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H_4 receptor antagonism exhibits anti-nociceptive effects in inflammatory and neuropathic pain models in rats

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ABSTRACT

The histamine H_4 receptor (H_4R) is expressed primarily on cells involved in inflammation and immune responses. To determine the potential role of H_4R in pain transmission, the effects of JNJ7777120, a potent and selective H_4 antagonist, were characterized in preclinical pain models. Administration of JNJ7777120 fully blocked neutrophil influx observed in a mouse zymosan-induced peritonitis model (ED_{50} = 17 mg/kg s.c., 95% CI = 8.5–26) in a mast cell-dependent manner. JNJ7777120 potently reversed thermal hyperalgesia observed following intraplantar carrageenan injection of acute inflammatory pain (ED_{50} = 22 mg/kg i.p., 95% CI = 10–35) in rats and significantly decreased the myeloperoxide activity in the carrageenan-injected paw. In contrast, no effects were produced by either H_1R antagonist diphenhydramine, H_2R antagonists ranitidine, or H_3R antagonist ABT-239. JNJ7777120 also exhibited robust anti-nociceptive activity in persistent inflammatory (CFA) pain with an ED_{50} of 29 mg/kg i.p. (95% CI = 19–40) and effectively reversed monoiodoacetate (MIA)-induced osteoarthritic joint pain. This compound also produced dose-dependent anti-allodynic effects in the spinal nerve ligation (ED_{50} = 60 mg/kg) and sciatic nerve constriction injury (ED_{50} = 88 mg/kg) models of chronic neuropathic pain, as well as in a skin-incision model of acute post-operative pain (ED_{50} = 68 mg/kg). In addition, the analgesic effects of JNJ7777120 were maintained following repeated administration and were evident at the doses that did not cause neurologic deficits in rotarod test. Our results demonstrate that selective blockade of H_4 receptors *in vivo* produces significant anti-nociception in animal models of inflammatory and neuropathic pain.

1. Introduction

Histamine (HA) is a biogenic amine that affects a variety of functions in the human body. It has been known to play a role in inflammation, gastric acid secretion, and neurotransmission [Passani et al., 2007; Parsons and Ganellin, 2006; Huang and Thurmond, 2008]. Multiple receptors exist for histamine in mammalian tissues and these have been classified into 4 distinct receptor types (H_1R, H_2R, H_3R, and H_4R), all of which are G-protein coupled receptors (GPCRs) [Schneider et al., 2002]. The four HA receptor subtypes are distinct in terms of their pharmacology and molecular biology and have been implicated in diverse biological effects of the neurotransmitter histamine [Haas et al., 2008; Thurmond et al., 2008]. H_1 receptors are expressed on multiple cell types including endothelial and smooth muscle cells, where they mediate vasodilation and bronchoconstriction [Simons, 2003]. In addition, H_1 receptors are expressed in the brain, where they mediate many of the CNS effects of histamine [Haas et al., 2008; Barbier and Bradbury, 2007]. Similar to H_1 receptors, H_2 receptors are also expressed on many cell types and have been demonstrated to function as a key modulator for gastric acid secretion [Barocelli and Ballabeni, 2003]. The H_3 receptor is predominantly expressed in the central nervous system, which acts as a presynaptic autoreceptor and plays a role in central and peripheral neurotransmissions [Esbenshade et al., 2008].

The H_4 receptor, identified in 2000, mediates its effects by coupling to Gαi/o G-proteins and has low homology with other histamine receptors, sharing only 35% amino acid identity with the H_3R (58% homology in its transmembrane regions) and a much lower homology to H_1R and H_2R [Oda et al., 2000; Nakamura et al., 2000; Liu et al., 2001]. This receptor has a distinct expression profile on immune cells including mast cells, eosinophils, dendritic cells, and T cells and has modulatory functions of these cells, such as, activation, migration, and production of cytokines and chemokines [Hofstra et al., 2003; de Esch et al., 2005], suggesting its role in inflammatory and immune responses. Although somewhat controversial, some studies have reported H_4R on neutrophils and monocytes, nevertheless functional

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data are lacking [Morse et al., 2001; Zhu et al., 2001]. Additionally, the H_4R appears to play a role in pruritus and inflammatory bowel disease [Varga et al., 2005; Dunford et al., 2007]. Interestingly, H_4R expression has also been reported in peripheral nerves and in the neurons of the submucous plexus [Nakaya et al., 2004; Breunig et al., 2007].

Histamine appears to play a complex role in pain modulation. Histamine released from mast cells is an established mediator of acute allergic reactions and chronic inflammation [Thurmond et al., 2008]. The close connection between mast cells, microvessels, and sensory fibers has been demonstrated [Freemont et al., 2002]; hence, histamine, in conjunction with other mast cell products like prostaglandins and nerve growth factor (Thacker et al., 2007), may contribute to pain sensation. Although rather controversial in the literature, several observations have demonstrated that the histamine receptor subtypes play roles in nociception. While central activation of histamine H_1R induces anti-nociception [Thoburn et al., 1994], peripheral histamine was found to stimulate nociceptive fibers in rodents through the activation of histamine H_1R [Malmberg-Aiello et al., 1998; Parada et al., 2001]. On the other hand, the anti-nociceptive effect of histamine H_3 receptor activation has been suggested [Cannon et al., 2007; Cannon and Hough 2005]. A recent study suggests that histamine H_4R may be involved in the early phase of acute inflammation induced by carrageenan in the rat [Coruzzi et al., 2007].

Since H_4R receptors have a broader distribution than just inflammatory cells, including areas of the central nervous system (CNS) (Strakhova et al., 2009; Connelly et al., 2009). H_4 antagonists may have broader utility in pain states than those secondary to inflammation. For these reasons, the present study used a potent and selective H_4 antagonist JNJ7777120 [Thurmond et al., 2004] to explore the role of the H_4R in preclinical inflammatory and neuropathic pain models.

2. Materials and Methods

2.1. Animals, compounds, and dosing

Male Sprague Dawley rats obtained from Charles River Laboratories (Wilmington, MA) were used for all experiments, unless indicated otherwise. The animals were housed in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved facilities at Abbott Laboratories in a temperature-regulated environment under a controlled 12-h light–dark cycle, with lights on at 6:00 a.m. Food and water were available *ad libitum* at all times except during testing. All testing was done following procedures outlined in protocols approved by Abbott's Institutional Animal Care and Use Committee and followed the Guidelines on Ethical Standards for Investigations of Experimental Pain in Conscious Animals laid down by the International Association for the Study of Pain (IASP) (Zimmermann, 1983).

JNJ7777120 [Jablonowski et al., 2003] is available through Sigma-Aldrich Chemical Co (catalog # J3770, St. Louis, MO). ABT-239 [Cowart et al., 2005] was synthesized at Abbott Laboratories (Abbott Park, IL). Gabapentin and celecoxib were purchased from ChemPacific (Baltimore, MD). [^{3}H]-mepyramine, [^{3}H]-tiotidine, and [^{3}H]-N-α-methylhistamine were obtained from PerkinElmer Life and Analytical Sciences (Boston, MA), and [^{3}H]-histamine was from Amersham Biosciences Inc. (Piscataway, NJ). All other chemicals including diphenhydramine, ranitidine HCl, indomethacin, sodium diclofenac, zymosan, λ-carrageenan, complete Freund's adjuvant (CFA), capsaicin, and sodium monoiodoacetate (MIA), were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO).

JNJ7777120 (dissolved in 10%DMSO/30%hydroxyl-β-cyclodextrin in water), gabapentin (prepared in water), diclofenac (prepared in water), celecoxib (dissolved in PEG-400), ABT-239 (prepared in saline), diphenhydramine (prepared in saline), ranitidine (dissolved in 10%DMSO/30%hydroxyl-β-cyclodextrin in water were administered intraperitoneally at a volume of 2 ml/kg 30 min before behavioral testing.

2.2. Radioligand binding assay

Standard radioligand binding techniques were utilized in competition binding assays for recombinant histamine H_1, H_2, H_3 and H_4 receptors stably expressed in membranes prepared from HEK cells, as previously described [Esbenshade et al., 2005]. The receptor membrane preparations were incubated with [^{3}H]-mepyramine, [^{3}H]-tiotidine, [^{3}H]-N-α-methylhistamine and [^{3}H]-histamine, respectively, in the presence or absence of ligand solutions for 30 min at 25 °C in a final volume of 0.5 ml of 50 mM Tris/5 mM EDTA, pH 7.4, buffer. All binding reactions were terminated by vacuum filtration onto polyethylenimine (0.3%) presoaked Unifilter plates (PerkinElmer Life and Analytical Sciences) followed by three brief washes with 2 ml of ice-cold 50 mM Tris/5 mM EDTA, pH 7.4, buffer. Liquid scintillation counting was used to determine bound radiolabel. All assays were performed in triplicates and IC_{50} values converted to K_i values [Cheng and Prusoff, 1973].

2.3. Mouse zymosan-induced peritonitis model

Male Balb/C (Charles River Laboratories, Wilmington, MA) and WBB6F1/J-kit<w> (mast cells-deficient, Jackson Laboratories, Bar Harbor, ME) mice were used to study the effects of JNJ 7777120 on zymosan-induced peritonitis. JNJ 7777120 was given at 8.3–70 mg/kg s.c. ($n = 8$ per group) in a vehicle containing 2%DMSO/3% Tween-80/95% (v/v, 10 ml/kg) 30 min prior to i.p. administration of 0.25 mg zymosan (Sigma-Aldrich, St. Louis, MO) in saline (0.5 ml per mouse). After 2 h, the animals were euthanized, and the peritoneal cavities were washed with 3 ml of ice-cold PBS (pH 7.2) and the lavage was collected in an EDTA-coated tube. The number of migrated polymorphonuclear leukocytes (PMNL) was determined with CellDyn (Abbott Laboratories, Abbott Park, IL) by taking an aliquot (10 µl) of the lavage fluid. Indomethacin at a dose of 10 mg/kg (prepared in 2% DMSO/3% Tween-80/95% HPMC, v/v, 10 ml/kg s.c.) was also included in the same study. The samples that were visibly red were not included in the analysis [Rao et al., 1994].

2.4. PMNL myeloperoxidase (MPO) activity

The lavage samples (1 ml) were centrifuged at 2000×*g* for 5 min in refrigerated micro-centrifuge tubes. The cell pellets were re-suspended in 1 ml of 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltimethylammonium bromide (HTAB), sonicated for 30 s, freeze-thawed three times, and centrifuged at 20,000×*g* for 10 min a refrigerated micro-centrifuge to collect supernatants which were used in MPO assays. MPO assays were performed as per the procedures previously [Bradley et al., 1982] adapted to a 96 well format. The reaction was initiated by the addition of assay buffer containing o-dainisidine (0.2 mg/ml) and hydrogen hydroperoxide (0.001%). The rate of change of absorbance was monitored at 450 nM in kinetic mode by a plate reader.

3. *In vivo* inflammatory pain models

3.1. Carrageenan-induced acute inflammatory pain

Thermal hyperalgesia was produced by intraplantar injection of 100 µl of 1% (w/v) λ-carrageenan in saline into the plantar surface of the right hind paw of the rat. The hyperalgesia to thermal stimulation was determined 2 h following carrageenan injection (see below). The hind paw volumes were measured by plethysmometry (Basile Ugo, Varese, Italy) 2 h after carrageenan injection using water displacement. Edema formation for each animal was determined from the

difference in ipsilateral and contralateral hind paw volumes. Paw tissues were then collected (after the animals were humanely euthanized with CO_2) and homogenized in a buffer solution. The paw MPO activities were determined as the procedures described above. JNJ7777120 was injected either 30 min before or 90 min (i.p.) after carrageenan injection.

Thermal hyperalgesia was determined using a commercially available thermal paw stimulator (UARDG, University of California, San Diego, CA) as described by Hargreaves et al. (1988). Rats were placed into individual plastic cubicles mounted on a glass surface maintained at 30 °C, and allowed a 20 min habituation period. A thermal stimulus, in the form of radiant heat emitted from a focused projection bulb, was then applied to the plantar surface of each hind paw. The stimulus current was maintained at 4.50 ± 0.05 amp, and the maximum time of exposure was set at 20.48 s to limit possible tissue damage. The elapsed time until a brisk withdrawal of the hind paw from the thermal stimulus was recorded automatically using photodiode motion sensors. The right and left hind paw of each rat was tested in 3 sequential trials at 5 min intervals. Paw withdrawal latency (PWL) was calculated as the mean of the two shortest latencies. PWL were measured 30 min post JNJ7777120 administration in both the carrageenan-inflamed and uninjected paw.

3.2. CFA-induced chronic inflammatory pain

Chronic inflammatory mechanical allodynia was induced by injection of 150 µl of a 50% solution of CFA in phosphate buffered saline (PBS) into the plantar surface of the right hind paw in rats; control animals received only PBS treatment. Mechanical allodynia was assessed 48 h post-CFA injection. On the day of testing, JNJ7777120 was injected 30 min (i.p.) before testing for mechanical allodynia (see below).

Mechanical allodynia was measured using calibrated von Frey filaments (Stoelting, Wood Dale, IL). Paw withdrawal threshold (PWT) was determined by increasing and decreasing stimulus intensity, and estimated using the Dixon's up-down method [Chaplan et al., 1994]. Rats were placed into inverted individual plastic containers ($20 \times 12.5 \times 20$ cm) on top of a suspended wire mesh with a 1 cm^2 grid to provide access to the ventral side of the hind paws, and acclimated to the test chambers for 20 min. The von Frey filaments were presented perpendicularly to the plantar surface of the selected hind paw, and then held in this position for approximately 8 s with enough force to cause a slight bend in the filament. Positive responses included an abrupt withdrawal of the hind paw from the stimulus, or flinching behavior immediately following removal of the stimulus. A 50% withdrawal threshold was determined using an up–down procedure [Dixon, 1980]. The strength of the maximum filament used for von Frey testing was 15.0 g. A percent maximal possible effect (% MPE) of testing compound was calculated according to the formula: ([compound-treated threshold] − [vehicle-treated threshold]) / ([maximum threshold] − [vehicle-treated threshold]) × 100%, where the maximum threshold was equal to 15 g.

3.3. Knee joint osteoarthritic model

Unilateral knee joint osteoarthritis was induced in the rats by a single intra-articular (i.a.) injection of sodium monoiodoacetate (MIA, 3 mg in 0.05 ml sterile isotonic saline) into the right knee joint cavity under light isoflurane anesthesia using a 26G needle, as previously described [Chandran et al., 2009]. The dose of the MIA (3 mg/i.a. injection) was selected based on results obtained from preliminary studies wherein an optimal pain behavior was observed at this dose. Pain behavioral assessment of hind limb grip force was conducted by recording the maximum compressive force exerted on the hind limb strain gauge setup, in a commercially available grip force measurement system (Columbus Instruments, Columbus, OH). The grip force data was converted to a maximum hindlimb cumulative compressive force (CFmax) (gram force)/kg body weight for each animal. The analgesic effects of JNJ7777120 were determined 20 days following the i.a. injection of MIA. The vehicle control group for each compound being tested was assigned 0% whereas the age matched naïve group was assigned as being 100% (normal). The % effect for each dose group was then expressed as % return to normalcy compared to the naïve group.

4. *In vivo* neuropathic pain models

4.1. Rat spinal L5–L6 nerve ligation (SNL) model of neuropathic pain

As previously described in detail by Kim and Chung (1992) rats were placed under isoflurane anesthesia and a 1.5 cm incision was made dorsal to the lumbosacral plexus. The paraspinal muscles (left side) were separated from the spinous processes, the L5 and L6 spinal nerves isolated, and tightly ligated with 5-0 silk sutures distal to the dorsal root ganglion. Care was taken to avoid ligating the L4 spinal nerve. Following spinal nerve ligation, a minimum of 7 days of recovery and no more than 3 weeks was allowed prior to behavioral testing for mechanical allodynia. Only rats with threshold scores $\leq$4.5 g were considered allodynic and utilized in compound testing experiments.

In another series of *in vivo* studies, repeated dosing experiments were also conducted in this neuropathic pain model. For these studies, rats were dosed with either vehicle or JNJ777720 (28 and 70 mg /kg i.p.) twice daily for 8 days. Animals were tested in the morning on days 1, 5, and 8, respectively, 30 min after drug dosing.

4.2. Rat chronic constriction injury-induced (CCI) model of neuropathic pain

As previously described in detail by the method of Bennett and Xie (1988), the right common sciatic nerve was isolated at mid-thigh level, and loosely ligated by 4 chromic gut (5-0) ties separated by an interval of 1 mm. All animals were left to recover for at least 2 weeks and no more than 4 weeks prior to testing of mechanical allodynia.

Mechanical testing was measured using calibrated von Frey filaments as the procedures described above. Only rats with a baseline threshold score of less that 4.5 g were used in this study, and animals demonstrating motor deficit were excluded.

4.3. Post-operative pain model

A model of post-operative pain was performed as described by Brennan et al. (1996). The plantar aspect of the rat left hind paw was exposed through a hole in a sterile plastic drape, and a 1-cm longitudinal incision was made through the skin and fascia, starting 0.5 cm from the proximal edge of the heel and extending towards the toes. The plantaris muscle was elevated and incised longitudinally leaving the muscle origin and insertion points intact. After homeostasis by application of gentle pressure, the skin was apposed with 2 mattress sutures using 5-0 nylon. Animals were then allowed to recover for 2 h after surgery, at which time tactile allodynia was assessed as the procedures described above.

4.4. Rat plasma and brain concentrations

JNJ7777120 was injected (i.p.) and the rats were humanely euthanized (with CO_2) and decapitated at 30 min. Brains were immediately removed and freed from blood vessels as much as possible. The resulting brain tissues were frozen at −80 °C, followed by weighing and homogenization before analysis. Plasma was separated from blood samples by centrifugation and frozen (−70 °C) until analysis. JNJ7777120 was extracted from the samples via liquid–liquid extraction and were quantified by liquid chromatography/mass spectroscopy.

4.5. Rotarod performance and locomotor activity

To determine the potential CNS side effects of JNJ7777120, rotarod performance was measured using an accelerating rotarod apparatus (Omnitech Electronics, Inc. Columbus, OH). Rats were allowed a 30 min acclimation period in the testing room and then placed on a 9 cm diameter rod that increased in speed from 0 to 20 rpm over a 60 s period. The time required for the rat to fall from the rod was recorded, with a maximum score of 60 s. Each rat was given 3 training sessions before testing. Rotarod performance (latencies to fall from the rotarod) was determined 30 min following the administration of H_4 antagonist JNJ7777120.

Spontaneous exploratory behavior was examined in naïve rats to determine the potential CNS side effects of JNJ7777120 (30 min post-compound administration). Rats were individually placed into an open field test chamber (40×40×36 cm) where they are permitted to move around freely. Horizontal (locomotion) activity was recorded by a photo beam detector system for 30 min (AccuScan Instruments, Columbus, OH). Time spent adjacent to wall and time in the central area of open field was also measured in this assay.

4.6. Data analysis

All *in vivo* behavioral studies were conducted in a randomized blinded fashion. The statistical analysis was carried out using GraphPad Prism (version 4.03; GraphPad Software, Inc., San Diego, CA). The values were represented as mean ± S.E.M. Statistical significance on group means was measured by one-way analysis of variance (ANOVA), followed by Bonferroni's post-hoc analysis. In all cases $p<0.05$ was assumed as the level for statistical significance. ED_{50} values (effective dose, 50%) (GraphPad Prism) were also calculated by linear regression analysis and reported with the 95% confidence interval (95%CI).

5. Results

JNJ7777120 exhibited potent and selective binding to H_4R subtype. It potently bound to human H_4 receptors (K_i = 12 nM) with approximately >250-fold selectivity over H_1 and H_3 receptors and >700-fold selectivity over H_2 subtypes (Table 1). JNJ7777120 also potently binds to rat H_4 with an affinity (K_i) of 2.5 nM, indicating that the binding properties of this H_4 antagonist on H_4R subtype are similar between rat and human (Table 1). The high selectivity of JNJ7777120 was also demonstrated in rat H_4 receptors over the binding affinities of H_1 (>3000 nM), H_2 (>3000 nM), and H_3 (>1000 nM) subtypes. JNJ7777120 also exhibits potent binding affinity (K_i = 4.6 nM) and functional antagonism (pA2 = 8.1) against the mouse H_4R subtype [Thurmond et al., 2004].

Mice injected intraperitoneally with zymosan develop peritonitis, resulting in significant leukocyte (predominantly neutrophils, >80% of total leukocytes) accumulation in the peritoneum. Administration of JNJ7777120 30 min prior to injection of zymosan produced dose-dependent inhibition (ED_{50} = 17 mg/kg, 95% CI = 8.5–26) in neutrophil accumulation in peritoneal lavages. A 40 ± 10%, 56 ± 5%, %, and 78 ± 6% decrease ($p<0.01$) in neutrophil counts was seen with JNJ7777120 at doses of 8.3, 28, and 70 mg/kg s.c., respectively (Fig. 1A). Under the same conditions, the levels of myeloperoxidase (MPO) an enzyme marker for neutrophils, in lavage samples were also assessed. Pretreatment with JNJ7777120 significantly inhibited the MPO levels with an 80 ± 8% effect ($p<0.001$ vs. vehicle control) at 70 mg/kg dose (Fig. 1B). The inhibitory effects of MPO enzymatic levels are consistent with the influx of neutrophil counts. In the same experiments, subcutaneous administration of a non-steroid anti-inflammatory drug (NSAID) indomethacin (10 mg/kg s.c) also elicited a statistically significant inhibition of the influx of neutrophils (49 ± 9%, $p<0.01$) and MPO activities (50 ± 13%, $p<0.01$). To determine the role of mast cells on H_4R-mediated neutrophil recruitment evoked by zymosan, the inhibitory effects of JNJ7777120 (70 mg/kg s.c.) were determined in mast cell-deficient (MCD) mice. The results shown in Fig. 2A and B demonstrate that zymosan-induced neutrophil influx is primarily (>60%) mast cells dependent. JNJ7777120 treatment (70 mg/kg s.c.) significantly blocked the neutrophil migration determined by cellular counts (60 ± 3%, $p<0.01$) as well as enzymatic MPO levels (64 ± 3%, $p<0.01$) in the mast cell-sufficient control colony but not in MCD mice, indicating the mast cell dependence of JNJ777120 effects. By contrast, indomethacin pretreatment (10 mg/kg s.c) significantly prevented the zymosan-induced reaction in both the control and MCD mice.

JNJ7777120 elicited significant anti-nociceptive effect in both acute (carrageenan) and chronic (complete Freund's adjuvant) inflammatory pain models in rats. Administration of carrageenan produced a significant decrease in paw withdrawal latencies (PWL), from 10.9 ± 0.8 to 3.6 ± 0.4 s, demonstrating inflammation-induced thermal hypersensitivity. JNJ7777120 (8.3–70 mg/kg i.p.) significantly reversed the carrageenan-induced decrease in paw withdrawal latencies to control

Table 1
Radioligand binding affinity (K_i, nM) for JNJ7777120, diphenhydramine, ranitidine, and ABT-239 on recombinant human and rat HA receptor subtypes.

Compound	hH_1	hH_2	hH_3	hH_4	rH_4
JNJ7777120	>2700	>9100	>3800	12 ± 0.7	2.48 ± 0.23
Diphenhydramine	10 ± 0.6	>2500	>2000	>10,000	>9100
Ranitidine	>10,000	60 ± 5	>1500	>8000	>8000
ABT-239	>1600	>6700	0.45 ± 0.1	>10,000	>7000

Competition binding with [^{3}H]-mepyramine, [^{3}H]-tiotidine, [^{3}H]-N-α-methylhistamine and [^{3}H]-histamine, respectively, for H_1, H_2, H_3, and H_4 antagonists. Data are presented as mean ± s.e.m. (n = 5).

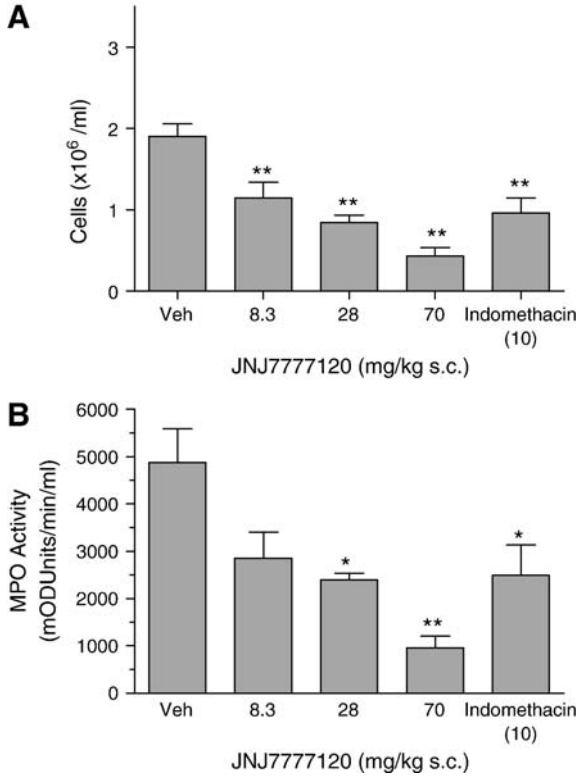

Fig. 1. Effects of H_4R antagonist JNJ7777120 (s.c.) on the zymosan-induced peritonitis model in Balb/C mice. (A) JNJ7777120 produced a dose-dependent reduction in neutrophil influx induced 2 h after injection of 2 mg of zymosan in Balb/C mice (n = 6). (B) Dose-related inhibition by JNJ7777120 of peritoneal myeloperoxidase (MPO) accumulation 2 h after injection of 2 mg (i.p.) of zymosan (n = 6). Indomethacin (10 mg/kg s.c.) was included as a positive control in the same experiments (n = 6). Data expressed as mean ± S.E.M. *$p<0.05$, **$p<0.01$ as compared to vehicle-treated mice.

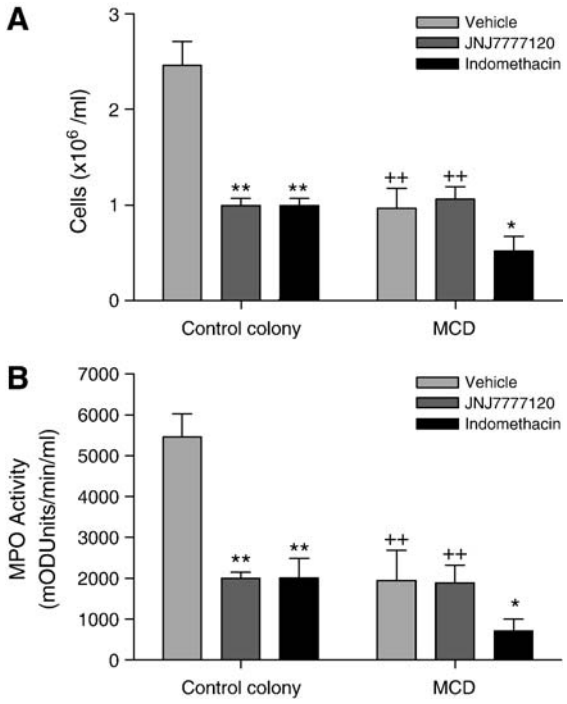

Fig. 2. Effects of H_4R antagonist JNJ7777120 (s.c.) on zymosan-induced peritonitis model in mast cell-deficient (MCD) mice. (A) Inhibitory effect of JNJ7777120 on the neutrophil influx induced 2 h after injection of 2 mg of zymosan in mast cell-deficient (MCD) mice (WBB6F1/J-kit<w>) or normal control colony mice (W/WB6F1/J-kit<w>-c) ($n=6$). (B) Inhibitory effect of JNJ7777120 on peritoneal myeloperoxidase (MPO) accumulation 2 h after injection of 2 mg of zymosan in MCD mice or their normal control colony (W/WB6F1-+/+) ($n=6$). Indomethacin (10 mg/kg s.c.) was included as a positive control in the same experiments ($n=6$). Data expressed as mean ± S.E.M. $^{*}p<0.05$, $^{**}p<0.01$ as compared to vehicle-treated mice. $^{++}$ $p<0.01$ as compared to normal control colony.

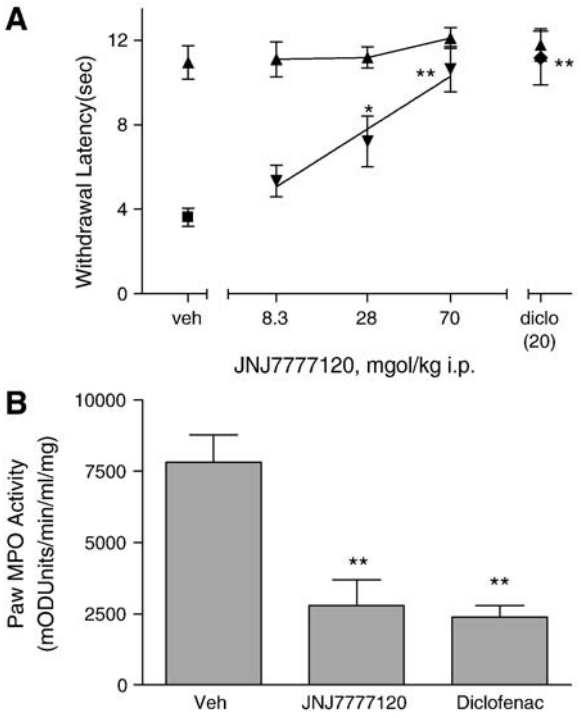

Fig. 3. Effects of H_4R antagonist JNJ7777120 (i.p.) on carrageenan-induced acute inflammatory pain model in rats. (A) JNJ7777120 dose-dependently reversed carrageenan-induced decrease in paw withdrawal latencies in the ipsilateral (▼) vs. vehicle (■) but not contralateral (▲) paws ($n=12$). 2 h following carrageenan injection, JNJ7777120 was injected 30 min before thermal hyperalgesia assessment. Diclofenac (♦, diclo, 20 mg/kg i.p., $n=12$) was included as a positive control in the same experiments. (B) Inhibitory effects of JNJ7777120 (70 mg/kg) of paw myeloperoxidase (MPO) accumulation 2 h after carrageenan ($n=6$). JNJ7777120 was administered 30 min before and 90 min after carrageenan injection. Diclofenac (20 mg/kg i.p. $n=6$) was included as a positive control in the same study. Data expressed as mean ± S.E.M. $^{*}p<0.05$, $^{**}p<0.01$ as compared to vehicle-treated animals.

levels in a dose-related fashion, resulting in a 96 ± 7% effect at the highest dose tested with an ED_{50} value of 22 mg/kg i.p. (95% CI = 10–35) (Fig. 3A). JNJ7777120 had no effect on PWL of the contralateral non-inflamed paw, indicative of a specific anti-hyperalgesic effect in this model (Fig. 3A). Under the same conditions, administration of a NSAID diclofenac showed similar anti-nociceptive activity with a dose of 20 mg/kg i.p. (Fig. 3A). JNJ7777120 (70 mg/kg i.p.) significantly decreased the MPO activity (64 ± 11%, $p<0.01$) in carrageenan paw tissue (Fig. 3B) but did not reduce paw edema volumes (14 ± 5%, $p>0.05$). In the same experiments, diclofenac (20 mg/kg i.p.) elicited a statistically significant inhibition of both MPO activities (70 ± 5%, $p<0.01$) and edema volumes (60 ± 4%, $p<0.01$) of carrageenan-injected paws.

Several experiments were also carried out to examine the differential effects of various HA receptor antagonists on the carrageenan-induced thermal hypersensitivity. The results showed that none of the selective H_1 receptor antagonist diphenhydramine, H_2 receptor antagonist ranitidine, or selective H_3 antagonist ABT-239 (Table 1) exhibited any effect on reversing the carrageenan-induced thermal hyperalgesia at the *in vivo* pharmacological relevant doses of each receptor subtype antagonist (Table 2). Diclofenac (20 mg/kg i.p.) was used as a positive control in these studies and showed 84% anti-nociceptive effect.

In the CFA-induced sub-chronic inflammatory pain model, acute systemic administration of JNJ7777120 30 min prior to testing dose-dependently reversed (ED_{50} = 29 mg/kg i.p., 95% CI: 19–40) mechanical allodynia by 15 ± 7%, 51 ± 8% ($p<0.01$), and 71 ± 5% ($p<0.01$), at 8.3, 28, and 70 mg/kg, i.p., respectively (Fig. 4A). In addition, the analgesic effects of JNJ7777120 at 28 mg/kg were maintained following repeated dosing (i.p., twice a day) where a 65 ± 14% ($p<0.01$ vs. vehicle) reversal effect in rats receiving acute dosing and 72 ± 14% ($p<0.01$ vs. vehicle) reversal of effect following a 3 day of repeated administration were seen (Fig. 4B). The plasma levels of JNJ7777120 were collected and measured after the behavioral testing, and showed no difference in exposure levels between the two treatment groups.

As the treatment of chronic joint pain secondary to OA remains an area of significant unmet need [Read and Dray, 2008], the analgesic effects of JNJ7777120 on activity-induced pain behavior were evaluated in a rat model of MIA-induced osteoarthritis joint pain, observed 20 days following the i.a. injection of MIA. Systemic i.p. administration of JNJ7777120 significantly reversed MIA-induced decreased grip force (47 ± 3% at 70 mg/kg, $p<0.01$ vs. vehicle). Under the same conditions, 62% reversal of MIA-induced decreased grip force

Table 2
Lack of anti-nociceptive effects of histaminergic antagonists diphenhydramine (H_1), ranitidine (H_2), and ABT-239 (H_3) in the carrageenan-induced inflammatory pain model. Two hours following carrageenan injection, the antagonist was injected 30 min (i.p.) before testing. Administration of carrageenan produced a significant decrease in paw withdrawal latencies (PWL). Paw withdrawal latencies in the vehicle group were significantly diminished from 10.1 ± 0.2 to 3.2 ± 0.2 s. Data are expressed as mean ± S.E.M., $^{**}p<0.01$ vs. vehicle treated group.

Treatment	Dose (mg/kg i.p.)	Percent reversal (%)
Diphenhydramine	1	0 ± 6, $n=6$
	3	22 ± 10, $n=6$
	10	0 ± 5, $n=6$
Ranitidine	3	6 ± 3, $n=6$
	10	8 ± 7, $n=6$
	30	16 ± 6, $n=6$
ABT-239	0.1	7 ± 6, $n=12$
	0.3	18 ± 5, $n=12$
	1	10 ± 4, $n=17$
	3	6 ± 8, $n=6$
Diclofenac	15	84 ± 3**, $n=29$

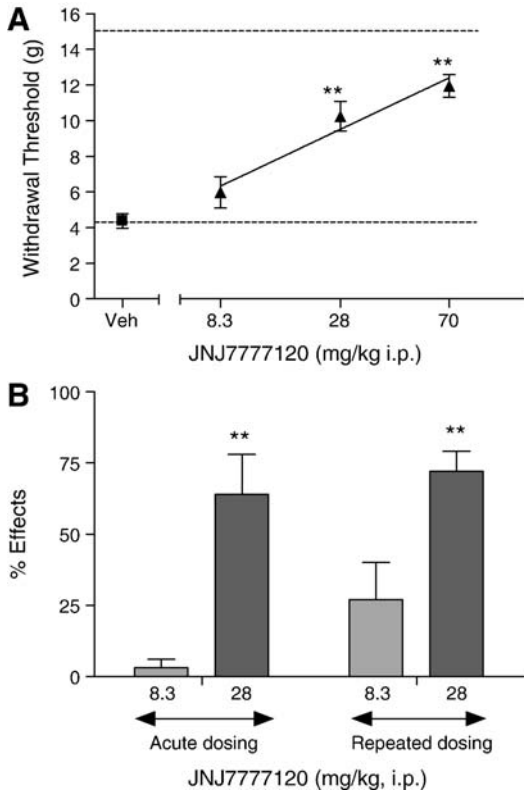

Fig. 4. Effects of H_4R antagonist JNJ7777120 (i.p.) on CFA-induced chronic inflammatory pain model in rats. (A) JNJ7777120 (▲) dose-dependently reversed CFA-induced decrease in paw withdrawal thresholds vs. vehicle treated group (■, veh) ($n=12$). 48 h following CFA injection, JNJ7777120 was injected 30 min before mechanical allodynia testing. (B) The analgesic effects of JNJ7777120 were maintained following repeated dosing (i.p., twice a day). Data represent mean ± S.E.M. $^{**}p<0.01$ as compared to vehicle-treated animals.

was elicited by 30 mg/kg i.p. of celecoxib, a clinically-used analgesic for OA pain (Fig. 5). In order to ensure that the doses of JNJ7777120 used were not enhancing hindlimb grip force assessment on their own, a group of naïve rats was administered JNJ7777120 to evaluate if the compound reduced grip force. None of the JNJ7777120-treated naïve rats demonstrated any increases on grip force readouts.

JNJ7777120 readily crossed the blood–brain barrier (brain/plasma ratio = 20, 30 min after i.p. administration at a dose of 30 mg l/kg). Separate studies were conducted to determine the potential analgesic action of JNJ7777120 in the neuropathic pain models. Administration of JNJ7777120 produced a significant reversal of nerve injury-induced tactile hypersensitivity in two rat models of neuropathic pain. In SNL animals, a reduction in paw withdrawal thresholds (PWT) was observed ipsilateral to the nerve injury (3.24 ± 0.10 g), demonstrating the development of mechanical allodynia. JNJ7777120 treatment attenuated SNL-induced mechanical allodynia in a dose-related manner with an ED_{50} of 60 mg/kg (95% CI: 51–68) and an efficacy of 94 ± 3% ($p<0.01$ vs. vehicle) at the highest dose tested (Fig. 6A). Under the same conditions, intraperitoneal administration of a clinically-used drug for neuropathic pain gabapentin (90 mg//kg i.p.) produced a statistically significant reversal (54% ± 3%, $p<0.01$). A separate study was conducted to determine whether the anti-allodynic effect of JNJ7777120 was maintained after repeated dosing for 8 days at 28 and 70 mg/kg i.p (Fig. 6B). The results demonstrated that there was no tolerance development to the effect of JNJ7777120. The brain and plasma levels of JNJ7777120 were collected and measured after the behavioral testing, and showed no difference in exposure levels between the repeated dosing and acute dosing group.

In rats, chronic constriction injury (CCI) of the sciatic nerve produced a decrease in PWT to mechanical stimulation with von Frey

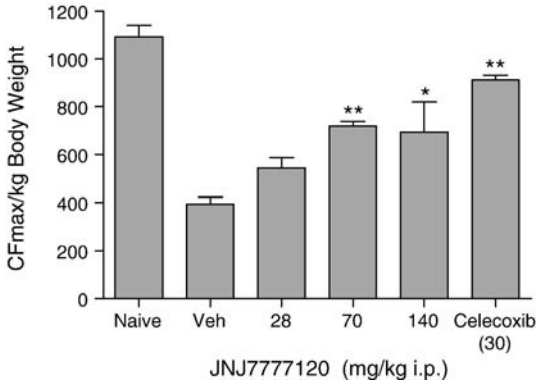

Fig. 5. Effects of H_4R antagonist JNJ7777120 (i.p.) on hindlimb grip force in rats, 20 days following injection of monoiodoacetate (MIA). JNJ7777120 demonstrated a reversal of activity-induced pain behavior in osteoarthritic (OA) rats with effects comparable to celecoxib (30 mg/kg, i.p.), a clinically-relevant analgesic for OA pain. Data are mean ± S.E.M. $^{*}p<0.05$, $^{**}p<0.01$ vs. vehicle treated group, $n=6$ per group.

monofilaments 2 weeks following surgery (PWT = 3.3 ± 0.2 g, Fig. 7), demonstrating the development of mechanical allodynia. Administration of JNJ7777120 attenuated CCI-induced mechanical allodynia in a dose-related manner with an ED_{50} of 88 mg/kg (95% CI: 65–120) and an efficacy of 73 ± 9% ($p<0.01$ vs. vehicle) at the highest dose (140 mg/kg) tested. Under the same conditions, intraperitoneal

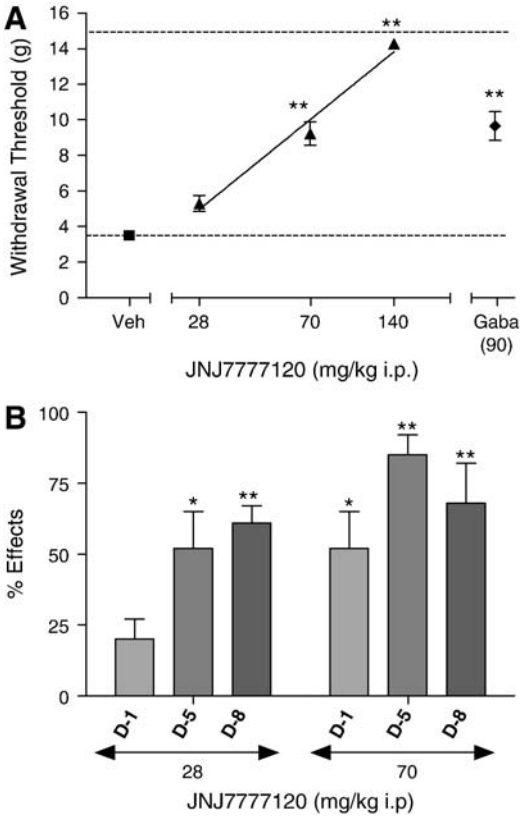

Fig. 6. Effects of H_4R antagonist JNJ7777120 (i.p.) on mechanical allodynia in spinal nerve ligation (SNL) model of neuropathic pain in rats. (A) JNJ7777120 (▲) dose-dependently attenuated neuropathic pain in SNL model ($n=12$), which is seen as an increase in the withdrawal threshold of the nerve injured paw, vs. vehicle treated group (■, veh). Two weeks following spinal nerve injury, JNJ7777120 was injected 30 min before testing. Gabapentin (◆, gaba, 90 mg/kg i.p. $n=6$) was included as a positive control in the same study. (B) The analgesic effects of JNJ7777120 were maintained following repeated dosing (i.p., twice a day). Data expressed as mean ± S.E.M. $^{*}p<0.05$, $^{**}p<0.01$ as compared to vehicle-treated animals.

administration of gabapentin (90 mg/kg) also produced a statistically significant reversal (52 ± 9%, $p<0.01$) of mechanical allodynia (Fig. 7).

The analgesic effects of JNJ7777120 were also evaluated in the skin-incision model of acute post-operative pain. Skin incision produced an acute (2 h after surgery) mechanical allodynia (PWT = 2.12 ± 0.10 g) (Fig. 8) but not affected mechanical paw withdrawal thresholds in the contralateral paw. JNJ7777120, administered 2 h post-surgery, dose-dependently attenuated mechanical allodynia (ED_{50} = 68 mg/kg, 95%CI: 53–88.) with a maximal efficacy of 79 ± 6% ($p<0.01$ vs. vehicle) at the highest dose tested (Fig. 8). Intraperitoneal administration of morphine (6 mg/kg) produced a full reversal of mechanical allodynia in the same study (Fig. 6).

JNJ7777120 had no effect on motor coordination in rat rotarod performance assay at the doses (30, 70, and 100 mg/kg i.p.) higher than the ED_{50}s of pain efficacy studies in inflammatory or neuropathic preclinical pain models. However, at 300 mg/kg i.p., JNJ7777120 reduced the time spent on the rotarod to 57 ± 9% of that seen in vehicle treated animals at the 30-min time point. Haloperidol (1 mg/kg i.p.), a dopamine receptor antagonist, was used as a positive control in these studies and showed 62% reduction ($p<0.01$ vs. vehicle). In the locomotor test, JNJ7777120 treated animals (30, 70, and 140 mg/kg i.p.) produced dose-related deficits of locomotion with reduction of activities by 27 ± 10%, 72 ± 11% ($p<0.01$ vs. vehicle), and 97 ± 1% ($p<0.01$ vs. vehicle), respectively, as compared to the vehicle treated animals. Haloperidol (1 mg/kg i.p.), used as a positive control, showed 98% reduction in locomotor activity. However, H_4 antagonist JNJ7777120 at the doses significantly impaired locomotion activity did not produce any effect (<5% difference as compared to the vehicle treated rats) on either time spent peripherally adjacent to wall or time in the central area of open field.

6. Discussion

The present study investigated the potential role of histaminergic H_4 receptor activation in pain transmission, using a potent and selective H_4R antagonist. JNJ7777120 exhibited nanomolar binding affinity for H_4 receptors and had high selectivity (>200-fold) compared to all other histamine receptor subtypes. JNJ7777120 exhibits analgesia across a wide range of preclinical inflammatory and neuropathic pain models in rats. These effects were maintained following repeated administration.

Systemic administration of JNJ7777120 fully blocked the acute inflammatory response observed in a mouse model of zymosan-induced peritonitis. The neutrophil influx in this model is primarily mast cell-dependent since the neutrophil influx is reduced (>60%) in mast cell-deficient mice. Furthermore, JNJ777120 treatment did not produce any anti-inflammatory effects in MCD mice, suggesting that JNJ7777120 anti-inflammatory effects are mediated through its action on mast cells. The effects of the selective H_4R antagonist in a mouse peritonitis model also point to a more general role for the H_4R in inflammation, making this receptor a potential target for treating inflammatory diseases. H_4 receptors are abundantly expressed in mast cells [Hofstra et al., 2003], in which activation of H_4R induces calcium mobilization and mediates mast cell migration towards histamine, but does not affect IgE cross linking-induced degranulation [Hofstra et al., 2003]. In addition, JNJ7777120 is also able to block histamine-induced leukocyte shape change as well as the upregulation of the adhesion molecules, while H_1, H_2, and H_3 selective receptor antagonists are not effective [Ling et al., 2004]. *In vivo* studies have shown that aerosolized histamine was capable of inducing sub-epithelial mast cell accumulation in mouse airways, which could be blocked by JNJ7777120 [Thurmond et al., 2004], further confirming the effect of H_4R on mast cells.

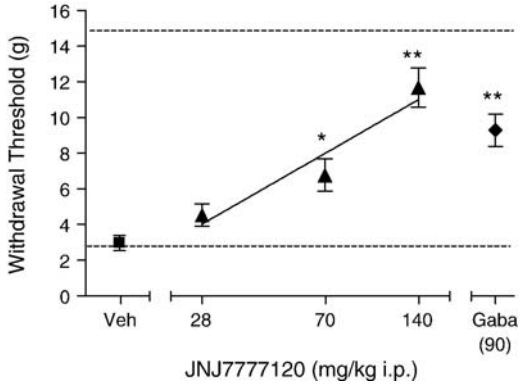

Fig. 7. Effects of H_4R antagonist JNJ7777120 (i.p.) on mechanical allodynia in sciatic nerve chronic constriction injury (CCI) model of neuropathic pain in rats. JNJ7777120 (▲) dose-dependently attenuated neuropathic pain in CCI model ($n=12$), vs. vehicle treated group (■, veh). Three weeks following sciatic nerve injury, JNJ7777120 was administered 30 min before testing. Gabapentin (♦, gaba, 90 mg kg i.p. $n=6$) was included as a positive control in the same study. Data expressed as mean ± S.E.M. $^{*}p<0.05$, $^{**}p<0.01$ as compared to vehicle-treated animals.

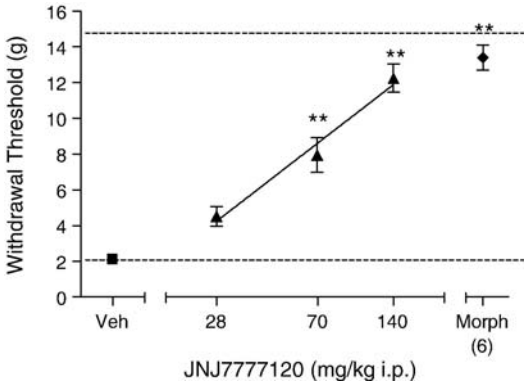

Fig. 8. Effects of H_4R antagonist JNJ7777120 (i.p.) on skin-incision induced acute post-operative pain. JNJ78777120 (▲), administered 2 h post-surgery, dose-dependently attenuated tactile allodynia, vs. vehicle treated group (■, veh). Morphine (♦, morph, 6 mg/kg, i.p.) was included in the same study. Data represent mean ± S.E.M. $^{**}p<0.01$ as compared to vehicle-treated animals ($n=12$ per group).

JNJ7777120 exhibited robust anti-hyperalgesic effects in carrageenan-induced acute and CFA-induced persistent inflammatory pain models in rats. Though the precise mechanisms underlying the role of H_4 in inflammatory pain need further study, in the present study JNJ7777120 pretreatment showed the significant attenuation of the elevated tissue MPO activity in the carrageenan-injected paw, suggesting that the anti-hyperalgesic effect of JNJ7777120 may be secondary to its anti-inflammatory action. The carrageenan-induced hyperalgesic response is primarily dependent on prostaglandins [Jett et al., 1999; Seibert et al., 1994]. We also observed that the inflammatory infiltrate of carragenan paw tissue contains significant recruitment of activated mast cells and neutrophils and there are also eosinophils, macrophages, and lymphocytes but in less considerable levels (Hsieh et al., unpublished histological observation). Histamine is a key mediator released by activated mast cells. It can sensitize nociceptors [Mizumura et al., 2000] and therefore the activated mast cells may contribute directly to the nociceptive responses by releasing mediators other than histamine, including tryptase, TNFα, prostaglandins, and IL-1 [Thacker et al., 2007; Bischoff 2007; Sommer et al., 2001; Syriatowicz et al., 1999; Theodosiou et al., 1999; Sommer et al, 1999]. These factors also promote leukocyte recruitment in non-neural models of inflammation [Kubes and Granger, 1996].

Given that the histamine receptors could be potentially involved in pain transmission [Farzin et al., 2002], several pharmacological experiments were conducted to investigate the relative contribution of the 4

known histamine receptors in carrageenan-induced thermal hypersensitivity using selective antagonists. None of the H_1R antagonist diphenhydramine, H_2R antagonist ranitidine, or H_3R antagonist ABT-239 (Esbenshade et al., 2005) exhibited any effects on carrageenan-induced thermal hyperalgesia when tested at the *in vivo* pharmacological relevant doses.

Intra-articular injection of sodium monoiodoacetate into the knee joint of rats has been used to induce morphological and behavioral changes that mimic OA pain in humans [Combe et al., 2004; Guingamp et al., 1997]. A behavioral endpoint that measures grip force in rats has been characterized for several classes of drugs with demonstrated clinical efficacy in the management of pain associated with OA [Chandran et al., 2009; Pomonis et al., 2005]. In this model, JNJ7777120 exhibited significant restoration of grip force performance. To our knowledge, this represents the first demonstration of the effectiveness of a H_4 antagonist in a model of chronic OA pain. In this model, immediately following MIA injection there is an elevation in the levels of prostaglandins, which return to basal levels by day 7 post MIA injection [Pulichino et al., 2006]. Histological assessment also demonstrates the presence of inflammation early on following MIA injection and a subsequent resolution of the inflammation [Chandran et al., 2009]. Thus the anti-nociceptive effects of JNJ7777120 could be attributed to the anti-inflammatory action of H_4 receptor antagonism. Additionally, the H_4 receptor expression is up-regulated in synovial cells obtained from rheumatoid arthritic patients [Ohki et al., 2007]. Osteoarthritis is by far the most common type of degenerative arthritis and afflicts millions of people worldwide (Read and Dray, 2008). Relief from chronic joint pain secondary to osteoarthritis, often refractory to currently available therapeutics, remains an area of significant unmet need (Read and Dray, 2008). Thus, our results provide evidence that selective H_4 receptor antagonists may be an attractive approach for the development of new drugs for the treatment of osteoarthritic pain.

Systemic administration of JNJ7777120 also produced dose-dependent anti-allodynic effects in two models of neuropathic pain and a model of acute post-operative pain. There are reports of H_4R expression in the CNS areas [Connelly et al., 2009; Liu et al., 2001; Cogé et al., 2001]. Our laboratories had also recently provided the evidence that the strong gene expression of H_4 mRNA was detected in the human and rat spinal cords, rat DRG, and these observations were confirmed by the immunohistochemical analysis of H_4 receptors expression on neurons in the rat lumbar DRG and in the lumbar spinal cord (Strakhova et al., 2009). However, the potencies in these models were weaker as compared to the inflammatory pain models. The reasons for differential potency of JNJ7777120 and the mechanisms of H_4R underlying these pain models are unknown. The extent of hyperalgesia may be related to the extent of the inflammatory response at the site of injury in animal models of neuropathic pain [Clatworthy et al., 1995]. Peripheral afferents can be sensitized by inflammatory mediators, a number of which are released by inflammatory leukocytes, which accumulate around the lesion site in an injured peripheral nerve [Perkins and Tracey, 2000; Clatworthy et al., 1995]. There is considerable evidence that inflammatory processes in the injured nerve contribute to neuropathic pain [Eliav et al., 1999; Cui et al., 2000]. Mast cells may all contribute to neuropathic symptoms in animal models, since their degranulation or depletion reduces neuropathic hyperalgesia [Thacker et al., 2007; Perkins and Tracey, 2000]. Interestingly, H_4R expression has also been reported in peripheral nerves [Nakaya et al., 2004].

Similarly in a skin-incision model of post-operative pain, JNJ7777120 was efficacious in reversing allodynia (2 h post-incision). Histamine release is recognized as a common event during surgery and our results provide additional support for the potential of H_4 antagonist in the management of post-surgical pain. These results provide the evidence of H_4 receptor as a potential target for acute pain.

JNJ7777120 readily crossed the blood–brain barrier. The efficacious CNS concentrations of systemic JNJ7777120 administration were higher than *in vitro* specific competition binding affinities, possibly due to nonspecific binding and unknown drug concentration at the receptor level in the CNS tissues. These pharmacokinetic/pharmacodynamic discrepancies are also observed in several *in vivo* behavioral models in rats (Medhurst et al., 2007). Further characterization to determine whether the central mechanism (at spinal or supra-spinal site) is involved in the anti-nociceptive action of H_4 receptor antagonism is currently being examined in our laboratory.

Rotarod performance test was employed to demonstrate that the increase of paw withdraw latency and paw withdraw threshold following the administration of JNJ7777120 in animal pain models were not due to impaired motor function of these animals. Impaired motor coordination in animals can affect the ability of animals to move their paws, and therefore, may influence the paw withdraw latency and paw withdraw threshold during the pain efficacy testing. However, JNJ7777120 at the doses (30, 70, and 100 mg/kg i.p.) that produced efficacy in both the inflammatory or neuropathic preclinical pain models were devoid of motor coordination deficit of movement in rotarod performance assay. The increase in paw withdraw latency and paw withdraw threshold following the administration of JNJ7777120 in pain models is likely due to specific anti-hyperalgesic and anti-allodynic effects mediated by H_4R antagonist. These are in agreement with the observations that JNJ7777120 had no effect on paw withdrawal latency of the contralateral non-inflamed paw in the carrageenan model (Fig. 3A), indicative of a specific anti-hyperalgesic action of JNJ7777120, in spite of its potential adverse effects observed in rotarod performance at high doses. Effects of JNJ7777120 on exploratory behavior as determined by locomotion activity were unexpected and the underlying mechanism is unclear. Exploration behavior is measured as a change in locomotion in response to a novel environment and there are a variety of factors influencing the level of exploratory behavior, including arousal level, attention, learning, memory, and fear of novelty a variety [Leussis and Bolivar, 2006], therefore, the reduction of exploratory behavior by JNJ7777120 might not be a "true" motor deficit and not necessarily affect paw withdrawal latencies or thresholds. As the effects of H_4 antagonist JNJ7777120 (at effective doses) on locomotion activity are the first of its kind and therefore, time spent adjacent to wall and time in the central area in the open field were also recorded in the present study. Lack of effects on these two measurements predictive of fear-avoidance in a novel environment [Leussis and Bolivar, 2006] would imply that JNJ7777120 might simply produce locomotor deficits as indicated by horizontal crossings.

In conclusion, results from the present *in vivo* behavioral studies, using a potent and selective antagonist of H_4 receptors, have demonstrated that this histaminergic receptor subtype is involved in pain transmission in rats and that H_4 receptor antagonism exhibits anti-nociceptive effects in a broad range of the preclinical pain models Although more work is needed, the current results show that H_4R antagonist may have therapeutic potential for the treatment of distinctly different types of mechanistically histamine-dependent pain.

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