Pharmacological characterization of JNJ-28583867, a histamine H3 receptor antagonist and serotonin reuptake inhibitor

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Pharmacological characterization of JNJ-28583867, a histamine H3 receptor antagonist and serotonin reuptake inhibitor

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Abstract

Wake-promoting agents such as modafinil are used in the clinic as adjuncts to antidepressant therapy in order to alleviate lethargy. The wake-promoting action of histamine H3 receptor antagonists has been evidenced in numerous animal studies. They may therefore be a viable strategy for use as an antidepressant therapy in conjunction with selective serotonin reuptake inhibitors. JNJ-28583867 (2-Methyl-4-(4-methylsulfanyl-phenyl)-7-(3-morpholin-4-yl-propoxy)-1,2,3,4-tetrahydro-isoquinoline) is a selective and potent histamine H3 receptor antagonist (Ki = 10.6 nM) and inhibitor of the serotonin transporter (SERT) (Ki = 3.7 nM), with 30-fold selectivity for SERT over the dopamine and norepinephrine transporters. After subcutaneous administration, JNJ-28583867 occupied both the histamine H3 receptor and the SERT in rat brain at low doses (<1 mg/kg). JNJ-28583867 blocked imetit-induced drinking (3–10 mg/kg i.p.), confirming in vivo functional activity at the histamine H3 receptor and also significantly increased cortical extracellular levels of serotonin at doses of 0.3 mg/kg (s.c.) and higher. Smaller increases in cortical extracellular levels of norepinephrine and dopamine were also observed. JNJ-28583867 (3–30 mg/kg p.o.) showed antidepressant-like activity in the mouse tail suspension test. JNJ-28583867 (1–3 mg/kg s.c.) caused a dose-dependent increase in the time spent awake mirrored by a decrease in NREM. Concomitantly, JNJ-28583867 produced a potent suppression of REM sleep from the dose of 1 mg/kg onwards. JNJ-28583867 has good oral bioavailability in the rat (32%), a half-life of 6.9 h and a Cmax of 260 ng/ml after 10 mg/kg p.o. In summary, JNJ-28583867 is a combined histamine H3 receptor antagonist-SERT inhibitor with in vivo efficacy in biochemical and behavioral models of depression and wakefulness.

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Keywords: Histamine; Serotonin; Depression; Wakefulness; Microdialysis

1. Introduction

Depression is the most prevalent psychiatric disease, affecting 340 million people worldwide (Jane-Llopis et al., 2003). For the last 20 years, selective serotonin reuptake inhibitors have been the drugs of choice to treat this debilitating disease. These drugs increase serotonin levels in the synaptic cleft by inhibiting its reuptake into the presynaptic neuron through blockade of the serotonin transporter (SERT). Although many patients experience relief after treatment with one of the many marketed selective serotonin reuptake inhibitors, efficacy is noticeable only after weeks of treatment. Many physicians are reported to co-prescribe stimulants with selective serotonin reuptake inhibitors to provide subjective relief during the first weeks of antidepressant therapy (Menza et al., 2000). Many of these stimulants are enhancers of dopamine release and as such produce robust behavioral activation. Such activation has been cautioned to be potentially detrimental due to the risk of allowing patients to act on their suicidal ideation. There are, however, other classes of molecules that have been shown to

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produce wakefulness in animals without releasing dopamine or producing behavioral activation. One such class of molecules is the histamine H3 receptor antagonists. Although wake-promoting actions of histamine H3 receptor antagonists have not yet to be demonstrated in humans, the pharmacology of the histamine H3 receptor makes it an attractive candidate for combination with SERT blockade in order to create a novel antidepressant strategy. The histamine H3 receptor was described in 1983 as a presynaptic, auto-inhibitory receptor on histaminergic neurons (Arrang et al., 1983). Many subsequent studies have shown histamine to be an important neurotransmitter for wakefulness (Lin et al., 1990). Activation of the histamine H3 receptor was shown to decrease the amount of histamine released from the nerve terminals (Arrang et al., 1983). The cloning and characterization of the human histamine H3 receptor has made it possible to further explore its pharmacology (Bonaventure et al., 2007; Letavie et al., 2006; Lovenberg et al., 1999).

We thus asked the question whether we could create a potential antidepressant molecule that would combine the wake-promoting effect of a histamine H3 receptor antagonist with the serotonin reuptake effects of a SERT inhibitor. The synthetic approach and structure–activity relationships associated with this effort have been described elsewhere (Keith et al., 2007; Letavie et al., 2007). This paper describes the pharmacology and pharmacokinetics of one such molecule, JNJ-28583867 (2-Methyl-4-(4-methylsulfanylphenyl)-7-(3-morpholin-4-yl-propoxy)-1,2,3,4-tetrahydro-isooquinoline), a novel and selective histamine H3 receptor antagonist/SERT inhibitor.

2. Materials and methods

2.1. In vitro assays

Histamine H3 receptor binding assays and functional assays: were performed as previously described (Barbier et al., 2004).

Rat brain serotonin transporter: a rat brain without cerebellum (Zivic Laboratories Inc. — Pittsburgh, PA) was homogenized in 52.6 mM Tris pH 8/126.4 mM NaCl/5.26 mM KCl and centrifuged at 207 g for 5 min. The supernatant was removed and recentrifuged at 32,572 g for 30 min. Pellets were rehomogenized in 52.6 mM Tris pH 8/126.4 mM NaCl/5.26 mM KCl. Membranes (protein concentration ~ 20 mg/ml) were incubated with 0.6 nM [3H]-Citalopram plus/minus test compounds (7 concentrations ranging for 10 μM to 0.1 nM) on ice or at room temperature, respectively, for 1 h in 50 mM Tris, 120 mM NaCl, 5 mM KCl (pH 7.4) (total volume 500 μl). Nonspecific binding was determined in the presence of 10 μM GBR-12909 (piperazine, 1-[2-[[4-(fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)], hydrochloride). Data were processed as described above, based on a Kd value of [3H]-WIN-35,428 and a ligand concentration of 10.9 nM.

Human norepinephrine (NET) and serotonin transporter (SERT): homogenized MDCK (Madin-Darby Canine Kidney) and HEK293 (Human Embryonic Kidney) membranes expressing the human NET (protein concentration 11.2 mg/ml) or SERT (protein concentration 15.6 mg/ml), respectively (Perkin-Elmer), were incubated with [3H]-nisoxetine (for NET) or [3H]-citalopram (SERT) plus/minus test compounds (7 concentrations ranging for 10 μM to 0.1 nM) on ice or at room temperature, respectively, for 1 h in 50 mM Tris, 120 mM NaCl, 5 mM KCl (pH 7.4) (total volume 500 μl). Nonspecific binding was determined in the presence of 10 μM desipramine for NET and 10 μM fluoxetine for SERT. The membranes were washed and the radioactivity was counted as above. Calculations for Ki at the NET values were done based on a Kd value for [3H]-nisoxetine and a ligand concentration of 5.4 nM; for the SERT a Kd value for [3H]-citalopram and a ligand concentration of 3.1 nM was used.

2.2. In vivo experiments

All animal work reported in this paper was performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health.

Animals were allowed to acclimate for at least 7 days after arrival in the facility prior to being used in experiments. Animals used for microdialysis and EEG/EMG studies were single housed, all other animals were group housed in accordance with institutional standards, provided food and water ad libitum, and were maintained on a 12-h light–dark cycle (lights on: 6:00 to 18:00).

2.2.1. Ex vivo receptor and transporter binding autoradiography

Male Sprague–Dawley rats (Charles River Laboratories, 300–350 g) were treated by s.c. administration of vehicle (sterile saline) or JNJ-28583867 (6 animals per dose and 4 animals per time point). The animals were euthanized using carbon dioxide and decapitated at different time points after drug administration. Tissue section for ex vivo receptor autoradiography was prepared as previously described (Langlois et al., 2001).

Ex vivo occupancy of histamine H3 receptor binding sites, SERT, DAT or NET binding sites was measured in the striatum (H3, DAT) or frontal cortex (SERT, NET) of each individual rat.
After thawing, the sections were dried under a stream of cold air and then incubated at room temperature for 1 min with the tracer (see Table 1 for detailed assay conditions). The sections were not washed prior to incubation, to avoid dissociation of the drug-receptor complex. Incubation was restricted to 1 min at room temperature to minimize dissociation of the drug from the receptor. Three adjacent brain slices from the same animal have been collected per slide. Two brain slices were used to measure non-specific binding. After the incubation, the slides were washed in ice-cold buffer (Table 1), followed by a quick rinse in ice-cold water. The sections were then dried under a stream of cold air.

Quantitative analysis was performed as previously described (Langlois et al., 2001) using the β-imager (BioSpace, Paris, France). Ex vivo receptor labeling was expressed as the percentage of receptor/transporter labeling in corresponding brain areas of saline-treated animals. The percentage of receptor occupancy was plotted against dosage. Percentage of receptor occupancy was also plotted against time.

2.2.2. Microdialysis in the cortex of freely moving rats

Male Sprague–Dawley rats (Charles River Laboratories) weighing 300–350 g were used. Each rat was given a 0.05 ml s.c. injection of Buprenex 0.06 mg/kg (buprenorphine hydrochloride) 5 min prior to anesthesia. Animals were anesthetized with an isoflurane/air mixture and stereotaxically implanted with a guide cannula (Eicom) in the prefrontal cortex (incisor bar = 3.5 mm, +3.2 mm anterior, 0.8 mm lateral and 1 mm ventral to Bregma) (Paxinos and Watson, 1997). The guide cannula was secured in place with skull screws and dental cement. Animals were allowed at least 3 days to recover from surgery prior to experimentation.

Dialysis experiments were conducted between 8:00 am and 4:00 am the following day, in a controlled environment. The animals remained in their home cage throughout experimentation. Dialysis probes (Eicom, 4 mm active membrane length) were perfused with artificial cerebral spinal fluid (aCSF, 147 mM NaCl, 4 mM KCl, 0.85 mM MgCl2, 2.3 mM CaCl2, pH 7.4) at a flow rate of 1 μl/min and implanted the afternoon prior to sample collection. The probe was connected via FEP tubing to a liquid swivel (QM, Instech) mounted on a counterbalance arm. The following morning 4 h of baseline samples were collected into a 96-well plate (Sarstedt, 96 well multiply PCR) via a four-channel fraction collector (Eicom). Samples were collected every 60 min for 20 h into the 96-well plate maintained at 4 °C containing 15 μl of the antioxidant (0.1 M acetic acid, 1 mM oxalic acid and 3 mM L-cysteine in sterile water). Samples were split into two plates and analyzed immediately following each experiment.

Microdialysates were analyzed by high-performance liquid chromatography with an ESA CouArray Coulometric detector and ESA 5014B Microdialysis cell. Serotonin separation was performed on a Hypersil (150×3.2 mm inner diameter) 5 μm C18 column at room temperature. The mobile phase consisted of 75 mM NaH2PO4, 0.5 mM disodium–EDTA, 350 mg/ml 1-octanesulfonic acid, pH adjusted to 3.1 with H3PO4 and following filtration through a 0.22 μm filter, 1.0% tetrahydroflorine and 9.0% acetonitrile were added. Flow rate was 0.4 ml/min. Of each sample 22.5 μl was injected onto the column using a model 540 Microtiter auto-sampler. Dopamine and norepinephrine separation was performed on a 150×2.0 mm I.D. 5 μm reverse phase column (MD-150, ESA) at room temperature. The mobile phase consisted of 75 mM lithium acetate, 100 μM disodium–EDTA, 350 mg/ml heptane sulfonic acid, 7.5% methanol and the pH was adjusted to 7.4 with acetic acid. Flow rate was 0.25 ml/min.

The concentration for each sample was calculated from the peak area of the chromatographic signal and the slope from the corresponding standard curve. The percent change from baseline values was calculated from the mean basal value of each neurotransmitter for each animal and presented in the figures as mean±S.E.M. The area under the curve (AUC) values were calculated by the summation of the difference between each neurotransmitter post drug administration and the mean percent of basal release value (100%). Statistical analyses were performed on the AUC values by a one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison test. Data was graphed and statistics were calculated using Prism software (GraphPad, San Diego, CA).

Table 1

<table>
<thead>
<tr>
<th>Transporter/receptor</th>
<th>Radioligand</th>
<th>Non-specific binding</th>
<th>Incubation medium</th>
<th>Rinses at 4 °C (min)</th>
<th>Rinse medium</th>
<th>β Imager exposure time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT</td>
<td>[125]I]RTI-55</td>
<td>0.05 nM</td>
<td>GBR-12935</td>
<td>2×10</td>
<td>B</td>
<td>1</td>
</tr>
<tr>
<td>SERT</td>
<td>[3H]Citalopram</td>
<td>1 nM</td>
<td>Fluoxetine</td>
<td>2×10</td>
<td>B</td>
<td>1</td>
</tr>
<tr>
<td>NET</td>
<td>[3H]Nisoxetine</td>
<td>3 nM</td>
<td>Maprotiline</td>
<td>3×5</td>
<td>C</td>
<td>1</td>
</tr>
<tr>
<td>H3</td>
<td>[3H]Methylhistamine</td>
<td>2 nM</td>
<td>Clobenropit</td>
<td>4×0.3</td>
<td>D</td>
<td>1</td>
</tr>
</tbody>
</table>

Incubation/rinse media:
A Tris–HCl buffer (50 mM, pH 7.4), 120 mM NaCl, 0.1 μM Citalopram.
B Tris–HCl buffer (50 mM, pH 7.4), 120 mM NaCl.
C Tris–HCl buffer (50 mM, pH 7.4), 300 mM NaCl, 5 mM KCl.
D Na/K Phosphate Buffer (50 mM, pH 7.4).
2.2.3. Imetit-induced drinking

Experimentally naïve, male, Sprague–Dawley rats (Charles River Laboratories) weighing 225–250 g at the time of arrival were used. Behavioral testing occurred during the light phase between 08.30 and 13.00 h.

An animal’s home cage (made of clear plastic) was placed inside a metal cage rack (46 × 24 cm) and a drinking spout was located on one wall of the animal’s cage. The drinking spout was connected to an automated watering system that delivered water to an animal. A McMillan turbine flow meter, located in the drinking spout, measured the volume of water consumed by an animal upon each lick. The BioPac MP 150 system was used to convert the analog signal of drinking into a digital signal, and these data were then analyzed using the AcqKnowledge software package (version 3.7.3). On the first day of the experiment, water intake was measured during a 60-min baseline session. The next day, animals were injected with JNJ-28583867 (1, 3, 10 mg/kg i.p.) followed 60 min later by an i.p. dose of 1 mg/kg imetit and water intake was measured. JNJ-285867 was formulated in 20% hydroxypropyl-β-cyclodextrin in water and injected i.p. (injection volume 1 ml/kg) for doses of 1, 3 and 10 mg/kg. Results were analyzed by an ANOVA followed by a Newman–Keuls test. Data was graphed and statistics were calculated using Prism software (GraphPad, San Diego, CA).

2.2.4. 5-Hydroxytryptophan potentiation test

Male Balb/c mice (Charles River Laboratories) weighing approximately 20 g were used. At t = 0, the animals were dosed s.c. with 10 mg/kg carbidopa and an oral dose of JNJ-28583867; 20 min later they received a s.c. injection of 40 mg/kg 5-hydroxytryptophan. Animals were observed and scored for the 5 min interval ending at t = 60 min. For the 24 h experiments, the animals were dosed with JNJ-28583867 or vehicle on day 1, and the next day carbidopa and 5-hydroxytryptophan were administered with the same time interval as for the 1 h experiments. The head twitch response was then scored for a period of 5 min 24 h after the dose of JNJ-28583867.

Testing was performed in a custom-built device based on the instrument described by Elliott and colleagues (Elliott et al., 2000). Data was graphed and statistics (unpaired t-test) were calculated using Prism software (GraphPad, San Diego, CA).

2.2.5. Mouse tail suspension test

Male Balb/c mice (Charles River Laboratories) weighing 25–30 g were used. JNJ-28583867 was tested at 1, 3, 10 or 30 mg/kg p.o. A vehicle control (5% dextrose) and a positive control (citalopram, 3 mg/kg i.p.) were also tested. All compounds were formulated in 5% dextrose on each study day. Dilutions were done in the same vehicle to prepare lower concentration dosing solutions. All doses were randomized and administered at 10 ml/kg, p.o. Mice were dosed 1 h prior to testing (30 min prior to testing in the citalopram group). The test was performed according to Steru and colleagues (Steru et al., 1985). The test was 6 min in duration. The time spent struggling was summed for each animal, averaged for the dose group, and then expressed as % of the vehicle control group. Groups were compared using an ANOVA followed by Dunnett’s test. Data was graphed and statistics were calculated using Prism software (GraphPad, San Diego, CA).

2.2.6. EEG/EMG recordings

A miniature telemetry device (Data Sciences TL10M3-F50-EET) was implanted into male Sprague–Dawley rats (n = 5, 400–500 g, Harlan, Inc.) in order to record EEG, EMG, and locomotor activity. Thirty min prior to injection, each implant was activated. The signals from the telemetry implant were transmitted via radio frequency to a receiver under the home cage of each animal. On the day of the experiment, animals were dosed with JNJ-28583867 (0.3, 1, or 3 mg/kg, s.c.) or vehicle (sterile saline) 2 h after light onset. EEG and EMG signals were remotely monitored continuously for 8 h following vehicle or compound administration. All data were sampled at 100 Hz. At the end of the recording period, an analyst who was blinded to the drug treatment used the SleepSign for Animals software package to visually score the polysomnographic traces within 10-s epochs as wake, non-REM, or REM. Elapsed time from injection to the onset of non-REM or REM sleep was measured and expressed as latency (min, mean ± S.E.M.) to each respective stage. In addition, the duration (min) of wake, non-REM, REM sleep, and spontaneous locomotor activity (activity counts) was averaged over the 8-h treatment period (mean ± S.E.M.). Data was graphed and statistics (one-way ANOVA followed by Dunnett’s test) were calculated using Prism software (GraphPad, San Diego, CA).

2.2.7. Pharmacokinetics and bioanalysis

One group of six male Sprague–Dawley Rats (three animals per route, Charles River Laboratories) approximately 300 g body weight was used. JNJ-28583867 was formulated at 1 mg/ml in 0.5% hydroxypropyl methyl cellulose and delivered in a volume of 10 ml/kg for oral dosing, and at 1 mg/ml in dextrose 5% in water for intravenous dosing in a volume of 1 ml/kg. The dosing solution was prepared immediately prior to injection. Animals received a bolus dose of the compound via a 16 gauge intragastric gavage at 10 mg/kg or a bolus intravenous dose via a 24-gauge Terumo® Surflo® catheter in the lateral tail vein. Blood samples (250 µl) were taken from the lateral tail vein into heparinized Natelson blood collection tubes and expelled into 1.5 ml microcentrifuge tubes. The blood samples were centrifuged for 5 min at 18,000 g in a microcentrifuge. Plasma was retained and kept in a −20 °C freezer until analysis by LC-MS/MS. The compound was analyzed on an API-4000 triple quadrupole mass spectrometer using an Agilent 1100 LC. Column: Ace C18, 2 × 50 mm (available from Mac-Mod). “A” buffer: 0.1% formic acid in water; “B” buffer: 0.1% formic acid in acetonitrile. Gradient: 5% B–70% B in 3 min. Compound was detected using MRM (multiple reaction monitoring) with a transition of 413.2 to 128.2.

2.3. Drugs

JNJ-28583867 was synthesized according to the method of Letavic and colleagues (Letavic et al., 2007). Requests for JNJ-28583867 should be addressed to Dr. M. Letavic.
α-methylhistamine (24 Ci/mmol), [3H]-nisoxetine (85 Ci/mmol), [3H]-WIN-35,428 (87 Ci/mmol), [125I]RTI (2200 Ci/mmol), and [3H]-citalopram (84 Ci/mmol) were from Perkin-Elmer (Wellesley, MA). Imetit, citalopram, 5-hydroxytryptophan, clobenpropit, maprotiline, GBR-12935, carbidopa, paroxetine, fluoxetine, and sertraline were purchased from Sigma, Saint Louis, MI. Duloxetine was synthesized at Johnson & Johnson Pharmaceutical Research & Development.

3. Results

3.1. Affinity of JNJ-28583867 at the human and rat histamine H3 receptor and at the rat and human SERT

The in vitro affinities of JNJ-28583867 (Fig. 1) at its two targets are shown in Table 2. The affinity of JNJ-28583867 for the human histamine H3 receptor is 10.6 nM, consistent with the pA2 value obtained in a functional cell-based assay of 8.05. JNJ-28583867 is a potent inhibitor at the human SERT (3.7 nM). For the SERT there was no species selectivity observed; for the histamine H3 receptor JNJ-28583867 was approximately 2-fold less potent at the rat receptor. The compound had approximately 30-fold selectivity for the SERT compared to the NET and DAT (Table 3). JNJ-28583867 was also tested in a commercial (CEREP, www.CEREP.com) panel of 50 targets (G-protein coupled receptors, ion channels and transporters) and had IC50 > 10 μM at all other targets.

3.2. Ex vivo histamine H3 receptor and SERT occupancy of JNJ-28583867 in rat brain

A study to investigate ex vivo H3 receptor and SERT occupancy with JNJ-28583867 (1 mg/kg s.c.) as a function of time was carried out in rat (Fig. 2A). Maximal levels of occupancies at both targets were observed 1 h following administration and lasted for a period of 18 h. At 1 mg/kg s.c., higher levels of occupancies were observed for the histamine H3 receptor compared to the SERT (~100% vs. ~80%, Fig. 2A).

The occupancy profile for the NET was similar to the SERT (~80% for 18 h, data not shown). Lower levels of occupancies of the DAT were measured throughout the entire time-course (~30%, data not shown).

Dose-dependency of H3/SERT occupancy 1 h after s.c. administration of JNJ-28583867 (0.3, 1 or 3 mg/kg) is presented in Fig. 2B. A low s.c. dose of JNJ-28583867 produced a maximal ex vivo occupancy of the histamine H3 receptor at 1 h post-dose, with an ED50 for occupancy being <0.3 mg/kg s.c.

Table 2

<table>
<thead>
<tr>
<th>Ki (nM)</th>
<th>JNJ-28583867</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human H3</td>
<td>10.6±1.8 (5)</td>
</tr>
<tr>
<td>Human SERT</td>
<td>3.7±0.4 (3)</td>
</tr>
<tr>
<td>Rat H3</td>
<td>20.3±1.1 (3)</td>
</tr>
<tr>
<td>Rat SERT</td>
<td>2.2±0.5 (5)</td>
</tr>
<tr>
<td>Human DAT</td>
<td>102.3±16.0 (3)</td>
</tr>
<tr>
<td>Human NET</td>
<td>121.0±56.0 (3)</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Selectivity</th>
<th>JNJ-28583867</th>
<th>Paroxetine</th>
<th>Fluoxetine</th>
<th>Citalopram</th>
<th>Duloxetine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERT/NET</td>
<td>3.7</td>
<td>0.3</td>
<td>1.9</td>
<td>3.0</td>
<td>0.3</td>
</tr>
<tr>
<td>SERT/DAT</td>
<td>212</td>
<td>98</td>
<td>156</td>
<td>2100</td>
<td>47</td>
</tr>
<tr>
<td>NET/DAT</td>
<td>2.2</td>
<td>792</td>
<td>450</td>
<td>9000</td>
<td>328</td>
</tr>
</tbody>
</table>

Results are represented as average±S.E.M. of n=3–5 experiments performed in triplicate.


Fig. 1. Structure of JNJ-28583867.

Fig. 2. Ex vivo histamine H3 receptor/SERT occupancy with JNJ-28583867 in rat striatum (H3) or cortex (SERT). A. Time-dependency after s.c. administration (1 mg/kg). B. Dose-dependency 1 h after s.c. administration (0.3, 1 or 3 mg/kg).

Results are represented as average±S.E.M. of n=4 for the time-course and n=6 for the dose–response.
JNJ-28583867 increased norepinephrine levels in the frontal cortex in a dose-dependent manner despite its relatively low affinity for the NET (Fig. 4). The effect on norepinephrine levels was less pronounced than that on 5-HT levels, with a statistically significant effect \((P<0.05 \text{ vs. vehicle})\) only at doses of 1 mg/kg and higher. At the highest dose tested, 3 mg/kg s.c., the maximal increase was approximately 4-fold of basal levels \((374\pm46\%)\), or about half of the maximal response observed in the 5-HT experiments.

JNJ-28583867 also had an effect on the levels of dopamine in the microdialysate. This effect was dose-dependent, with a 3-fold maximum increase in dopamine levels being observed at the highest \((3 \text{ mg/kg s.c.) dose} \) (Fig. 4B, D).

3.4. Efficacy of JNJ-28583867 in the imetit-induced drinking model

An in vivo functional measure of histamine H3 receptor antagonism of JNJ-28583867 was investigated using the blockade of imetit-induced drinking model (Fox et al., 2002). On the first day of this two-day experiment, baseline drinking was recorded. All groups had comparable levels of water intake prior to treatment with the histamine H3 receptor antagonist or agonists (data not shown). Treatment with 1 mg/kg i.p. imetit caused a significant increase in drinking \((3.9\pm0.8 \text{ ml} \text{ vs. } 1.5\pm0.6 \text{ ml}; P<0.05)\) which was blocked by pretreatment with JNJ-28583867 (Fig. 5). The dose–response curve was steep, with no effect at 1 mg/kg \((3.6\pm0.9 \text{ ml})\) but complete blockade at 3 and 10 mg/kg i.p. \((0.5\pm0.3 \text{ and } 0.7\pm0.6 \text{ ml}, \text{ respectively}; P<0.01)\). In contrast, in a separate experiment, citalopram did not significantly affect imetit-induced drinking \((3.80\pm1.07 \text{ ml after vehicle + imetit vs. } 4.30\pm1.20 \text{ ml after i.p. treatment with } 40 \text{ mg/kg citalopram + imetit})\) (data not shown).

3.5. Efficacy of JNJ-28583867 in the 5-HTP induced head twitch model

The combination of 5-hydroxytryptophan and carbidopa induces a typical head twitch response (Darmani and Reeves, 1996), which can be potenated by inhibitors of SERT by allowing more 5-HT to activate 5-HT2A receptors (Rogoz et al., 1996), which can be potentiated by inhibitors of SERT by allowing more 5-HT to activate 5-HT2A receptors (Rogoz et al., 1996). JNJ-28583867, administered orally at 10 mg/kg, induced a clear increase of the head twitch response (Fig. 6A). Interestingly, this potentiation was observed both at 1 h or 24 h after dosing with JNJ-28583867, indicating a long duration of action, in agreement with the microdialysis data (Fig. 3). The response at 24 h \((353\pm38\%\) was slightly less than that at 1 h \((425\pm71\%), \text{ but both were highly significantly different from vehicle (} P<0.001 \text{ at either time point}). An oral dose–response curve measuring the head twitch response potentiation 1 h after dosing with JNJ-28583867 confirmed the effect of 10 mg/kg JNJ-28583867 \((357\% \text{ of vehicle}, P<0.001)\) and showed that lower doses had no effect in this model. The maximal response obtained at 30 mg/kg was \(397\pm38\%\) of vehicle. Alternatively, a selective histamine H3 receptor antagonist, JNJ-10181457 \((10 \text{ mg/kg i.p.)}, \text{ did not have any effect in this model (} 67\pm27\% \text{ at } 1 \text{ h}, 159\pm70\% \text{ at } 24 \text{ h post-dosing})\) (data not shown).
3.6. Efficacy of JNJ-28583867 in the tail suspension model

JNJ-28583867, dosed orally 1 h prior to testing, dose-dependently increased the struggling time in male Balb/c mice, with statistically significant differences noted at doses of 3 mg/kg and higher ($P<0.05$, Fig. 7). Maximal efficacy (an increase in struggling time of 32% over vehicle controls) was reached at 30 mg/kg p.o. The reference compound citalopram...
(3 mg/kg i.p.), induced an average increase in struggling time of 25%.

3.7. Effect of JNJ-28583867 on sleep/wake states during the light phase

JNJ-28583867 was administered (0.3, 1 and 3 mg/kg, s.c.) to male adult rats chronically implanted with EEG and EMG electrodes for evaluation of vigilance states. The latency to the onset of NREM sleep was significantly delayed at the dose of 3 mg/kg JNJ-28583867 whereas REM sleep latency was significantly prolonged from the dose of 1 mg/kg onwards (Table 4). For 8 h following compound injection, JNJ-28583867 caused a dose-dependent increase in the time spent awake (Fig. 8). The increase in wake was mirrored by a decrease in NREM sleep amount (Fig. 8). Concomitantly, JNJ-28583867 produced a potent reduction of REM sleep at the dose of 1 mg/kg and virtually abolished this sleep stage at the dose of 3 mg/kg (Fig. 8).

Effect of subcutaneous administration of JNJ-28583867 on locomotor activity in the rat during the light phase is shown in Fig. 9. For 8 h following compound injection, locomotor activity was not changed at 0.3 and 1 mg/kg doses. At 3 mg/kg, a significant increase in locomotor activity was observed (Fig. 9).

3.8. Pharmacokinetics and tissue distribution of JNJ-28583867

JNJ-28583867 was administered to rats by the oral (10 mg/kg), intravenous (1 mg/kg) and subcutaneous (10 mg/kg) route and pharmacokinetic parameters were determined (Fig. 10, Table 5). The compound had a moderately slow oral absorption ($T_{max}=3$ h) with good oral bioavailability (32%) and reached a $C_{max}$ of 260 ng/ml after oral dosing. The volume of distribution after intravenous dosing was 7.9 l/kg. The $T_{1/2}$ after oral dosing was 9.4 h and 6.5 h after intravenous dosing. Subcutaneous dosing, which was the preferred route for several of the models described in this paper, also yielded good plasma levels ($C_{max}$: 256 ng/ml) and fast absorption with a similar half-life.

4. Discussion

We set out to identify and characterize molecules combining both SERT inhibitory and histamine H$_3$ receptor antagonist activity in a single chemical entity. Several papers have been published detailing the structure–activity relationships of the medicinal chemistry involved in this effort (Keith et al., 2007;
Table 5

Pharmacokinetic parameters of JNJ-28583867 after oral (10 mg/kg), intravenous (1 mg/kg) and subcutaneous dosing (1 mg/kg) in the rat

<table>
<thead>
<tr>
<th>Dose</th>
<th>Cmax (ng/ml)</th>
<th>Tmax (h)</th>
<th>AUCinf (h ng/ml)</th>
<th>Vd (l/kg)</th>
<th>CI (ml/min kg)</th>
<th>T1/2 (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p o.</td>
<td>260</td>
<td>3</td>
<td>3837</td>
<td>36</td>
<td>43</td>
<td>9.4</td>
</tr>
<tr>
<td>i.v.</td>
<td>1196</td>
<td>8</td>
<td>14</td>
<td>14</td>
<td>25</td>
<td>6.5</td>
</tr>
<tr>
<td>s.c.</td>
<td>256</td>
<td>0.25</td>
<td>817</td>
<td>51</td>
<td>61</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Cmax = maximum plasma concentration; Tmax = time to reach the maximum plasma concentration; AUC = area under the plasma concentration–time curve; Vd = apparent volume of distribution; CI = clearance; T1/2 = apparent elimination half-life.

low doses. The time-course analysis showed that the maximal level of occupancy for both targets was maintained for 18 h. The ex vivo receptor/transporter occupancy study was followed up by experiments designed to confirm in vivo efficacy in models specific for either of the compound’s two biochemical targets. The histamine H3 receptor-specific model we selected is based on the well-established observation that histamine H3 receptor agonists induce a drinking response, which can be blocked by pretreatment with histamine H3 receptor antagonists (Fox et al., 2002). JNJ-28583867 decreased the drinking response induced by imetit, with complete blockade observed at 3 mg/kg i.p. This effect can be contributed solely to blockade of the histamine H3 receptors by JNJ-28583867, as we have shown that the selective serotonin reuptake inhibitor citalopram does not block the imetit-induced drinking (data not shown). These experiments confirmed that at doses of 3–10 mg/kg, in vivo efficacy could be expected, at least as far as the histamine H3 receptor was concerned. We followed up with an experiment aimed at confirming efficacy at the SERT using the 5-HTP induced head twitch test, which is a well-established model of serotoninergic neurotransmission. When injected systemically in the presence of a peripheral inhibitor of aromatic L-amino acid decarboxylase such as carbidopa, 5-hydroxytryptophan (5-HTP), a 5-HT precursor, will be converted to 5-HT in the brain. In mice this combination will elicit a behavioral phenotype characterized by head twitches that is attributed to activation of postsynaptic serotonin 5-HT2A receptors (Darmani and Reeves, 1996). Inhibitors of the SERT potentiate this head twitch response by allowing 5-HT to activate serotonin5-HT2A receptors. An oral dose of JNJ-28583867 at 10 mg/kg induced a highly significant increase in head twitch response, both when given approximately 1 and 24 h prior to the start of the experiment. A steep dose–response curve was observed, with no efficacy at 3 mg/kg, and almost maximal efficacy at 10 mg/kg p.o. In contrast, even a high dose (10 mg/kg i.p.) of a selective histamine H3 receptor antagonist, JNJ-1018457 did not induce any potentiation of the head twitch response, confirming that the potentiating effect of JNJ-28583867 can be attributed to its SERT inhibitory properties. This experiment also indicated that JNJ-28583867 is orally active.

Microdialysis experiments confirmed that JNJ-28583867 was capable of inducing a robust and persistent increase in 5-HT levels over baseline. This effect was visible in the dose range 0.3–3 mg/kg s.c. in a dose-dependent manner. The maximal effect, observed at 3 mg/kg, was around 8-fold of basal levels.

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Fig. 9. Effect of subcutaneous administration of JNJ-28583867 on locomotor activity in the rat during the light phase. Results are represented as average activity counts (±S.E.M. of n=5 animals) during 8 h post-dosing. **P<0.05 compared to vehicle based on one-way ANOVA followed by Dunnett’s test.

Letavic et al., 2007). One compound, JNJ-28583867, was selected for further profiling. In vitro characterization of the compound indicated high affinity for both the human SERT and histamine H3 receptor (3.7 and 10.6 nM, respectively), with no apparent affinity for any of the 50 drug targets included in a CEREP screening panel, with the exception of the NET and DAT, as detailed below. No major species selectivity was observed between humans and rats. The compound had much lower affinity for the NET (121 nM) and DAT (102 nM), leading to a 30-fold selectivity for the SERT over these two neurotransmitter transporters. This degree of selectivity is in the same range as that achieved by many so-called selective serotonin reuptake inhibitors, as shown in Table 3. For instance, in our hands, fluoxetine has an 82-fold selectivity for the SERT over the DAT and sertraline has a 61-fold selectivity for the SERT over the NET. The compound had much higher affinity for both the human SERT and receptor antagonist/SERT inhibitor in vivo, autoradiography was performed ex vivo after s.c. dosing. The ex vivo receptor/transporter occupancy method has limitations such as dissociation from the ex vivo binding site during the incubation, however, when ex vivo receptor/transporter occupancy study was followed up with an experiment aimed at confirming efficacy at the SERT using the 5-HTP induced head twitch test, which is a well-established model of serotoninergic neurotransmission. When injected systemically in the presence of a peripheral inhibitor of aromatic L-amino acid decarboxylase such as carbidopa, 5-hydroxytryptophan (5-HTP), a 5-HT precursor, will be converted to 5-HT in the brain. In mice this combination will elicit a behavioral phenotype characterized by head twitches that is attributed to activation of postsynaptic serotonin 5-HT2A receptors (Langlois et al., 2001; Li et al., 2006). Excellent occupancy at both targets (histamine H3 receptor, SERT) was achieved after 3 mg/kg s.c. in a dose-dependent manner. The maximal level of occupancy for both targets was maintained for 18 h. The ex vivo receptor/transporter occupancy study was followed up by experiments designed to confirm in vivo efficacy in models specific for either of the compound’s two biochemical targets. The histamine H3 receptor-specific model we selected is based on the well-established observation that histamine H3 receptor agonists induce a drinking response, which can be blocked by pretreatment with histamine H3 receptor antagonists (Fox et al., 2002). JNJ-28583867 decreased the drinking response induced by imetit, with complete blockade observed at 3 mg/kg i.p. This effect can be contributed solely to blockade of the histamine H3 receptors by JNJ-28583867, as we have shown that the selective serotonin reuptake inhibitor citalopram does not block the imetit-induced drinking (data not shown). These experiments confirmed that at doses of 3–10 mg/kg, in vivo efficacy could be expected, at least as far as the histamine H3 receptor was concerned. We followed up with an experiment aimed at confirming efficacy at the SERT using the 5-HTP induced head twitch test, which is a well-established model of serotoninergic neurotransmission. When injected systemically in the presence of a peripheral inhibitor of aromatic L-amino acid decarboxylase such as carbidopa, 5-hydroxytryptophan (5-HTP), a 5-HT precursor, will be converted to 5-HT in the brain. In mice this combination will elicit a behavioral phenotype characterized by head twitches that is attributed to activation of postsynaptic serotonin 5-HT2A receptors (Darmani and Reeves, 1996). Inhibitors of the SERT potentiate this head twitch response by allowing 5-HT to activate serotonin5-HT2A receptors. An oral dose of JNJ-28583867 at 10 mg/kg induced a highly significant increase in head twitch response, both when given approximately 1 and 24 h prior to the start of the experiment. A steep dose–response curve was observed, with no efficacy at 3 mg/kg, and almost maximal efficacy at 10 mg/kg p.o. In contrast, even a high dose (10 mg/kg i.p.) of a selective histamine H3 receptor antagonist, JNJ-1018457 did not induce any potentiation of the head twitch response, confirming that the potentiating effect of JNJ-28583867 can be attributed to its SERT inhibitory properties. This experiment also indicated that JNJ-28583867 is orally active.

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The elevated 5-HT levels were maintained throughout the duration of the experiment consistent with the level of SERT occupancy observed in the ex vivo autoradiography study.

As expected based on its low affinity for the DAT and NET (102 and 121 nM, respectively), the effect of JNJ-28583867 on dopamine and norepinephrine levels was much less pronounced than that on 5-HT. For dopamine, a maximal increase of 3-fold was observed at the highest dose of 3 mg/kg. Noteworthy, only low levels of ex vivo DAT occupancy were observed in the ex vivo autoradiography study.

Because JNJ-28583867 was administered at doses higher than 3 mg/kg (up to 30 mg/kg) in several of the animal models described in this paper, it is highly probable that dopamine overflow increased by more than 3-fold under such conditions. It is therefore possible that increased dopamine levels might have contributed to some of the observed effects. The effect of JNJ-2883867 on norepinephrine was somewhat stronger, with levels increasing up to 4-fold. Here too, the increase in neurotransmitter levels tended to plateau and remain elevated for the duration of the experiment in agreement with the ex vivo NET occupancy profile.

The ability to increase levels of norepinephrine is considered a desirable characteristic, as there is clinical evidence that compounds selectively blocking the reuptake of norepinephrine, such as reboxetine, are effective antidepressants (Dubini et al., 1997; Hajos et al., 2004). The fact that selective inhibitors of SERT and NET are both equally effective antidepressant strategies has been attributed to possible interactions between noradrenergic and serotoninergic neurotransmission. Yet it appears that the two systems influence mood by parallel, independent pathways (Eriksson, 2000). Preliminary data indicate that selective serotonin reuptake inhibitors are superior for treating depressed patients with symptoms of anxiety, obsessive–compulsive behavior and aggression, while compounds with a predominantly noradrenergic profile could be more effective in correcting depressed mood, reduced vigilance and reduced social functioning (Eriksson, 2000). Support for this hypothesis has been provided by a study in which a selective norepinephrine uptake inhibitor (maprotiline) was less effective than the selective serotonin reuptake inhibitors paroxetine in reducing premenstrual irritability, while being equally effective in elevating mood (Eriksson, 2000). This renewed interest in the role of NE as a mood-determining neurotransmitter has culminated in the successful marketing of a number of compounds that are described as selective norepinephrine uptake inhibitors such as reboxetine (Hajos et al., 2004), or as dual 5-HT/norepinephrine uptake inhibitors or SNRIs, such as venlafaxine and duloxetine (Stahl et al., 2005). Among the additional claims for this latter type of antidepressant is preliminary evidence indicating they might exhibit a faster onset of action and a higher therapeutic efficacy than the selective serotonin reuptake inhibitors, while having a similar side effect profile (Tran et al., 2003). Thus, we consider that the effects of JNJ-28583867 on norepinephrine levels may contribute to its antidepressant efficacy.

The imetit-induced drinking test, the head twitch potentiation test and microdialysis data provided assurance that JNJ-28583867 did indeed possess both histamine H3 receptor antagonist activity and SERT blockade activity in vivo. These tests, while very relevant from a mechanistic point of view, do not offer face or predictive validity as far as antidepressant efficacy is concerned. Thus we followed up with a number of experiments intended to probe whether these neurochemical effects would translate to in vivo efficacy in animal models of depression and associated symptoms as well.

Among the prominent symptoms of depression are disturbed sleep and the daytime fatigue associated with it. Polysomnographic investigations have shown severe disturbances in the sleep architecture of depressed patients. Among the typical abnormalities observed are: discontinuous sleep, decreased slow-wave sleep, shorter latency to REM sleep and an increased intensity and duration of REM sleep (Riemann et al., 2002). It is hypothesized that suppression of REM sleep is involved in antidepressant efficacy. This is illustrated by the dramatic success of overnight deprivation of (REM) sleep (Riemann et al., 2002). Another non-pharmacological treatment for depression, electroconvulsant therapy, likewise decreases REM sleep. A majority of antidepressant drugs, regardless of their neurochemical mechanism of action, suppress REM sleep with nefazodone, trazodone (in some studies), trimipramine (at low doses) and bupropion being notable exceptions (Sharpley and Cowen, 1995). Antidepressant drugs also affect slow-wave sleep, although in a less clear-cut manner.

Administration of histamine H3 receptor antagonists has been shown to increase wakefulness and to decrease non-REM sleep in several animal species as well as REM sleep with a number of histamine H3 antagonists. For instance, the histamine H3 receptor antagonist carboperamide induces waking in rats (Monti et al., 1996). Another histamine H3 receptor antagonist, thioperamide, decreased both REM and non-REM sleep in rats (Monti et al., 1991) and cats (Lin et al., 1990). It is of interest to note that although histamine H3 receptor antagonists promote wakefulness, they do so without the locomotor stimulant effects associated with amphetamine derivatives (Barbier et al., 2004). They may therefore be considered mild stimulants.

The behavioral profile of histamine H3 receptor antagonists (suppression of sleep with no stimulation of locomotor activity and limited addictive potential) is much like that of modafinil. Therefore, we expect that a combined histamine H3 receptor/ selective serotonin reuptake inhibitor compound would provide symptomatic relief for the fatigue during the first weeks of treatment, before the mood-elevating effect of the selective serotonin reuptake inhibitor can be noticed. EEG experiments were conducted in order to observe the effects of JNJ-28583867 on sleep/wake states. We found that JNJ-28583867 was very effective at suppressing REM sleep, with almost complete suppression at 1 mg/kg s.c. At the same dose wake-promoting effects were also observed. These effects were noticeable almost immediately after dosing and remained throughout the observation period. The EEG profile of JNJ-28583867, which was characterized by increased wakefulness and decreased REM and NREM sleep, was entirely compatible with its neurochemical mechanism of action. JNJ-28583867 combines, in a single molecule, several mechanisms which are expected to affect sleep/waking behavior: histamine H3 receptor antagonism (Monti et al., 1996), increased serotonin

**References:**

- Eriksson, 2000
- Eriksson et al., 1997
- Hajos et al., 2004
- Monti et al., 1991
- Monti et al., 1996
- Lin et al., 1990
- Stahl et al., 2005
- Tran et al., 2003
- Riemann et al., 2002
- Sharpely and Cowen, 1995
(Cape and Jones, 1998), increased dopamine (Isaac and Berridge, 2003) and increased norepinephrine (Jones, 2005).

JNJ-28583867 was also tested in a classical test of antidepressant activity, the mouse tail suspension model (Porsolt, 2000; Steru et al., 1985). As was expected based on the neurochemical profile of JNJ-28583867, an increase in struggling time was observed. The increase was significant at oral doses of 3 mg/kg p.o. and higher, and reached a maximum at 30 mg/kg, where the observed increase in struggling time was slightly higher than that caused by 3 mg/kg i.p. of the reference selective serotonin reuptake inhibitor, citalopram. Additional studies are required to assess the antidepressant-like effect of JNJ-28583867 in other tests such as chronic mild-stress or learned helplessness.

The effects on anhedonia assessed by sucrose solution intake or effects on chronic stress-induced alteration in circadian rhythm, for example, will have to be considered for detailed comparison between JNJ-28583867 and antidepressants currently available.

Finally, some pharmacokinetic characterization of JNJ-28583867 was carried out in the rat. The behavioral experiments had indicated good oral bioavailability and this was confirmed, as JNJ-28583867 has a C\textsubscript{max} of 260 ng/ml after an oral dose of 10 mg/ kg. Clearance was 14 ml/min/kg after intravenous dosing and the volume of distribution was high (7.9 l/kg), indicating extensive tissue distribution. The half-life was 6.5 h after intravenous dosing and up to more than 9 h after oral dosing. The half-life correlates well with the observation that effects could be observed up to 24 h after a single oral dose, as was the case in the head twitch test. The metabolism of JNJ-28583867 has not been studied but plasma and brain levels of parent drug are sustained and correlate reasonably well with efficacy for an extended period of time.

In summary, we have shown that the combination of histamine H\textsubscript{3} receptor antagonist with selective serotonin reuptake inhibitor activity in a single molecule results in a pharmacology consistent with the combination of either class of molecule alone. JNJ-28583867 is a prototype of such a compound, with demonstrated oral efficacy in a behavioral model of depression (tail suspension test) and a typical neurochemical profile.

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References


