demonstrates that JNJ-5207852 is a potent and selective H_3 antagonist with excellent drug-like properties and manifest wake-promoting effects in rodents.

Methods

H_3 receptor binding

Binding of compounds to the cloned human and rat H_3 receptor, stably expressed in SK-N-MC cells, was performed as described earlier (Lovenberg *et al*., 2000). IC_{50} values were determined by a single-site curve-fitting program (GraphPad, San Diego, CA, U.S.A.) and converted to K_i values based on a *N*-[^{3}H]-α-methylhistamine K_d of 800 pM and a ligand concentration of 800 pM (Cheng & Prusoff, 1973).

For the experiments to determine inverse agonism/neutral antagonism of the compounds, K_i determinations were performed in the presence or absence of 25 μM 5′-guanylyl-imidodiphosphate (GppNHp) and 100 mM NaCl (Martin *et al*., 2002).

^{3}H-JNJ-5207852 (20–30 Ci mmol^{-1}) was prepared through a contract with Sibtech (Newington, CT, U.S.A.). Saturation-binding experiments were performed at concentrations of radioactive ligand between 0.5 and 65 nM. Nonspecific binding was determined in the presence of 10 μM histamine.

Cyclic AMP accumulation

Sublines of SK-N-MC cells that expressed a reporter construct and either the human or rat H_3 receptor were created. The reporter gene (β-galactosidase) is under the control of multiple cyclic AMP responsive elements. In 96-well plates, agonists were added directly to the cell media, followed 5 min later by an addition of forskolin (5 μM final concentration). When appropriate, antagonists were added 10 min prior to agonist addition. After a 6-h incubation at 37°C, the media was aspirated and the cells washed with 200 μl of phosphate-buffered saline, followed by a second aspiration. Cells were lysed with 25 μl 0.1 × assay buffer (10 mM Na-phosphate, pH 8, 0.2 mM $MgSO_4$, 0.01 mM $MnCl_2$) and incubated at room temperature for 10 min. Cells were then incubated for 10 min with 100 μl of 1 × assay buffer containing 0.5% Triton and 40 mM β-mercaptoethanol. Color was developed using 25 μl of 1 mg ml^{-1} substrate solution (chlorophenol red β-D galactopyranoside; Roche Molecular Biochemicals, Indianapolis, IN, U.S.A.). Color was quantitated on a microplate reader at absorbance 570 nM. The pA_2 values were calculated by Schild regression analysis of the pEC_{50} values.

Effect of JNJ-5207852 on sleep and waking in rats

All animal work reported in this paper was performed in accordance with the Declaration of Helsinki.

Animals and surgery Male Sprague–Dawley rats (Charles River, Wilmington, MA, U.S.A.) weighing 280–350 g were used in all studies. Stereotaxic surgery was performed using halothane anaesthesia administered through a nose cone, with the incisor bar set at 11.5 mm below ear bar zero, and body temperature was maintained at approximately 36°C. Animals were housed in pairs prior to and following surgery. The post-surgical recovery time was 5–7 days. All procedures conformed to the University, U.S. Department of Agriculture and National Institutes of Health guidelines for the care and use of laboratory animals. Electroencephalography and electromyography (EEG and EMG) electrodes were implanted as described below. EEG/EMG electrodes were cemented in position with acrylic cement (Plastics One, Roanoke, VA, U.S.A.). Animals were maintained on a 11 : 13 h cycle, with lights on at 06:00 h.

EEG and EMG recording and analysis EEG and EMG were recorded as described previously (Berridge & Foote,

1996). Briefly, a bipolar EEG electrode was implanted into the frontal cortex ($A+3.0$; $L\pm1.5$), two EMG electrodes were implanted into the neck muscle, and a ground screw electrode was placed over the cerebellum. The free ends of the EEG, EMG and ground electrodes were inserted into a five-pin plastic connector that was cemented in place, using acrylic cement (Plastics One). EEG and EMG signals were amplified, filtered (0.3–50.0 Hz bandpass), and continuously recorded on a polygraph and on a VCR recording tape using a four-channel headstage FET amplifier connected to standard EEG amplifiers.

On the day prior to testing, pairs of animals were weighed and transferred to testing chambers where they were housed individually (see Berridge & Foote (1996) for a description of the testing chambers.) The animals had free access to food and water.

On the day of testing, animals were connected to an EEG/EMG recording FET headstage. Following the return to a resting state, testing was initiated. Testing was conducted between 10:00 and 15:00 h. Following 60 min of collection of baseline data, animals were injected subcutaneously (s.c.) with vehicle, 1.0 or 10.0 mg kg^{-1} JNJ-5207852. Recording of behavior and EEG/EMG data was continued for the subsequent 90 min. Each animal was tested with both doses of JNJ-5207852. Testing sessions were separated by 3–4 days. All compounds were dissolved in artificial extracellular fluid (147 mM NaCl, 1.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, 2.5 mM KCl, 5.0 mM NaH_2P0_4, pH 7.4).

EEG and EMG were manually scored for the following behavioral state categories: (1) SWS (high-voltage EEG, low-voltage EMG); (2) REM sleep (low-voltage EEG combined with EMG activity of approximately 50% lower amplitude than that observed in SWS, with occasional short-duration, large-amplitude deflections due to muscle twitches); (3) quiet waking (low-voltage EEG with EMG activity of an average amplitude twice that observed in SWS); active waking (low-voltage EEG, sustained high-voltage EMG of approximately twice that observed in quiet waking, with occasional movement deflections). To be scored as a distinct epoch, the appropriate EEG and EMG activity patterns needed to persist for a minimum of 15 s. The time spent in each state was scored and totaled for the five 30-min epochs of the observation period. These included the two segments immediately prior to injection (PRE1: 0–30 min; PRE2: 30–60 min) and the three segments that followed the injections (POST1 = 60–90 min; POST2 = 90–120 min; POST3 = 120–150 min). All measurements were conducted by observers blind to experimental conditions.

Statistical analysis EEG/EMG effects were analyzed using a one-way or two-way ANOVA with time as a repeated-measures variable (five levels corresponding to five one-half-hour epochs: the first two epochs corresponding to the pre-injection portion and the last three epochs corresponding to the post-injection portion of the experiment) and, when appropriate, treatment as a between-subjects variable. When ANOVA indicated a statistically significant effect of time, treatment or a significant treatment × time interaction ($P<0.05$), *post-hoc* analyses were conducted using Tukey's HSD test (between-subject analyses) or means-comparison contrasts (within-subject analyses).

Effect of JNJ-5207852 on sleep-waking and body temperature in mice lacking the H_3 receptor

Electrode and transducer implantation At 3 months of age, $H_3^{+/+}$ ($n=6$) and $H_3^{-/-}$ ($n=6$) male (24–34 g) mice were surgically implanted with chronic electrodes for EEG/EMG recording as described previously (Toyota *et al*., 2002). In addition, transducers (PDT-4000 E-Mitter, Mini-Mitter) were inserted in the peritoneal cavity for biotelemetric recording of body temperature. Following surgery, mice were individually housed and given 2 weeks to recover from the procedure.

Challenge with JNJ-5207852 Mice were connected to a cable/rotating swivel system for EEG/EMG recording and allowed 1 week of adaptation to a sleep-recording chamber. The recording environment was temperature (23–24°C) and light (12L : 12D) controlled, with food and water available *ad libitum*. Each mouse was injected 1 day with saline (4 ml kg^{-1} body weight) and the next day with 10 mg kg^{-1} of JNJ-5207852 (dissolved in saline), followed by 24-h of EEG/EMG recording. Injections were made s.c. and performed at the onset of the circadian light phase (08:00 h). EEG/EMG signals were fed into amplifiers (Grass Model 12, Astro-Med Grass Instrument Division, West Warwick, RI, U.S.A.), filtered (0.3–50 Hz bandpass), and then digitized and stored on an on-line computer data acquisition program (Multisleep 5.01, Actimetrics, Evanston, IL, U.S.A.).

Recordings of body temperature EEG/EMG and body temperature data were collected simultaneously. The biotelemetry transducers (PDT-4000 E-Mitter, Mini-Mitter) were pre-calibrated to produce radiofrequency signals indicating body temperature (accurate to 0.1°C). Data were collected using a software package developed in our laboratory (Multisleep 5.01, Actimetrics, Evanston, IL, U.S.A.).

Data analysis Polygraphic recordings were visually scored by classifying each 10 s epoch as either wake (W), SWS or REM sleep, according to standard criteria (Toyota *et al*., 2002). Sleep–wake parameters were averaged into 2-h time intervals for each 24-h recording. The amount of each vigilance state was expressed as a percentage of recording time. For all epochs of SWS, the EEG power in the delta frequency range (1–4 Hz) was calculated using fast Fourier transform analysis, yielding a measurement of slow-wave delta power. Sleep consolidation was determined by measuring the number of stage shifts and sleep/wake bouts, as well as calculating the average duration of sleep–wake bouts. Stage shifts represented the number of transitions between 10-s epoch scores of wake, slow-wave and REM. A bout of wake or sleep was defined as at least 20 s (two consecutive 10-s epochs) of the respective stage until being interrupted by 20 consecutive seconds of another stage.

Data were analyzed individually for each genotype using a 2×12 repeated-measures ANOVA for treatment (vehicle vs compound) and time (2-h intervals over 24-h) effects. Interactions were clarified using *post hoc* (Tukey HSD) testing. Significance levels were set at $P<0.05$ for all analyses.

In vitro *autoradiography*

Tissue slices for autoradiography were prepared as described by Bonaventure *et al*. (2002). Male C57BL/6 $H_3^{-/-}$ mice weighing 20–25 g were used. The concentration of *N*-[^{3}H]-α-methylhistamine was 2.4 nM, whereas that of ^{3}H-JNJ-5207852 was 3 nM. The slides were then washed three times for 20 s with agitation in ice-cold 0.5 × PBS. After dipping in water, the slides were dried and put on Fujifilm BAS TR2025 for 5 days prior to developing. Nonspecific binding was determined in the presence of 100 μM histamine.

Ex vivo *autoradiography for determination of receptor occupancy*

Male Wistar rats (200 g) were treated by s.c. administration of vehicle or JNJ-5207852 at four dosages ranging from 0.16 to 2.5 mg kg^{-1} body weight (dosages: 0.16, 0.63, 2.5; three animals per dose). Thioperamide was administered at doses from 0.16 to 10 mg kg^{-1} (0.16, 0.63, 2.5, 10). The animals were decapitated 1 h after compound administration. Brains were immediately removed from the skull and rapidly frozen in dry-ice-cooled 2-methylbutane (−40°C). Sections (20 μm thick) were cut using a Leica CM 3050 cryostat-microtome (van Hopplynus, Belgium), and thaw-mounted on microscope slides (SuperFrost Plus Slides, LaboNord, France). The sections were then kept at −20°C until use.

Occupancy of H_3 receptors was measured in the striatum of each individual rat. After thawing, the sections were dried under a stream of cold air and then incubated at RT for 10 min in 50 mM Na/K phosphate buffer (pH 7.4) containing 2 nM [^{3}H]-R-α-methylhistamine. Nonspecific binding was measured on adjacent sections in the presence of 1 μM clobenpropit. After the incubation, the slides were washed (4 × 20 s) in ice-cold buffer, followed by a quick rinse in ice-cold water. The sections were then dried under a stream of cold air.

Quantitative autoradiography analysis was performed after 1 h acquisition with the β-imager (Biospace, Paris) according to our standard protocol (Langlois *et al*., 2001). The ED_{50} values (dose of compound producing 50% of H_3 receptor occupancy) were calculated by nonlinear regression analysis, using the GraphPad Prism program (San Diego, CA, U.S.A.).

Effect of JNJ-5207852 on body weight in control and ob/ob *mice*

Male mice (C57BL/6 and *ob*/*ob*) were obtained from Jackson Labs and individually housed with a 12L : 12D photoperiod. They had unlimited access to food (a standard diet; LABDIET #5008) and water. The experiment was started when the mice were 5 weeks of age and continued for 4 weeks. Each group comprised 8–10 mice. The *ob*/*ob* mice received daily intraperitoneal (i.p.) injections of vehicle (saline) or 3–10 mg kg^{-1} JNJ-5207852; the mice in the 10 mg kg^{-1} JNJ-5207852 group received a single loading dose of 30 mg kg^{-1} JNJ-5207852 on the first day of the experiment. The C57BL/6 mice received either saline or 10 mg kg^{-1} JNJ-5207852. Dosing was started at 09:00 h every day. Body weights were measured using a Sartorius BL1500 scale daily just prior to dosing.

Effect of JNJ-5207852 on locomotor activity in rats

Experimentally naïve, male, Sprague–Dawley rats (Charles River) weighing 282–334 g were used. The animals were individually housed with free access to food and water. The animal colony was maintained at 22 ± 2°C during a 12-h light/12-h dark illumination cycle with lights on from 06:00 to 18:00 h. Behavioral testing occurred during the light phase between 08:30 and 14:30 h.

Locomotor activity (LMA) was measured with the Motor-Monitor System (Hamilton Kinder software © 2000) by placing an animal's home cage (made of clear plastic) inside a metal cage rack (46 × 24 cm) that contained two photocell arrays. The first photocell array contained infrared-emitting diodes located 6.5 cm above the cage floor every 5 cm along the *X* and *Y* axes. These infrared-emitting diodes detected basic movements, fine movements and XY ambulations by measuring the number of beam breaks made by an animal during a given test session.

Locomotor-stimulating effects were reflected by an increase in basic movements (defined as $X+Y$ movements plus fine movements). Stereotypy (e.g., intense gnawing, grooming, sniffing) was reflected by an increase in fine movements, and a corresponding decrease in *XY* ambulations. Photodetectors were connected to an IBM computer, and data were automatically recorded for the entire test session. Each photocell beam interruption constituted one activity count and total counts per 10-min period were tabulated for a 360 min test session.

Prior to starting the experiment, animals were handled and given a 1-week acclimation period to the animal colony. At the time of testing, animals were placed into activity chambers for a 6 h test session. The test session consisted of a 2 h habituation period, followed by a 4 h observation period. To assure that there were no pre-existing group differences in activity levels prior to the initiation of treatment, LMA was monitored and recorded during a 2 h habituation period. Following the 2 h habituation period, testing was briefly interrupted and animals were s.c. injected with either saline (1 ml kg^{-1}; $n=6$), JNJ-5207852 (3, 10, 30 mg kg^{-1}; $n=6$–7 animals/group) or D-amphetamine (0.75 mg kg^{-1}; $n=6$). Testing was immediately resumed following the compound injection and LMA was continuously monitored during the remainder of the test session.

Pharmacokinetics of JNJ-5207852

A total of 16 male and female Sprague–Dawley rats (four rats/gender/formulation) exhibiting good general health were selected for this study and were assigned to two dose groups. The intravenous (i.v.) formulation was prepared as a solution in 10% solutol/5% dextrose at a nominal concentration of 5.0 mg ml^{-1}. The oral formulation was prepared as a suspension in 0.5% methocel at a nominal concentration of 15 mg ml^{-1}. On day 1 and following an overnight fast, animals were weighed (body weight range: 258–307 g) and each formulation was administered to a group of four male and four female rats. The i.v. formulation was administered by jugular venipuncture at a dose volume of 2 ml kg^{-1}. The oral formulation was administered by gavage at a dose volume of 2 ml kg^{-1}. Following dose administration, blood samples (0.25–0.40 ml) were collected from each animal by jugular

venipuncture under isoflurane anesthesia. Blood samples were collected (using lithium heparin as the anticoagulant) at predose and again at 0.08 (i.p. only), 0.25 (oral only), 0.33 (i.p. only), 0.5 (oral only), 1, 2, 4, 8, and 24 h post-dose. Blood samples were placed on ice, pending centrifugation. Following centrifugation, plasma was harvested and stored at approximately $-20^{\circ}C$, pending analysis of JNJ-5207852 using an LC assay with a lower limit of quantification of $5\,ng\,ml^{-1}$. In addition to the above, brains were collected from all animals (snap frozen in liquid nitrogen or in methanol/dry ice mixture) following collection of the last blood sample. Animals were killed by exsanguination and brains were stored at $-20^{\circ}C$ pending analysis for JNJ-5207852. The oral bioavailability was calculated relative to the mean AUC value calculated for the i.v.-treated animals.

Compounds

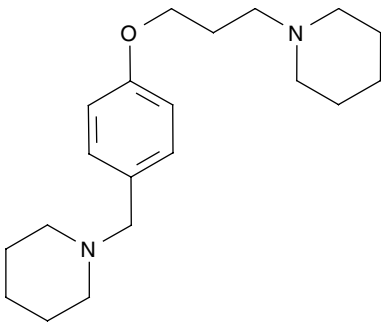
JNJ-5207852 and D-amphetamine (Sigma Chemical, St Louis, MO, U.S.A.) were freshly prepared and dissolved in sterile physiological saline. All compounds were administered s.c. or i.p. and all drug dosages refer to the compound salts. For the *in vivo* pharmacology studies, JNJ-5207852 was used as a hydrochloride salt, except for the locomotor studies, where the fumarate salt was used. For the pharmacokinetics studies, the fumarate salt was also used and a salt correction factor was applied. Requests for JNJ-5207852 should be addressed to Dr T.W. Lovenberg.

Results

In vitro *characterization of JNJ-5207852*

The structure of JNJ-5207852 (1-[4-(3-piperidin-1-yl-propoxy)-benzyl]-piperidine) is shown in Figure 1. In radioligand-binding assays, JNJ-5207852 shows high affinity for both the human and rat H_3 receptor with pK_i values of 9.24 ± 0.21 and 8.90 ± 0.17, respectively (average $\pm$ s.d. of at least two triplicates). The corresponding values for the reference antagonist, thioperamide, were 7.40 ± 0.33 and 8.40 ± 0.20, respectively. Thus, JNJ-5207852 had three- and 100-fold higher affinities for the rat and human receptor compared to the reference compound. The functional cell-based assay for antagonist potency measured the ability of the compound to cause a rightward shift in the concentration–response curves of histamine-mediated inhibition of forskolin-induced cAMP accumulation. The results show good concordance with the pK_i values obtained in binding experiments, with pA_2 values of 8.94 and 9.84 at the rat H_3 and human H_3 receptor. Slopes of Schild regression analysis were not significantly different from unity.

Constitutive activity has been described for the H_3 receptor (Wieland *et al*., 2001), opening up the possibility that some compounds might function as inverse agonists. However, in our functional assays, we were not able to detect consistent and pronounced constitutive activity of the H_3 receptor, which made it difficult to evaluate neutral antagonism or inverse agonism directly. Instead, we utilized a variant of the binding assay, where K_i values were determined in the presence or absence of GppNHp and high concentrations of NaCl. JNJ-5207852 and the reference agonist imetit and the reference inverse agonist thioperamide were evaluated. Imetit, as expected, showed a K_i ratio of <1 (0.47 ± 0.11), while thioperamide had a ratio of >1 (3.39 ± 0.50). JNJ-5207852 had a K_i ratio close to 1 (1.25 ± 0.16), which is indicative of neutral antagonism.



JNJ-5207852

Figure 1 Structure of JNJ-5207852, 1-[4-(3-piperidin-1-yl-propoxy)-benzyl]-piperidine.

JNJ-5207852 did not bind to human H_1, H_2 or H_4 histamine receptors ($pK_i < 5$ for all), despite the significant sequence homology between the H_3 and H_4 receptors. Thioperamide, in contrast, had a pK_i of 7.1 at the human H_4 receptor. JNJ-5207852 was also tested in a commercial (CEREP, Rueil-Malmaison, France) battery of approximately 50 G-protein-coupled receptors, ion channels and other drug targets. At a concentration of $1\,\mu M$, the compound had no inhibitory effect greater than 50% at any of these targets.

In vitro *autoradiography*

JNJ-5207852 was radiolabeled with tritium (^{3}H-JNJ-5207852); so we could assess the direct binding interactions with the H_3 receptor. Scatchard analysis of experiments with ^{3}H-JNJ-5207852 yielded pK_d values of 8.69 and 8.84 at the human receptor and rat receptor, respectively (Figure 2a and not shown). To further confirm the specificity of the binding, *in vitro* autoradiography with ^{3}H-JNJ-5207852 was performed on brain slices from wild-type or H_3 receptor knockout ($H_3^{-/-}$) mice. The high-affinity H_3 receptor agonist ^{3}H-*N*-α-methylhistamine was evaluated as a reference. Neither ^{3}H-*N*-α-methylhistamine (not shown) nor ^{3}H-JNJ-5207852 showed any appreciable binding in the brains of H_3 receptor-deficient mice, whereas the wild-type mice showed a normal patttern of H_3 receptor binding with ^{3}H-JNJ-5207852, with extensive labeling in the cortex, hypothalamus and striatum (Figure 2b).

Ex vivo *autordiography*

In order to evaluate whether JNJ-5207852 would be a suitable tool to study central H_3 receptor function *in vivo*, we performed *ex vivo* autoradiography to measure receptor occupancy after peripheral administration of unlabeled compound. *Ex vivo* brain autoradiography was performed after s.c. administration of $0.04–2.5\,mg\,kg^{-1}$ of JNJ-5207852. The results indicated that JNJ-5207852 penetrates rapidly into the brain and achieves good receptor occupancy, as measured by the competition for ^{3}H-*N*-α-methylhistamine-binding sites. The ED_{50} for receptor occupancy 1 h after dosing was $0.13\,mg\,kg^{-1}$, compared to $2\,mg\,kg^{-1}$ for thioperamide (Figure 3).

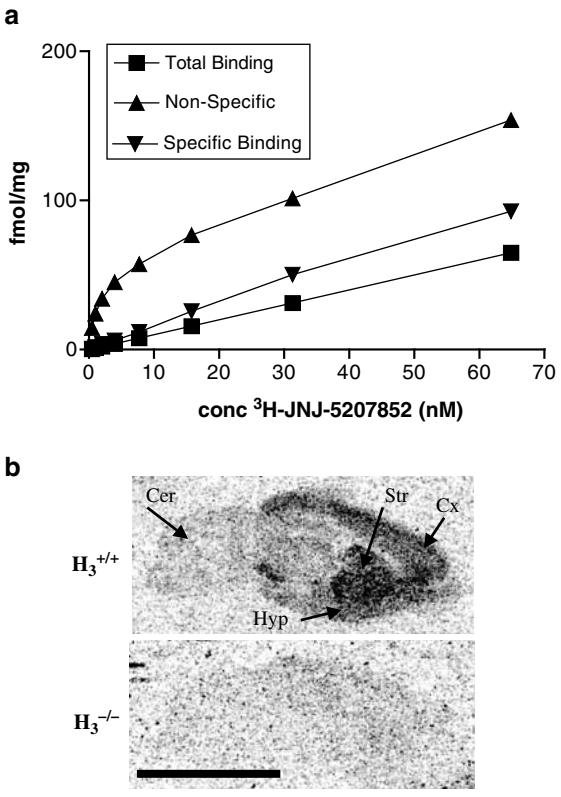


Figure 2 (a) Binding of ^{3}H-JNJ-5207852 to rat H_3 receptor. Membranes from rat brain were incubated with increasing concentrations of ^{3}H-JNJ-5207852. Nonspecific binding was determined in the presence of 10 μM histamine. (b) *In vitro* autoradiography of histamine H_3 receptors in sagittal sections from mouse brain. Sections from wild-type ($H_3^{+/+}$, top) and knockout ($H_3^{-/-}$, bottom) mice were incubated with 3 nM ^{3}H-JNJ-5207852 for 10 min prior to washing. Cortex (Cx), striatum (Str), hypothalamus (Hyp) and cerebellum (Cer) are indicated by arrows. Bar indicates 1 cm.

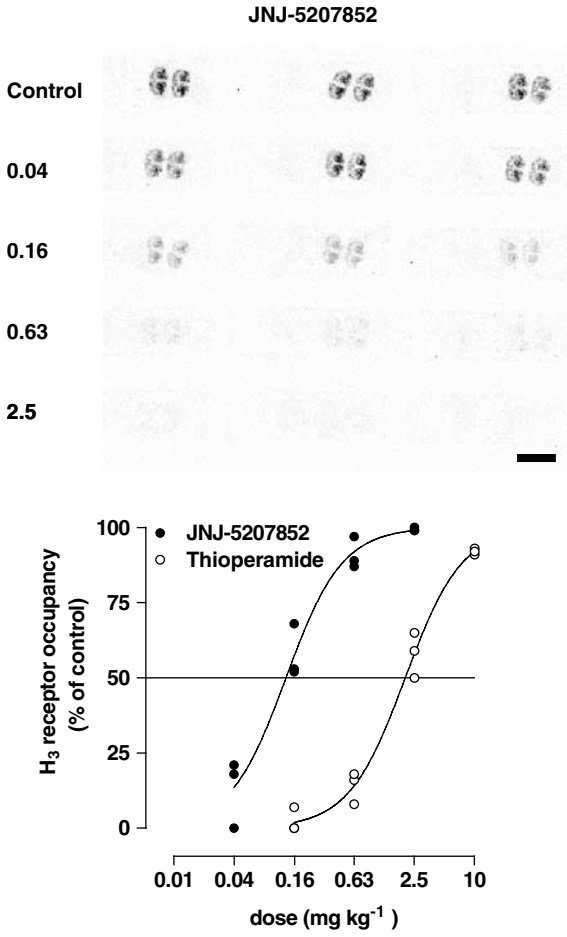


Figure 3 Occupancy of H_3 receptors by JNJ-5207852 and thioperamide in the rat striatum 1 h after s.c. adminstration ($n=3$ animals per dose group). Top, digital image obtained after 1-h acquisition with a β-imager, showing the dose-dependent inhibition of [^{3}H]-R-α-methylhistamine binding by JNJ-5207852 in rat forebrain sections. Bottom, individual values and mean curves illustrating the dose-dependent occupancy of H_3 receptors by JNJ-5207852 ($ED_{50}=0.13$ mg kg^{-1}) and thioperamide ($ED_{50}=2$ mg kg^{-1}). Bar indicates 2 cm.

Provigilant effects of JNJ-5207852 in rats

Based on the receptor occupancy data, doses of 1 and 10 mg kg^{-1} JNJ-5207852 would be expected to elicit pharmacological effects. These two doses of JNJ-5207852 (1 and 10 mg kg^{-1}) or vehicle were administered s.c. to rats. The animals' various sleep stages were monitored for two intervals of 30 min prior to, and three intervals of 30 min after administration of vehicle or test compound. JNJ-5207852 elicited a dose-dependent increase in the total time spent awake (Figure 4a). This wake-promoting effect was most noticeable at the higher dose, where 10 mg kg^{-1} JNJ-5207852 caused an increase in time spent awake that manifested within the first 30 min after dosing and remained present throughout the observation period (1580$\pm$103 vs 640$\pm$122 s in the vehicle-treated animals in the 30–60 min post-dosing interval, $P<0.05$; 1525$\pm$241 vs 577$\pm$184 s in the vehicle-treated animals in the 60–90 min post-dosing observation interval, $P<0.05$). At 1 mg kg^{-1}, a trend for increased waking in treated vs vehicle animals was apparent, which did not reach statistical significance.

The increase in waking was accompanied by significant decreases in the amount of time spent in SWS, as shown in Figure 4b (e.g. for the 60–90 min observation interval: 253$\pm$219 s in rats treated with 10 mg kg^{-1} JNJ-5207852 vs 1026$\pm$189 s in vehicle-treated rats, $P<0.05$). REM sleep was likewise decreased, although the difference did not reach statistical significance (Figure 4e). The increase in waking was apparent both as quiet and active waking (Figure 4c, d).

Sleep–wake effects of JNJ-5207852 in wild-type and H_3 receptor knockout mice

The effects of JNJ-5207852 (10 mg kg^{-1}) on sleep/wake were measured following s.c. injections in $H_3^{+/+}$ ($n=6$) and $H_3^{-/-}$ ($n=6$) mice at light onset. Administration of JNJ-5207852 (10 mg kg^{-1}) to $H_3^{+/+}$ mice significantly increased wakefulness ($P<0.03$, treatment $\times$ time), in hours 1–2 (+143%, $P<0.01$), hours 7–8 (+153%, $P<0.001$), hours 11–12 (+118%, $P<0.05$) and hours 22–24 (+125%, $P<0.01$) compared to the vehicle condition (Figure 5, top panel). Consequently, the amount of 24-h accumulated wakefulness was greater following JNJ-5207852 administration ($P<0.001$). The increase in wakefulness was accompanied by decreased SWS during corresponding time

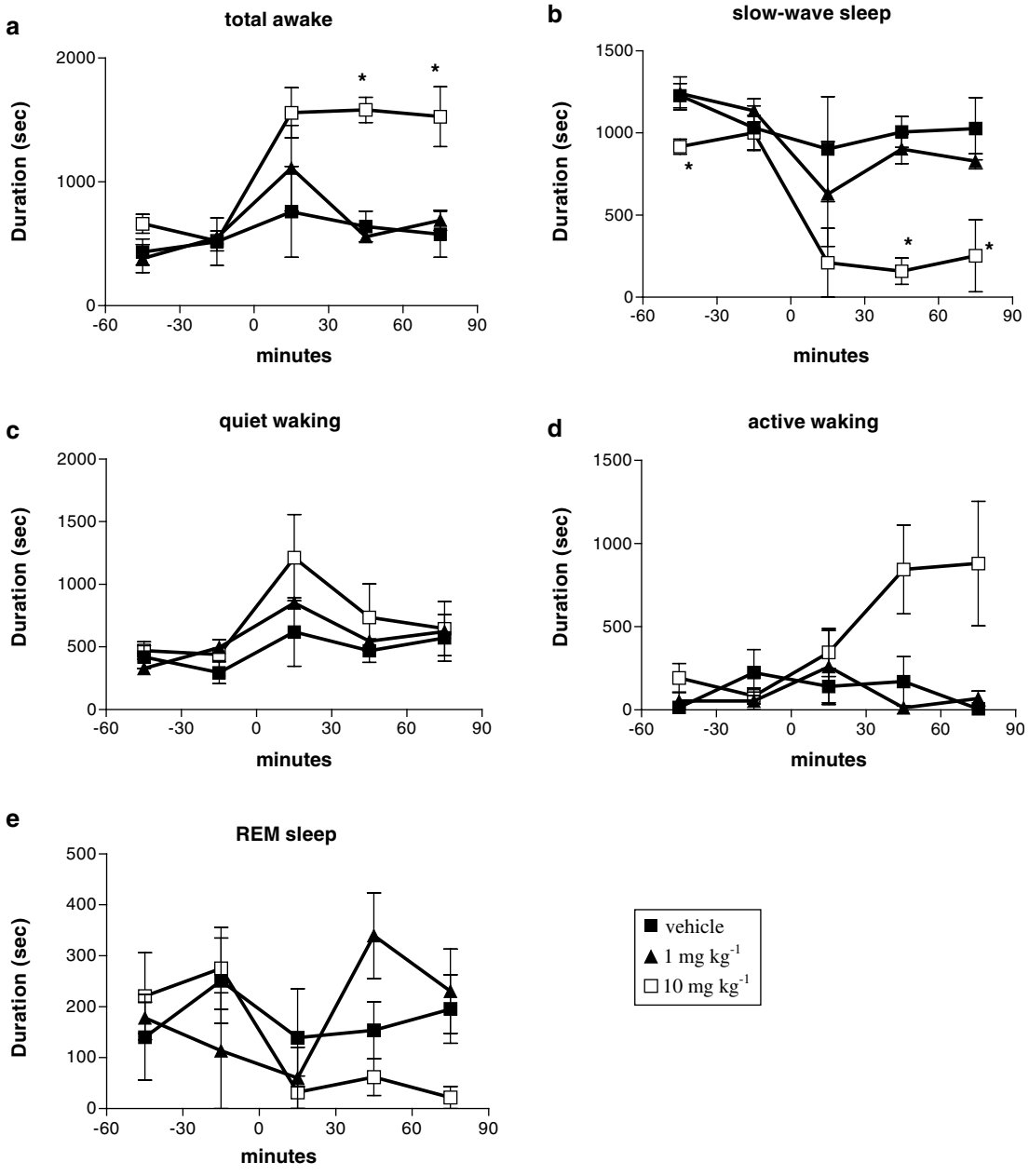


Figure 4 Arousal response to 1 and 10 mg kg^{-1} (s.c.) of JNJ-5207852 in the rat. Black square: vehicle; black triangle: 1 mg kg^{-1} JNJ-5207852; white square: 10 mg kg^{-1} JNJ-15207852. The compound was administered s.c. at $t=0$ min. Sleep–wake behavior was monitored for two epochs of 30 min prior to and three epochs after dosing, and expressed as average time spent in each of the sleep/waking states. (a) total time awake; (b) SWS; (c) quiet waking; (d) active waking; (e) REM sleep. Data are shown as average ± s.e.m. of 3–4 animals. *$P<0.05$, statistically significant from the response in the vehicle-treated animals.

intervals ($P<0.04$, treatment × time), leading to an overall 24-h reduction in SWS ($P<0.001$) (Figure 5, middle panel). REM was nonsignificantly attenuated during the same times that SWS was decreased (data not shown). Similarly, JNJ-5207852 reduced total slow-wave delta power ($P<0.05$), with significant effects during particular 2-h intervals ($P<0.001$, treatment × time), as shown in Figure 5, bottom panel.

The structure of sleep–wake cycles was also affected in $H_3^{+/+}$ mice by JNJ-5207852, as evidenced by time-dependent increases in the number of stage shifts ($P<0.001$), wake bouts ($P<0.001$) and SWS bouts ($P<0.001$) (Figure 6, top panels). The duration (min) of individual bouts was decreased for wakefulness ($P<0.001$, treatment × time) and SWS ($P<0.001$, treatment effect) (Figure 6, bottom panels). In combination, these measures indicate that, in $H_3^{+/+}$ mice, JNJ-5207853 significantly increased wake time and decreased the continuity of the sleep–wake cycle.

In contrast, the $H_3^{-/-}$ mice were insensitive to the wake-promoting effect of 10 mg kg^{-1} JNJ-5207852 and sleep–wake amounts were comparable to the vehicle condition (Figure 5). Sleep latencies for slow-wave and REM sleep were not affected by JNJ-5207852 in either $H_3^{+/+}$ or $H_3^{-/-}$ mice. Mean body temperature during the post-injection period was similar regardless of genotype or injection condition (data not shown).

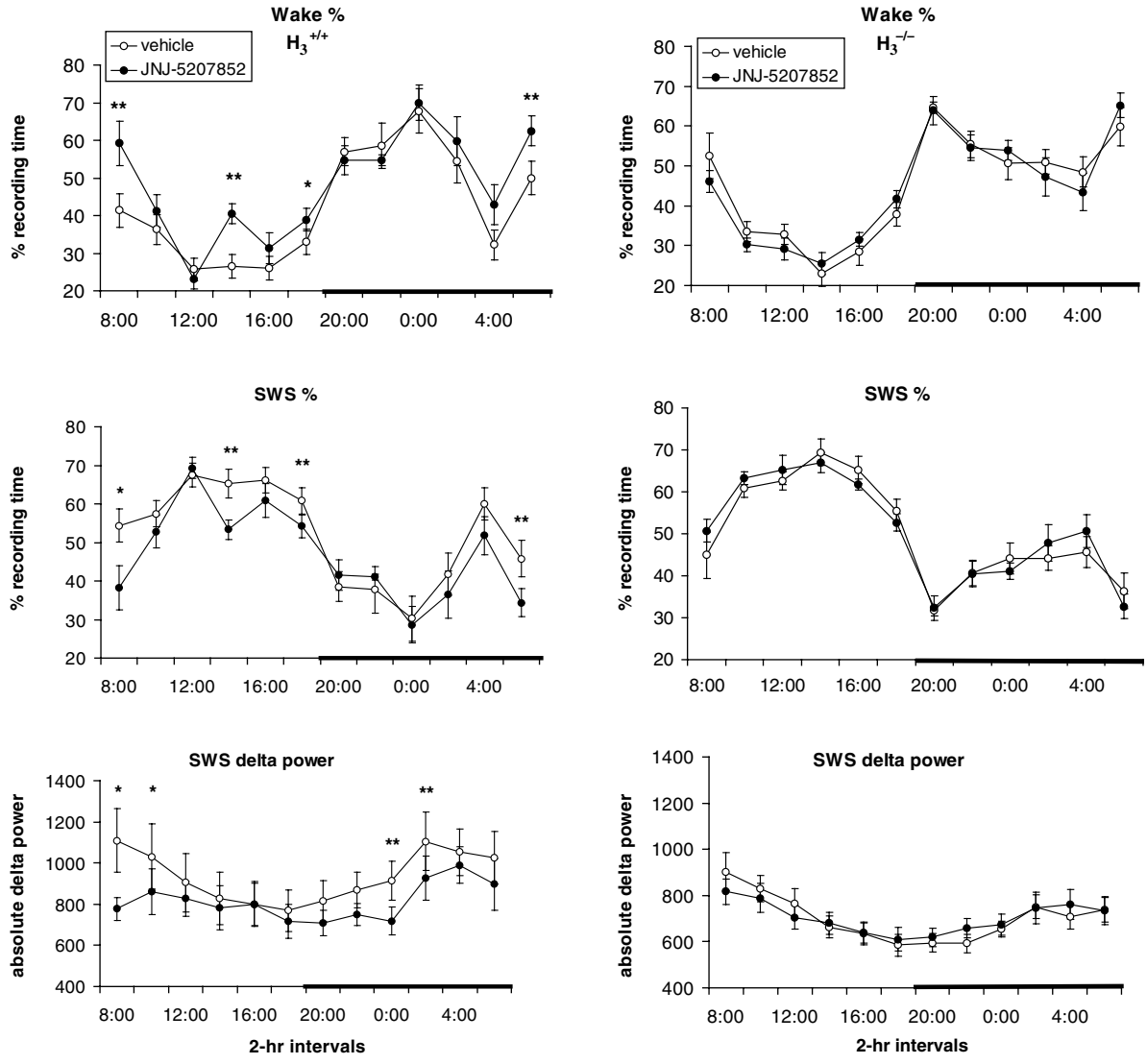


Figure 5 Effect of JNJ-5207852 on amounts of sleep and wakefulness in wild type $H_3^{+/+}$ ($n=6$, left column) and $H_3^{-/-}$ ($n=6$, right column) mice. Time (% of total recording time) spent in wakefulness (top), SWS sleep (middle) and SWS delta power (bottom) in 2-h intervals over 24 h of recording. Each animal received an s.c. injection of vehicle or 10 mg kg^{-1} JNJ-5207852 at light onset. The dark bar on the axis represents the 12-h dark period. In wild-type mice, JNJ-5207852 (10 mg kg^{-1}) significantly increased wakefulness and decreased SWS sleep time and SWS delta power compared to the vehicle condition (*$P<0.05$, **$P<0.01$, ***$P<0.001$), whereas JNJ-5207852 had no effect on wake or sleep times in $H_3^{-/-}$ mice.

Absence of locomotor stimulant effects of JNJ-5207852 in rats

In order to differentiate the wake-promoting effects of JNJ-5207852 from other classes of stimulants, we tested the effect of JNJ-5207852 on locomotor activity. Animals were injected s.c. with 3, 10, or 30 mg kg^{-1} JNJ-5207852 and observed for 4 h. A separate group of animals received 0.75 mg kg^{-1} D-amphetamine s.c. Figure 7 shows the locomotor activity for the first 90 min after administration. This observation period was chosen to mirror the studies of the effect of JNJ-5207852 on the rat EEG, which ran over three consecutive periods of 30 min. As shown in Figure 7, amphetamine induced a clear increase in locomotion within the first 90 min after injection. In contrast, JNJ-5207852, at doses up to 30 mg kg^{-1} (s.c.), was devoid of locomotor stimulant effects. Fine movements and rearing were likewise unchanged in the animals treated with JNJ-5207852, indicating the absence of stereotypy. The results were identical when the same doses were administered i.p. (not shown).

Lack of effect of JNJ-5207852 on body weight in control and ob/ob *mice*

Control and *ob*/*ob* mice received daily i.p. injections of saline, 3 or 10 mg kg^{-1} JNJ-5207852, and their body weights were measured daily for 28 days. The *ob*/*ob* mice displayed the rapid increase in body weight that is typical for this mutant strain with a starting body weight of 40.4±1.6 g to an end of study body weight of 52.2±1.0 g. Treatment of *ob*/*ob* mice with JNJ-5207852 did not affect the increase in body weight over time. The *ob*/*ob* mice treated with 3 mg kg^{-1} JNJ-5207852 started the study with a body weight of 41.7±2.4 g and ended it with a body weight of 51.5±1.1 g. The group receiving 10 mg kg^{-1} i.p.

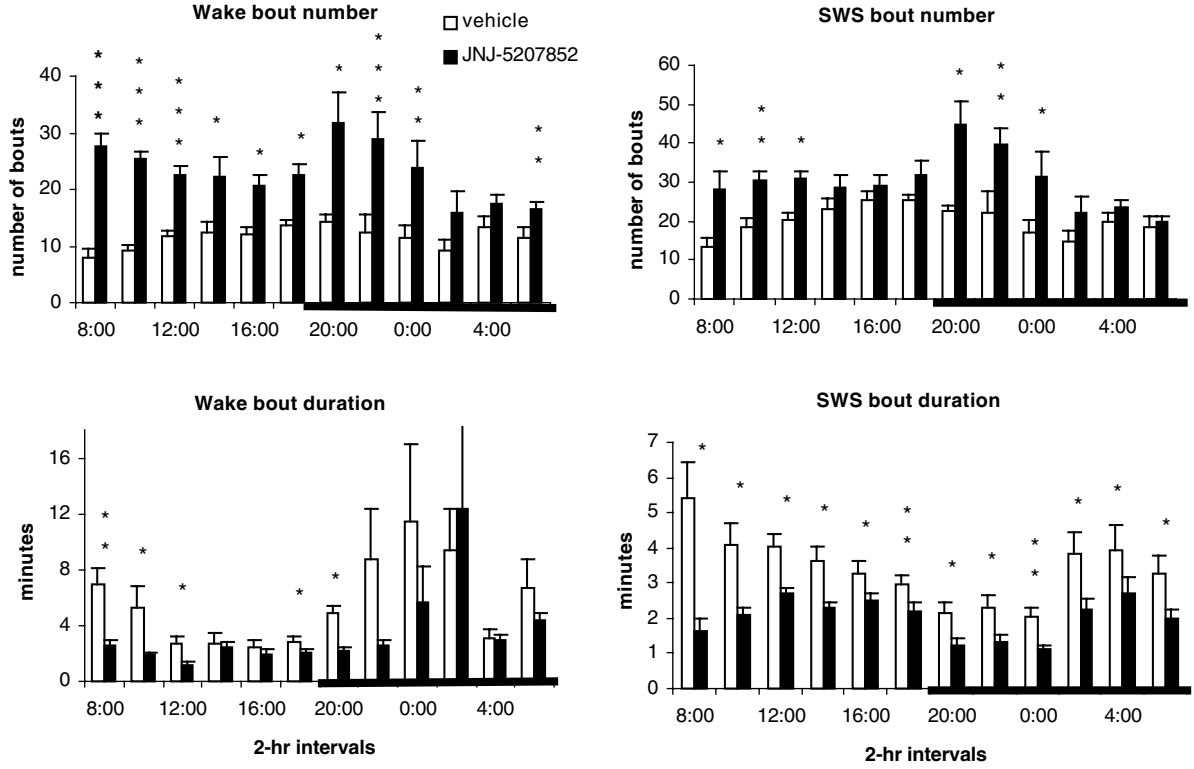


Figure 6 Effect of JNJ-5207852 on the structure of sleep and wakefulness in wild-type $H_3^{+/+}$ ($n=6$) mice. Data are averaged into 2-h intervals across the 24-h recording following injections of vehicle or 10 mg kg^{-1} JNJ-5207852 at light onset. The dark bar on the axis represents the 12-h dark period. These data show that the wake-promoting effect of JNJ-5207852 in wild-type mice was due to an increase in the number of wake and sleep bouts (top panels) and a decrease in the average duration of individual wake and SWS bouts (bottom panels) (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

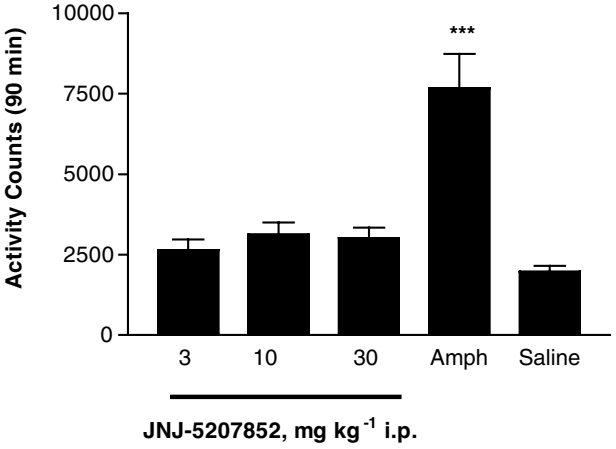


Figure 7 Effect of JNJ-5207852 on locomotor activity in rats. The animals were observed for 90 min after s.c. administration of 3, 10 or 30 mg kg^{-1} JNJ-5207852 or 0.75 mg kg^{-1} D-amphetamine (AMPH). Results are shown as the average ± s.e.m. of 6–7 animals. *** $P<0.001$ from vehicle.

daily went from 40.5 ± 0.7 g at the start of the study to 51.9 ± 1.1 g after 28 days. The control mice treated with vehicle gained weight at a more moderate pace (from 23.0 ± 0.6 to 27.1 ± 0.6 g). The control mice treated with 10 mg kg^{-1} JNJ-5207852 started out at 22.9 ± 0.8 g and ended the study at 28.4 ± 0.9 g. Daily inspection of the JNJ-5207852-treated mice did not reveal any gross changes in general health or behavior.

Oral and i.v. pharmacokinetics of JNJ-5207852

JNJ-5207852 was dosed orally at 30 mg kg^{-1} and i.p. at 10 mg kg^{-1} to four male and female rats. The average plasma concentrations are shown in Figure 8. JNJ-5207852 appeared to be orally absorbed in a moderately fast manner, with T_{max} values of 4.5 and 4.0 h for males and females, respectively. A slow elimination was suggested by average half-life values of 14.6 and 16.8 h for males and females, respectively. Following i.p. administration, half-life values were calculated at 13.2 and 20.1 h for males and females, respectively. The mean volume of distribution was 100070 and 105737 ml kg^{-1} for males and females, respectively, which suggests extensive distribution outside the plasma. The average oral bioavailability of JNJ-5207852 was high in both sexes at 107 and 85% for males and females, respectively.

The concentration of JNJ-5207852 in the brains was also determined at the 24-h time point. The concentrations after oral dosing were 5306 ± 282 ng ml^{-1} in males and 6726 ± 826 ng ml^{-1} in females. After i.p. dosing brain levels of 2096 ± 46 and 2483 ± 54 ng ml^{-1} were reached in males and females, respectively. These data suggest extensive brain penetration and retention.

Discussion

We describe here the *in vitro* and *in vivo* pharmacology of a novel, nonimidazole H_3 antagonist, JNJ-5207852, which

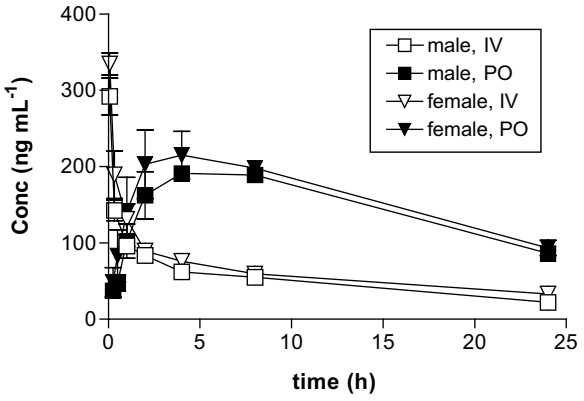


Figure 8 Average plasma concentrations of JNJ-5207852 in male and female rats following administration of a single i.p. bolus ($10\,mg\,kg^{-1}$) or oral ($30\,mg\,kg^{-1}$) dose of JNJ-5207852. Results are represented as average $\pm$ s.e.m. of $n=4$.

demonstrates clear wake-promoting effects that can be specifically attributed to H_3 receptor blockade.

JNJ-5207852 is a selective, high-affinity antagonist at both the human and rat recombinant receptor (pK_i of 9.24 and 8.90, respectively). Although the specificity of JNJ-5207852 was demonstrated with respect to known binding sites, we wished to explore whether JNJ-5207852 demonstrated any unpredictable binding. To accomplish this, we prepared ^{3}H-JNJ-5207852 and conducted a series of binding experiments. First, we demonstrated that ^{3}H-JNJ-5207852 displayed high specific binding to membranes prepared from the brains of rats and normal mice. We found high-affinity, saturable binding with a B_{max} value of $57.1\,fmol\,mg^{-1}$ protein in the rat brain, which compares well to the value reported in the literature for this tissue ($73\,fmol\,mg^{-1}$ protein, Alves-Rodrigues *et al*., 1996). Second, we performed autoradiographic analyses of ^{3}H-JNJ-5207852-binding sites. The binding pattern of ^{3}H-JNJ-5207852 in mouse brains was identical to that of the structurally distinct reference ligand, ^{3}H-*N*-α-methylhistamine, with distinct labeling in the cortex, striatum and hypothalamus and low levels in the cerebellum. This pattern corresponds to the known distribution of the H_3 receptor-binding sites (Pillot *et al*., 2002). Moreover, the binding of ^{3}H-JNJ-5207852 was abolished in the presence of $10\,\mu M$ histamine. Brains of $H_3^{-/-}$ mice have been shown to have no residual H_3 mRNA or ^{3}H-*N*-methylhistamine binding (Toyota *et al*., 2002). In this current study, the binding of ^{3}H-JNJ5207852 was abolished in brains from $H_3^{-/-}$ mice. These studies definitively demonstrate the selectivity and potency of JNJ-5207852 at H_3 receptors in recombinant and native systems. *In vivo* occupation of histamine H_3 receptors was determined by *ex vivo* autoradiography. The results showed good brain H_3 receptor occupancy by both thioperamide and JNJ-5207852 1 h after s.c. administration of the compounds (ED_{50}: $0.13\,mg\,kg^{-1}$; full receptor occupancy at $1\,mg\,kg^{-1}$). This was confirmed by bioanalysis of plasma and brain levels of the compound after s.c., i.p. and oral administration (not shown). Thus, JNJ-5207852 displayed good bioavailability and brain penetration, suitable for *in vivo* experimentation.

The best-known function of histamine in the brain is the regulation of wakefulness. H_3 antagonists increase waking and suppress sleep in rats (Monti *et al*., 1991; 1996), cats (Lin *et al*., 1990; Vanni-Mercier *et al*., 2003) and mice (Parmentier *et al*., 2002; Toyota *et al*., 2002). We used both a rat and a mouse model to evaluate the effect of JNJ-5207852 on sleep–waking behavior. JNJ-5207852 induced a clear state of increased vigilance in rats with concomitant decreases in both REM and SWS. JNJ-5207852 also increased the amount of wakefulness and decreased SWS time in mice. This occurred immediately in the 2 h post-injection, continued into subsequent intervals of the light phase and reappeared during the last few hours of the dark phase. SWS time was reduced during these corresponding intervals, whereas slow-wave delta power was generally reduced during the entire recording period. Slow-wave delta power is the primary measure of EEG sleep intensity; therefore, no indication occurred, during any point of the recording, of a rebound hypersomnolence in response to prior episodes of JNJ-5207852-induced wakefulness. The overall increase in wakefulness by JNJ-5207852 was due to more frequent and shorter duration wake episodes throughout the recording period, rather than due to a prolonged sleep latency or a few long-lasting episodes of wake.

Although full receptor occupancy was seen in the mice after s.c. doses of $1\,mg\,kg^{-1}$ JNJ-5207852, we observed statistically significant effects on waking only at the $10\,mg\,kg^{-1}$ dose. A possible explanation is that the effect at $1\,mg\,kg^{-1}$ JNJ-5207852 failed to show statistically significant differences because, as seen Figure 4, the vehicle-treated animals also responded to the manipulation and s.c. injection by a short episode of waking. Indeed, in follow-up experiments, when the wake-inducing effect of $1\,mg\,kg^{-1}$ JNJ-5207852 30 min after administration was compared to baseline in the same animals, statistically significant differences could be achieved (not shown). Another possibility is that *ex vivo* autoradiography is likely to overestimate receptor occupancy when the brain concentrations of the compound are high. A compound that is present in the brain but not occupying the receptor may be mobilized during the autoradiography procedure and bind to the receptor, leading to incorrectly high estimates of receptor occupancy. Preliminary experiments in our laboratory support this hypothesis. Thus, it is possible that after an s.c. dose of $1\,mg\,kg^{-1}$ JNJ-5207852 the receptor occupancy may actually be lower than 100% and that doses as high as $10\,mg\,kg^{-1}$ may be required to evoke a strong, lasting waking response.

The wake-promoting effect of JNJ-5207852 is consistent with previous rodent and cat studies using H_3 receptor antagonists, such as thioperamide (Lin *et al*., 1990; Toyota *et al*., 2002), carboperamide (Monti *et al*., 1996) and ciproxifan (Parmentier *et al*., 2002). Sleep latency was not consistently altered by JNJ-5207852, thioperamide (Toyota *et al*., 2002), or carboperamide (Monti *et al*., 1996), but was prolonged by ciproxifan (Parmentier *et al*., 2002).

In studies of the wake-promoting actions of JNJ-5207852, the compound was administered in the first half of the light phase. The effects of JNJ-5207852 administration at different times of the circadian cycle were not assessed in these studies. However, it is known that the concentration of extracellular histamine is low during the light (rest) phase and increases in the dark (active) phase in rodents (Mochizuki *et al*., 1992). The reappearance of high wakefulness at the end of the dark phase, 20–24 h following JNJ-5207852 injection, in $H_3^{+/+}$ mice could be the result of an interaction with the circadian pattern of histamine release. For instance, the natural rhythm may have been suppressed by the acute effects, leading to a rebound effect at the start of the next cycle. This may be assessed in

future experiments. Another possibility is the continued presence of JNJ-5207852 in the brain. Our pharmacokinetic and biodistribution experiments indicated that high brain levels of JNJ-5207852 can still be observed 24 h after dosing.

The most likely mechanism for the wake-promoting effects of H_3 antagonists is an increase in histamine release and subsequent activation of H_1 receptor. Administration of histamine and H_1 receptor agonists (Monti *et al*., 1986; Lin *et al*., 1994), increases wakefulness, whereas a decrease in histamine levels (Monti *et al*., 1988; Parmentier *et al*., 2002) and blockade of H_1 receptors (Monti *et al*., 1986) have sedative effects. It should also be noted that H_3 receptors modulate other neurotransmitter systems (reviewed in Brown *et al*., 2001), many of which are involved in the regulation of sleep and could participate in the downstream effects of H_3 receptor antagonists. Future studies may investigate the wake-promoting effects of JNJ-5207852 using antagonists of these various neurotransmitter systems or mice with suitable genetic alterations.

We have previously demonstrated that $H_3^{-/-}$ mice are capable of responding to the stimulant amphetamine but not to the H_3 antagonist thioperamide (Toyota *et al*., 2002). Here we demonstrate that JNJ-5207852 is devoid of wake-promoting effects in $H_3^{-/-}$ mice. This provides strong evidence that the provigilant effect of JNJ-5207852 is indeed due to an interaction with the H_3 receptor and is most likely not accounted for by a nonspecific stimulatory effect. Additionally, the compound was devoid of locomotor-activating and stereotypy-inducing actions at doses well above those that promoted waking in normal mice and rats. This is in contrast to amphetamine-like stimulants, which induce locomotor effects at higher doses. In this respect, JNJ-5207852's behavioral profile resembles that of modafinil, a wake-promoting compound of unknown mechanism, which increases wakefulness without increasing locomotor activity or rebound hypersomnolence (Edgar & Seidel, 1997). Modafinil was tested in the H_3 receptor-binding assay, where it had no affinity for the receptor (not shown).

The H_3 inverse agonist, ciproxifan, has been shown to increase the fast cortical rhythms (25–45 Hz) in cats, which are associated with increased attention, and to improve performance in a number of cognition tests (Ligneau *et al*., 1998). Fox *et al*. (2002) also reported cognition-enhancing effects of GT-2331 and ciproxifan in a repeated acquisition avoidance test in spontaneously hypertensive rats. Since both these compounds are described as inverse agonists, it is conceivable that neutral antagonists and inverse agonists may have distinct therapeutic applications in cognition and memory, as well as for obesity (see also below). We are currently attempting to define the differences in *in vivo* effects of neutral antagonists and inverse agonists in a number of animal models.

The role of the H_3 receptor in the regulation of body weight has been a topic of recent investigations. The H_3 knockout mouse produced by Takahashi *et al*. (2002) displayed a mildly obese phenotype, wheareas our H_3 knockout mice had normal weights, despite a clear lack of the H_3 receptor (Toyota *et al*., 2002). Pharmacological evidence for the role of the H_3 receptor in feeding has been ambiguous. For instance, i.p. administration of thioperamide in rats reduced the food intake (Attoub *et al*., 2001), but direct i.c.v injection of thioperamide had no inhibitory effect on fasting-induced food and water intake, while higher doses actually increased the food intake (Itoh *et al*., 1999). Another H_3 antagonist, A331440, decreased body weight in C57BL/6 J mice with diet-induced obesity after chronic oral administration (Esbenshade *et al*., 2001), as well as in *ob*/*ob* mice (Hancock *et al*., 2003). GT-2394 attentuated food intake and body weight gain in normal Sprague–Dawley and obese male Zucker rats, when administered orally once daily for 15 days (Yates *et al*., 2003).

Histamine has been proposed as a downstream target of leptin (Yoshimatsu *et al*., 1999). Therefore, it was of interest to test the effect of an H_3 antagonist on body weight in leptin-deficient *ob*/*ob* mice as well as in normal mice. Chronic dosing with JNJ-5207852 did not have any effect on the evolution of body weight in *ob*/*ob* mice or wild-type mice. We believe that this lack of appetite suppressant effect may be due to the fact that JNJ-5207852 behaves as a neutral antagonist. Recent data indicate that neutral antagonists and inverse agonists at the H_3 receptor may have differing effects on food intake, with only the inverse agonists being capable of decreasing food intake and normalizing metabolic abnormalities (Hancock, 2003; Yates *et al*., 2003, Hancock *et al*., 2004).

We addressed the possibility that JNJ-5207852 might be an inverse agonist. Due to the difficulty in obtaining a consistent degree of constitutive activity in our cell-based system, we were not able to answer this question definitively using a functional signal transduction assay. Instead, we used a method which relies on the differential binding behavior of neutral antagonists vs inverse agonists to coupled and uncoupled receptors (Childers & Snyder, 1980). Inverse agonists have a higher affinity for the uncoupled state of the receptor, agonists have a lower affinity for the uncoupled state, and the affinity of neutral antagonists is unaffected by the coupling state. Thus, the ratio of the K_i value obtained in the absence of GppNHp/NaCl vs in the presence of these uncoupling conditions is expected to be around 1 for a neutral antagonist, <1 for an agonist and >2 for an inverse agonist. Using this method, we determined that JNJ-5207852, with a K_i ratio of 1.25, is most likely to be a neutral antagonist at the H_3 receptor.

Pharmacokinetics experiments revealed that JNJ-5207852 is extensively absorbed after oral administration. It has excellent brain penetration and a relatively long half-life (13–20 h after i.v. administration). One of the most striking characteristics of JNJ-5207852 is its high volume of distribution (more than 100,000 ml kg^{-1}), suggestive of extensive tissue distribution. Thus, JNJ-5207852 may be an excellent tool for probing H_3 receptor function.

In summary, JNJ-5207852 is a potent, selective nonimidazole H_3 antagonist with clear *in vivo* efficacy in rodent arousal models and lack of appetite-suppressant effects. It suppresses SWS and increases waking without inducing rebound hypersomnolence, or increasing locomotor activity. JNJ-5207852 thus represents a new pharmacological tool as a neutral antagonist to explore the role of H_3 receptors in the regulation of sleep and wakefulness and possibly in related systems such as circadian rhythms, as well as opening up new avenues for the treatment of conditions associated with excessive daytime sleepiness, such as sleep-apnea, multiple sclerosis and fibromyalgia.

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