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JNJ-20788560 [9-(8-Azabicyclo[3.2.1]oct-3-ylidene)-9H-xanthene-3-carboxylic Acid Diethylamide], a Selective Delta Opioid Receptor Agonist, Is a Potent and Efficacious Antihyperalgesic Agent That Does Not Produce Respiratory Depression, Pharmacologic Tolerance, or Physical Dependence

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ABSTRACT

μ-Opioid analgesics are a mainstay in the treatment of acute and chronic pain of multiple origins, but their side effects, such as constipation, respiratory depression, and abuse liability, adversely affect patients. The recent demonstration of the upregulation and membrane targeting of the δ-opioid receptor (DOR) following inflammation and the consequent enhanced therapeutic effect of δ-opioid agonists have enlivened the search for δ-opioid analgesic agents. JNJ-20788560 [9-(8-azabicyclo-[3.2.1]oct-3-ylidene)-9H-xanthene-3-carboxylic acid diethylamide] had an affinity of 2.0 nM for DOR (rat brain cortex binding assay) and a naltrindole sensitive DOR potency of 5.6 nM (5′-O-(3-[³⁵S]thio)triphosphate assay). The compound had a potency of 7.6 mg/kg p.o. in a rat zymosan radiant heat test and of 13.5 mg/kg p.o. in a rat Complete Freund's adjuvant RH test but was virtually inactive in an uninflamed radiant heat test. In limited studies, tolerance was not observed to the antihyperalgesic or antinociceptive effects of the compound. Unlike ibuprofen, JNJ-20788560 did not produce gastrointestinal (GI) erosion. Although morphine reduced GI motility at all doses tested and reached nearly full effect at the highest dose, JNJ-20788560 did not retard transit at the lowest dose and reached only 11% reduction at the highest dose administered. Unlike morphine, JNJ-20788560 did not exhibit respiratory depression (blood gas analysis), and no withdrawal signs were precipitated by the administration of opioid (μ or δ) antagonists. Coupled with the previously published lack of self-administration behavior of the compound by alfentanil-trained primates, these findings strongly recommend δ-opioid agonists such as JNJ-20788560 for the relief of inflammatory hyperalgesia.

The μ-opioid receptor (MOR) is the core target of marketed opioid analgesics, but the side effect profile and abuse liability propensity of these compounds impels the search for better and safer analgesic agents. Although the δ-opioid receptor (DOR) has long been known to mediate at least a modest level of antinociception (Heyman et al., 1986; Rodriguez et al., 1986), recent work on its regulation during inflammation has provided new impetus to its targeting for the relief of inflammatory pain. Under basal conditions, DOR is located predominantly intracellularly, whereas MOR is localized

ABBREVIATIONS: MOR, μ-opioid receptor; DOR, δ-opioid receptor; CFA, complete Freund's adjuvant; SB-235863, [8R-(4bS*,8aa,8ab,12bb)]-7,10-dimethyl-1-methoxy-11-(2-methylpropyl)oxycarbonyl 5,6,7,8,12,12b-hexahydro-(9h)-4,8-methanobenzofuro[3,2-e]pyrrolo[2,3-g]isoquinoline hydrochloride; SNC-80, (+)-4-[α-[(2S,5R)-2,5-dimethyl-4-(2-propenyl)-1-piperazinyl]-3-methyl]-N,N-diethylbenzamide; NSAID, nonsteroidal anti-inflammatory drug; GI, gastrointestinal; JNJ-20788560, 9-(8-azabicyclo[3.2.1]oct-3-ylidene)-9H-xanthene-3-carboxylic acid diethylamide; DPDPE, [D-Pen²,D-Pen⁵]-enkephalin; [³⁵S]GTPγS, 5′-O-(3-[³⁵S]thio)triphosphate; SNL, spinal nerve ligation; ANOVA, analysis of variance; MAIT, mouse abdominal irritant test; KOR, κ-opioid receptor; U-69593, (+)-(5α,7α,8β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide; CNS, central nervous system; ADL5859, N,N-diethyl-4-(5-hydroxyspiro[chromene-2,4′-piperidine]-4-yl)benzamide.

mainly on the cell membrane. Induction of inflammation produces a dramatic change in DOR; 18 h after the administration of Complete Freund's adjuvant (CFA) into rat paw, DOR immunogold labeling in laminae III to V of the ipsilateral lumbar spinal cord revealed up-regulation and membrane targeting of the receptor (Cahill et al., 2003). Importantly, these anatomical changes are accompanied by an increase in the potency of the δ opioid D-[Ala^2,Glu^4]-deltorphin II, administered intrathecally (Cahill et al., 2003). Furthermore, such potency increases in δ (but not μ)-agonist-mediated antihyperalgesic effects are seen with deltorphin administration into the rat rostral ventromedial medulla (Hurley and Hammond, 2000) as well as the oral dosing of SB-235863 (Petrillo et al., 2003). The enhanced amelioration of inflammatory pain by δ opioids was extended to primates in a thermal study in which inflammogens (CFA or prostaglandin E2) were administered into the tail, resulting in a 5- to 7-fold increase in the potency of the δ-opioid agonist SNC-80 after administration of the inflammatory agents (Brandt et al., 2001).

In addition to mediating potent analgesia, MOR agonists are associated with numerous side effects, such as constipation and respiratory depression. Their use is further associated with the potential for abuse, and their discontinuation gives rise to withdrawal symptoms. These μ-opioid-mediated side effects limit the effective treatment of patients in pain (Sundaresan et al., 1989; Gebhart et al., 2000). Some studies suggest that DOR agonists elicit milder side effects [constipation (Petrillo et al., 2003) and respiratory depression (Dondio et al., 2001)] or attenuate certain of the undesirable effects (Lee et al., 1993; O'Neill et al., 1997; Su et al., 1998) of μ opioids. Another class of widely used analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), have a propensity to produce gastrointestinal (GI) irritation, ulceration, and bleeding, leading to numerous deaths (http://www.healthsentinel.com/files/research/WordFile/ToxicAndDeadlyNSAIDs.doc).

The present study is an investigation of the antinociceptive and antihyperalgesic properties of a small-molecule δ-opioid agonist and an examination of its side effect properties. JNJ-20788560 was found to be antihyperalgesic in several preclinical models of inflammatory hyperalgesia. In limited studies, tolerance to this effect did not develop, nor were withdrawal signs precipitated upon antagonist administration. The compound did not induce GI erosion, elicit convulsions, or induce self-administration behavior in alfentanil-trained primates, and produced only a moderate decrease in GI motility and respiratory depression at high doses, suggesting a vastly improved therapeutic index for this δ-opioid agonist.

Materials and Methods

Materials

Chemicals. JNJ-20788560, shown in Fig. 1, was synthesized in Johnson & Johnson Pharmaceutical Research and Development Laboratories, and its hydrochloride salt was used in all of the present studies. Radiolabeled compounds were obtained from PerkinElmer Life and Analytical Sciences (Boston, MA); MgCl$_2$ and Tris were from Thermo Fisher Scientific (Pittsburgh, PA); sucrose was from EM Scientific (Gibbstown, NJ); [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin, DPDPE, GDP, CFA, and carrageenan were from Sigma-Aldrich (St. Louis, MO); SNC-80, TAN67, naloxone, and naltrindole were from
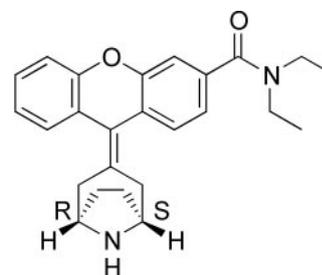

Fig. 1. Structure of JNJ-20788560.

Tocris Bioscience (Ellisville, MO); and morphine was from Spectrum Pharmaceuticals, Inc. (Irvine, CA).

Animals. All experimental procedures were approved by the Animal Care and Use Committee of Johnson & Johnson Pharmaceutical Research and Development and were in accordance with the *Guidelines for the Care and Use of Laboratory Animals* of the National Research Council and The International Association for the Study of Pain. Details regarding species and strain used are given with each separate procedure.

In Vitro Opioid Receptor Binding and Functional Assays

Opioid Receptor Binding. These assays were performed essentially as described previously. Male, Wistar rats (150–250 g, VAF; Charles River Laboratories, Inc., Kingston, NY) were killed by cervical dislocation, and their brains were removed and placed immediately on dry ice. On the day of the experiment, brains were removed from their −80°C storage and placed in ice-cold Tris HCl buffer (50 mM, pH 7.4). The forebrains were separated from the remainder of the brain by a coronal transection, beginning dorsally at the colliculi and passing ventrally through the midbrain-pontine junction. After dissection, the forebrains were homogenized in Tris buffer in a Teflon-glass homogenizer. The homogenate was diluted to a concentration of 1 g of forebrain tissue per 80 ml of Tris and centrifuged at 39,000g for 10 min. The pellet was resuspended in the same volume of Tris buffer containing 5 mM MgCl$_2$ with several brief pulses from a Polytron homogenizer. This particulate preparation was used for the opioid binding assays. After incubation with 2 to 3 nM of the δ-selective ligand [^{3}H]DPDPE or 0.8 nM of the μ-selective ligand [^{3}H][D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin at 25°C for 2.5 h in a 96-well plate with a total volume of 1 ml, the plate contents were filtered through Wallac filtermat B sheets on a Tomtec 96-well harvester. The filters were rinsed three times with 2 ml of 10 mM HEPES, pH 7.4, and dried in a microwave oven. To each sample area, BetaPlate Scint scintillation fluid (LKB) was added, and the resulting radioactivity was quantified on an LKB (PerkinElmer Life and Analytical Sciences–Wallac Oy, Turku, Finland) 1205 BetaPlate liquid scintillation counter. K_d and K_i values were calculated using GraphPad Prism (GraphPad Software Inc., San Diego, CA).

Agonist-Stimulated GTPγS Binding Assays. These assays were performed essentially as described previously (Codd et al., 2006). Membranes from endogenously DOR expressing NG-108 cells or recombinant μ-opioid receptor expressing Chinese hamster ovary cells (CHO-hMOR) were purchased from Receptor Biology, Inc. (Baltimore, MD). A vial containing 1 ml of membrane was added to 15 ml of cold binding assay buffer comprising 50 mM HEPES, pH 7.6, 5 mM MgCl$_2$, 100 mM NaCl, 1 mM dithiothreitol, and 1 mM EDTA, homogenized with a Polytron homogenizer, and centrifuged at 3000 rpm for 10 min. The supernatant was then centrifuged at 18,000 rpm for 20 min. The pellet was resuspended with a Polytron homogenizer in 10 ml of assay buffer.

The receptor-containing membranes were preincubated in the assay buffer with wheat germ agglutinin-coated SPA beads (Amersham, Chalfont St. Giles, UK) at 25°C for 45 min. The SPA bead-coupled membranes were then incubated with 0.5 nM [^{35}S]GTPγS; each assay well contained 1 mg of SPA beads and 3 to 5 μg of

membrane protein. The basal binding is that taking place in the absence of added test compound; this unmodulated binding is considered as 100%, with agonist-stimulated binding rising to levels significantly above this value. A range of concentrations of receptor agonists was used to stimulate [^{35}S]GTPγS binding. Both basal and nonspecific binding were tested in the absence of agonist; the nonspecific binding determination included 10 μM unlabeled GTPγS. Radioactivity was quantified on a Packard TopCount, and the percent stimulation was calculated as:

$$\frac{\text{(Test compound counts per minute} - \text{nonspecific counts per minute)}}{\text{(Basal counts per minute} - \text{nonspecific counts per minute)}} \quad (1)$$

EC$_{50}$ values were calculated using GraphPad Prism.

Hamster Vas Deferens Functional Assay. The field-stimulated vas deferens has been used as an in vitro functional assay for δ-opioid activity (McKnight et al., 1985). Segments of hamster vas deferens were suspended in 20-ml organ baths containing an oxygenated (95% O$_2$ and 5% CO$_2$) and prewarmed (37°C) physiological salt solution containing 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM KH$_2$PO$_4$, 25 mM NaHCO$_3$, and 11 mM glucose, pH 7.4. Yohimbine (1 μM) and atropine (1 μM) were also present throughout the experiments to block α$_2$-adrenergic and muscarinic receptors, respectively. The tissues were connected to force transducers for isometric tension recordings. They were stretched to a resting tension of 0.5 g and then allowed to equilibrate for 60 min, during which time they were washed repeatedly, and the tension was readjusted. Thereafter, appropriate electrical stimulation was applied to the tissues using a constant current stimulator. The experiments were carried out using semiautomated isolated organ systems possessing eight organ baths, with multichannel data acquisition. JNJ-20788560 was studied for its effect on this tissue, and SNC-80 and the peptidic δ agonist DPDPE were studied as δ-opioid reference compounds.

Antinociceptive Studies: Acute Studies

Rat Zymosan and CFA Radiant Heat Tests. Rats were injected with either zymosan A (100 μl of 25 mg/ml) or CFA (100 μl of 1:1 CFA/saline, 100 μl) subcutaneously into the subplantar tissue of the left hind paw to stimulate an inflammatory reaction. Three (zymosan) or 24 h (CFA) later, the response time of the animal to a thermal stimulus (Hargreaves et al., 1988) was re-evaluated and compared to the animal's baseline response time (preceding zymosan or CFA treatment). Only rats that exhibited at least a 25% reduction in response latency from baseline were included in further analysis. After post-treatment latency assessment, rats were orally administered test compound or vehicle (hydroxypropylmethylcellulose). Percentage reversal of hyperalgesia was calculated for each animal as (treatment response − postzymosan or CFA response)/(prezymosan or CFA response − postzymosan or CFA response) × 100. Therefore, a return to normal pretreatment thresholds was equal to 100% reversal. Average percentage reversal of hyperalgesia was then calculated for each treatment group ($n = 6-8$ rats/group). ED$_{50}$ values and associated statistics were calculated using PharmTools Plus software (The McCary Group, Inc., Schnecksville, PA).

Rat Yeast-Induced Mechanical Hyperalgesia Test. Inflammation was induced by subplantar injection of a 3% brewer yeast suspension (0.1 ml) into the right hind paw of male, Sprague-Dawley rats. Three hours later, mechanical nociceptive threshold (Randall and Selitto, 1957) was assessed with the paw pressure test with a vocalization endpoint. Compounds were orally administered 1 h before testing.

Mouse Abdominal Irritant Test. The procedure used was that described by Collier et al. (1968), with minor modifications. Thirty minutes after the oral administration of test drug, CD-1 mice received an intraperitoneal injection of 5.5 mg/kg acetylcholine bromide. The mice were then placed into large glass jars and were

continuously observed for the first occurrence of a characteristic behavioral response (twisting and elongation of the body extending throughout the hind limbs) within a 10-min observation period. The percent inhibition of this response was calculated as follows:

$$(\%) \text{ inhibition} = 100 \times \frac{\text{(number of nonresponders)}}{\text{(number of animals in group)}} \quad (2)$$

To query the mechanism underlying the activity of μ and δ opioids in this test, an antagonist study was conducted. Saline (vehicle), naloxone (1 mg/kg s.c.), or naltrindole (3 mg/kg s.c.) was administered 20 min before the oral administration of the test compound. The effect of agonists in the presence of vehicle was compared to the effect in the presence of antagonists.

Rat Spinal Nerve Ligation. The SNL model is one of several established animal models of neuropathic pain (Kim and Chung, 1992). Ligation of the L$_5$ dorsal root induces long-lasting allodynia in the corresponding hindpaw, which can be measured with von Frey probes. The time course and dose dependence of JNJ-20788560 was evaluated in the rat SNL model of neuropathic pain.

Repeated Dose Studies/Tolerance Evaluation

Rat CFA Radiant Heat Test. The development of tolerance is a common sequel to the repeated administration of μ-opioid agonists. To evaluate whether tolerance develops to the analgesic effect of the δ-opioid agonist JNJ-20788560, a multiple-day study design was undertaken using the CFA radiant heat hyperalgesia model. Study design in this model is challenging, as testing of the antihyperalgesic effect of a compound is typically carried out approximately 24 h after CFA injection. The compromise study design was that vehicle or compound (30 mg/kg p.o.) was administered to Sprague-Dawley rats for 5 days, and the level of their response compared to that when compound was dosed for only 2 days, days 4 and 5. CFA was administered i.pl. on day 3, after the administration of compound or vehicle. Thermal latency measures were obtained before dosing on each days 1 and 4 and after dosing on day 5.

Mouse Abdominal Irritant Test. The mouse abdominal irritant test was used as a second model to probe the potential for tolerance development to the antinociceptive effect of JNJ-20788560. Groups of 20 CD-1 mice were treated in one of the following four sequences: 1) no treatment for 4 days, followed by vehicle on day 5; 2) no treatment for 4 days, followed by JNJ-20788560 on day 5; 3) vehicle for 4 days, followed by JNJ-20788560 on day 5; or 4) JNJ-20788560 for 5 days. The abdominal constriction response of each group of mice was assessed 30 min after drug administration on day 5, and the responses of the groups were compared.

Side Effect Studies

Potential for GI Erosion. Because some nonopioid analgesics, particularly NSAIDs, have a propensity to produce GI irritation, ulceration, and bleeding, JNJ-20788560 was evaluated in a rat model of GI erosion, using ibuprofen as the positive control and celecoxib as a comparator. JNJ-20788560 (test compound) and celecoxib (comparator compound) were studied at several doses across their pharmacologically effective dose range, whereas ibuprofen (positive control) was included at a single dose within its therapeutic range, known from previous work to induce lesions. Lesion scores, a composite index that includes both the number and size of gastric lesions, were determined for each animal in each treatment group.

Potential for Effect on GI Motility. μ-Opioid agonists have a well known propensity to reduce GI motility, an effect quantifiable in animal models. The mouse upper GI transit test was conducted to evaluate the effects of JNJ-20788560 as well as μ- and δ-opioid reference compounds on the movement of chyme in the mouse small intestine (Nagakura et al., 1996; Baldrick et al., 1998). Thirty minutes after compound dosing, a carmine dye solution was orally administered as a marker. One hour later, mice were euthanized and the small intestine was removed. The length of the intestine and the

distance traversed by the carmine dye were measured. The mean values were used to calculate the percentage of the upper GI tract that was traversed.

Because the fecal output of rodents is produced in discrete pellets, their output can be easily quantified as a test of the effect of compounds on colonic motility. After acclimation to the test cage, CD-1 mice were dosed with vehicle (Methocel), morphine, or JNJ-20788560, and the number of pellets produced was quantified.

Potential for Respiratory Depression. Acute administration of μ-opioid agonists dose-dependently reduces respiration, with consequent blood gas and pH changes. To evaluate the respiratory effects of JNJ-20788560 in comparison to those of morphine, a blood gas study was conducted in rats. Measured as indices of respiratory status were blood pH, PCO_2, and PO_2, sampled at 1 and 2 h after dosing.

Other Opioid Side Effects. In rats administered JNJ-20788560 or morphine orally, the cornea and pinna reflexes were scored every 30 min on a scale of 0 to 3, in which a score of 0 indicated normal muscle tone, whereas a score of 3 indicated the absence of the reflex.

Abuse Liability Evaluation

Precipitated Withdrawal. The administration of a μ-opioid antagonist such as naloxone to human or rodent subjects that have repeatedly received μ-opioid agonists induces characteristic withdrawal symptoms. JNJ-20788560 and morphine were evaluated in parallel in mouse and rat withdrawal models to determine whether JNJ-20788560 exhibited morphine-like withdrawal signs or any other withdrawal behaviors, the term "any unusual" behaviors being used to include both of these behavioral categories.

Naloxone-precipitated jumping in mice receiving a single, relatively high dose of morphine, is a well established model of μ-opioid withdrawal (Smits, 1975). This model was employed in a design in which saline, the μ-opioid agonist morphine, or the δ-opioid agonist JNJ-20788560 was administered subcutaneously (50 mg/kg). Three hours later, saline, naloxone (10 mg/kg i.p.), or naltrindole (30 mg/kg i.p.) was administered. The CD-1 mice were placed in glass bell jars wherein withdrawal jumping was assessed for 10 min, and behavioral observations were made for 15 min postantagonist administration.

Precipitated withdrawal was also elicited and characterized in Sprague-Dawley rats following a subchronic dosing paradigm for each saline, morphine, and JNJ-20788560. Agonists were administered subcutaneously in an ascending dosing schedule from 5 to 20 mg/kg, administered twice daily for 4 days. Three hours after the last agonist dose, naloxone or naltrindole was administered subcutaneously (1 mg/kg); wet dog shakes were counted for 10 min, and behavioral observations were made for 15 min postantagonist administration.

Statistics

Data are expressed as the means ± S.E.M. where possible. The zymosan, CFA, and nociceptive radiant heat data were analyzed by ANOVA, followed by a Bonferroni post hoc test. Multidose antihyperalgesic (CFA) test results were analyzed by repeated measures ANOVA, followed by Bonferroni's Multiple Comparison Test. The multidose mouse abdominal irritant test (MAIT) study was analyzed by Chi square. The MAIT antagonist study was analyzed with Fisher's Exact Test. The GI erosion data were analyzed with the Kruskal-Wallis test, and the difference from vehicle was evaluated with Dunn's multiple comparison test. The GI transit test results were analyzed with ANOVA followed by a Tukey-Kramer post hoc test. Comparison of the fecal pellet output in vehicle- and drug-treated groups was made with an ANOVA followed by a post test for linear trend. The blood gas data were analyzed by ANOVA followed by Dunnett's post hoc test. The minimal criterion for significance was taken as $P < 0.05$.

Results

In Vitro Studies

Opioid Receptor Binding and Agonist-Stimulated GTPγS Binding Functional Evaluation. Table 1 contains the δ- and μ-opioid receptor binding affinity as well as GTPγS potency and relative efficacy for JNJ-20788560 and the δ-opioid reference compounds DPDPE, TAN67, and SNC-80. All four compounds exhibited low nanomolar affinity for DOR (SNC-80 > TAN67 > JNJ-20788560 > DPDPE) and were DOR selective over MOR (~85–900-fold). Moreover, in the DOR GTPγS assay, JNJ-20788560 exhibited a relative efficacy close to that of the full agonist SNC-80, whereas DPDPE and TAN67 were partial agonists (Table 1). Both JNJ-20788560- and SNC-80-stimulated GTPγS binding were reduced to basal levels by the relatively nonselective opioid antagonist naloxone and the δ-selective antagonist naltrindole (data not shown).

JNJ-20788560 was also evaluated at the κ-opioid receptor (KOR), for which it had a binding affinity of approximately 1 μM. In a GTPγS functional assay, its KOR EC_{50} was approximately 10 μM. Thus, JNJ-20788560 was DOR selective over KOR as well as MOR.

Hamster Vas Deferens Functional Assay. The δ-agonist reference compounds SNC-80 and DPDPE functioned as agonists in the hamster vas deferens assay, and their inhibition of field-stimulated contraction was blocked by the δ-selective antagonist naltrindole (Fig. 2). Because its inhibition appeared to reach a plateau lower than that of SNC-80, JNJ-20788560 appeared to be a partial agonist in this assay. The inhibition of electrically stimulated contractions by JNJ-20788560 was partially blocked by naltrindole.

Antinociceptive Studies

Acute Studies: Rat Zymosan Radiant Heat Test. Evaluated at an oral dose of 30 mg/kg, JNJ-20788560 significantly lengthened postzymosan thermal response latencies,

TABLE 1

Binding affinity and GTPγ S potency and relative efficacy of JNJ-20788560 and selected δ-opioid reference compounds

Binding assays were conducted in membranes prepared from rat brain cortex, whereas the functional assay was conducted in NG-108 cell membranes.

Compound	K_i			δ Opioid GTPγS	
	δ Opioid	μ Opioid	δ Selectivity	EC_{50}	Relative Efficacy[a]
	nM			*nM*	
JNJ-20788560[b]	2.0	1191	596	5.6	0.92
DPDPE	4.5	380	84	38	0.62
TAN 67	1.3	282	216	6.0	0.61
SNC-80	0.38	341	897	5.4	1.0

[a] The maximal effect of each compound is expressed relative to the maximal effect of SNC-80.
[b] The κ opioid K_i value for JNJ-20788560 (vs. [^{3}H]U-69593) was ~1 μM, and the κ opioid GTPγ S EC_{50} was ~10 μM. Both assays used membranes prepared from hKOR-CHO-K1 cells in a filtration assay.
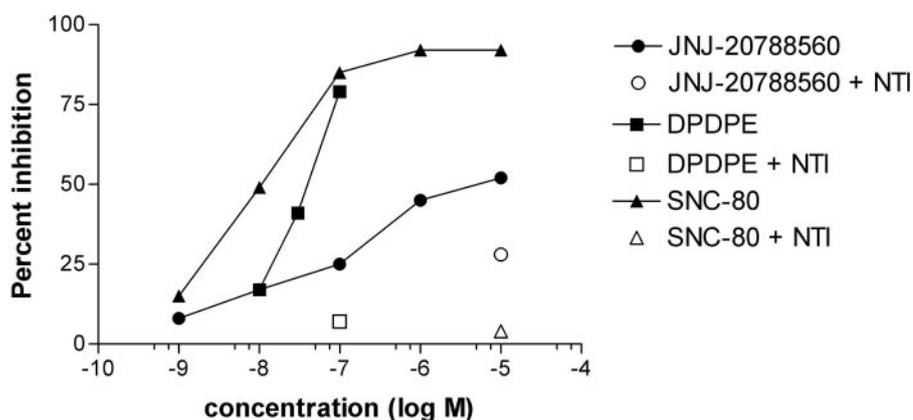
Fig. 2. Hamster vas deferens bioassay of JNJ-20788560 and δ opioid reference agonists DPDPE and SNC-80. Naltrindole was used at a concentration of 30 nM. Shown is the maximal change in the amplitude of the electrically evoked twitch contractions induced by each compound concentration. The values shown are the mean of duplicate determinations.

whereas the effect of morphine at the same dose did not reach significance. The time of peak effect for JNJ-20788560 was 30 min (Fig. 3A), and its ED_{50} value was 7.64 ± 6.48 mg/kg p.o. (Table 2).

Rat CFA Radiant Heat Test. JNJ-20788560 exhibited a similar time of peak effect in the rat CFA radiant heat test, reaching a near full antihyperalgesic effect (return to baseline latency) at a dose of 30 mg/kg p.o., as did indomethacin

(Fig. 3B). The reversal of inflammatory hyperalgesia by JNJ-20788560 was dose-dependent (Fig. 3C), with an ED_{50} value of 13.5 ± 5.7 mg/kg p.o. (Table 2).

Rat Yeast-Induced Mechanical Hyperalgesia Test. JNJ-20788560 dose-dependently reversed mechanical hyperalgesia induced by yeast pretreatment, with an ED_{50} value of approximately 0.3 mg/kg (Table 2); full reversal of the hyperalgesia was accomplished at a dose of 30 mg/kg. At a dose of
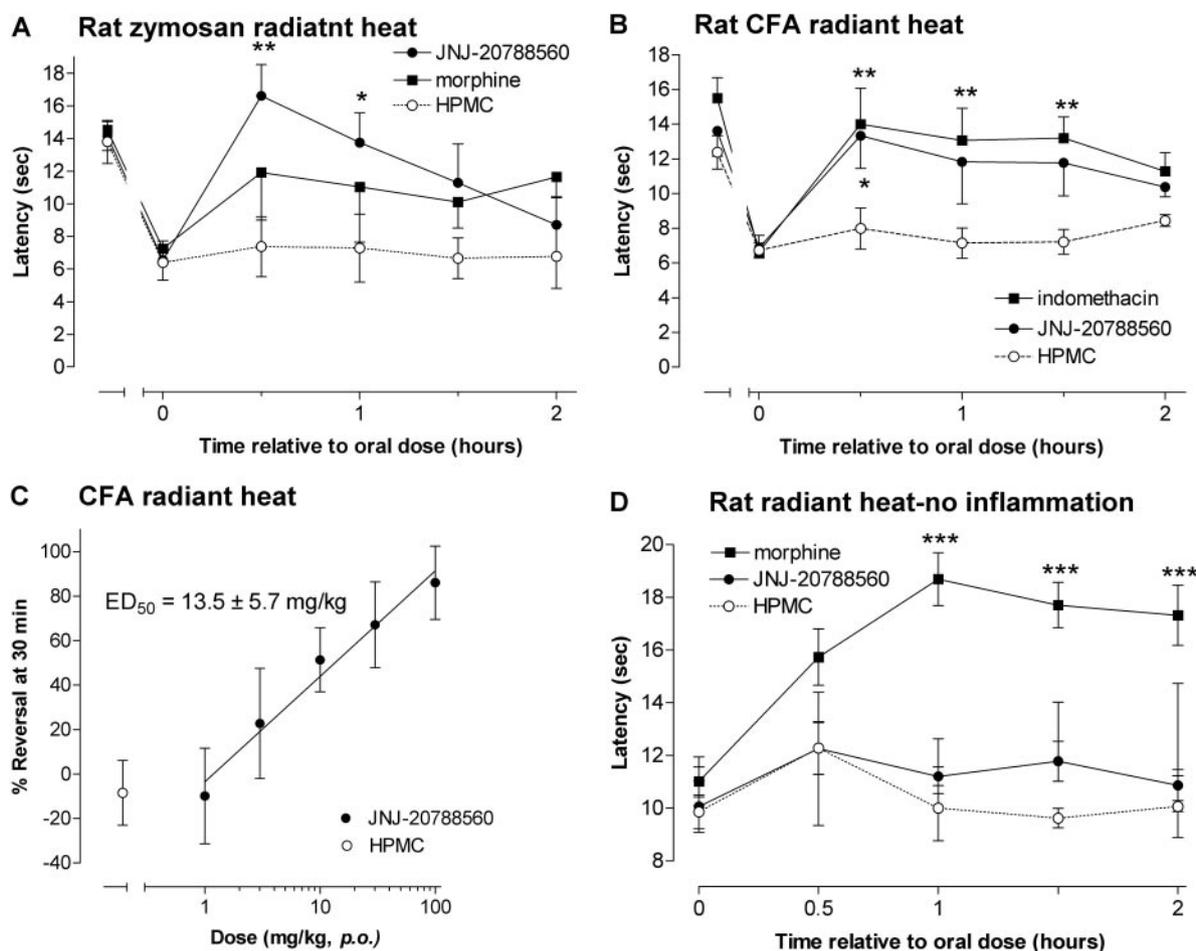

Fig. 3. Antihyperalgesic effect of JNJ-20788560 in several animal models of pain. A, time course of antihyperalgesic effect of JNJ-20788560 and morphine (each at 30 mg/kg p.o.) in the rat zymosan radiant heat test; ($n = 4$–6 rats/group). B, time course of antihyperalgesic effect of JNJ-20788560 and indomethacin (each at 30 mg/kg p.o.) in the rat CFA radiant heat test ($n = 5$–6 12 rats/group). C, dose response of JNJ-20788560 in the rat CFA radiant heat test 30 min after oral administration of the compound ($n = 8$–10 rats per dose group). D, time course of JNJ-20788560 and morphine (each at 30 mg/kg p.o.) in the radiant heat test of acute nociception ($n = 6$–8 rats per dose group). For each model, the values shown represent the means $\pm$ S.E.M. Significant difference from hydroxypropylmethylcellulose vehicle noted as: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

TABLE 2
Potency of JNJ-20788560 in various animal models

Model, Endpoint	Species	Time of Peak Effect	ED$_{50}$ ($\pm$S.E.M.)
			mg/kg p.o.
Zymosan, radiant heat	Rat	30 min	7.64 $\pm$ 6.48
CFA, radiant heat	Rat	30 min	13.5 $\pm$ 5.7
Yeast-induced hyperalgesia, paw pressure	Rat	60 min	~0.3
Abdominal irritant test, stretching	Mouse	30 min	14.9 $\pm$ 3.8
Spinal nerve ligation, tactile allodynia	Rat	60 min	~100

30 mg/kg p.o., celecoxib also fully reversed the yeast-induced hyperalgesia, whereas indomethacin was analgesic, elevating response thresholds above baseline at an oral dose of 10 mg/kg p.o.

Rat Nociceptive Radiant Heat Test (Uninflamed). JNJ-20788560 and morphine were also evaluated in a nociceptive radiant heat test, i.e., a test in which inflammation was not induced before drug administration. Whereas morphine was antinociceptive in this model, JNJ-20788560 only minimally elevated the response latency (Fig. 3D).

MAIT. In a time course study, JNJ-20788560 exhibited its peak effect in the acetylcholine bromide-induced abdominal irritant test at 30 min following its oral dosing, with the percent inhibition declining at 60 and 120 min. The effect was dose-dependent (Fig. 4A), with an ED$_{50}$ value of 14.9 $\pm$ 3.8 mg/kg p.o. (Table 2). At the highest dose tested, 116 mg/kg p.o., no unusual behavioral responses were observed.

In MAIT, the antinociceptive effect of morphine was significantly reduced by the opioid antagonist naloxone (Fig. 4B), whereas the action of SNC-80 was only slightly and nonsignificantly reduced by either naloxone or by the δ-selective antagonist naltrindole. The antinociception induced by JNJ-20788560 was not significantly attenuated by either naloxone or naltrindole. These data suggest that SNC-80 and JNJ-20788560 have an additional, extra-δ-opioid mechanism of action that contributes to their antinociceptive effect in
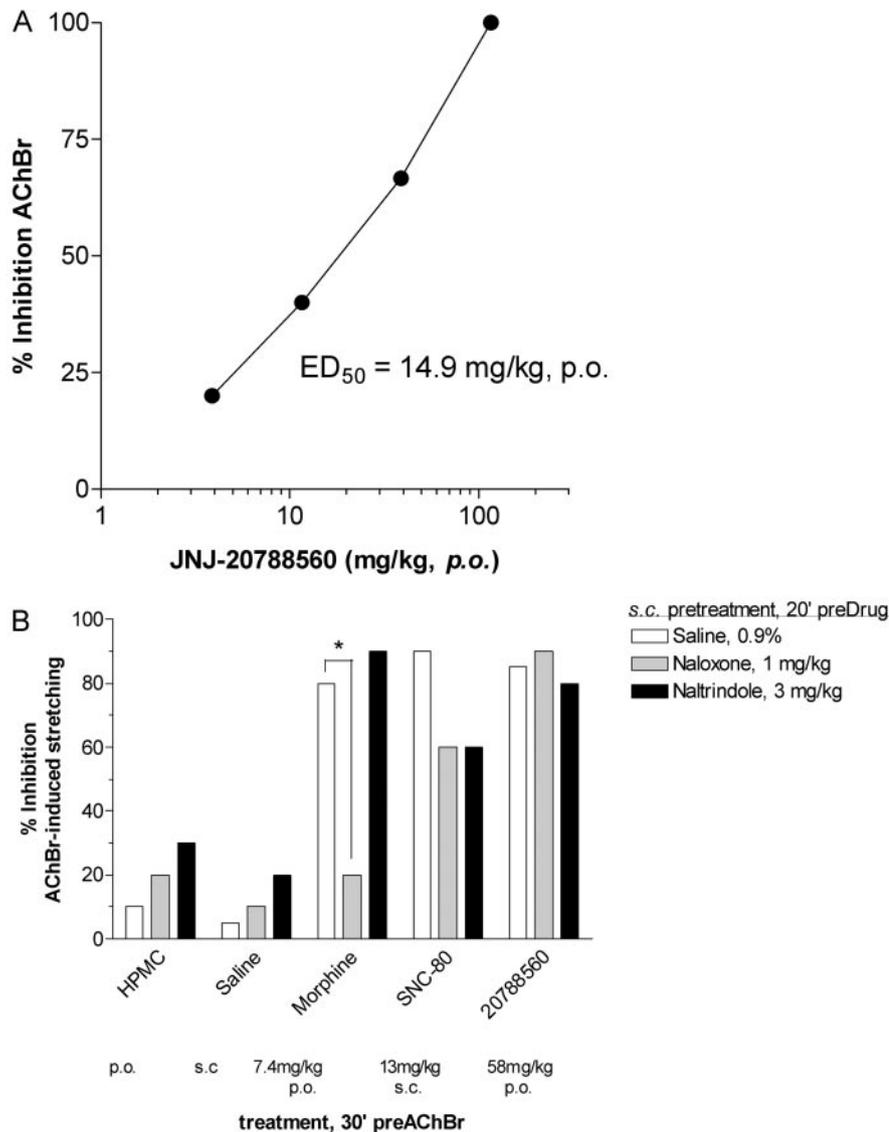

Fig. 4. Effect of JNJ-20788560 in the MAIT. A, dose-response relationship of JNJ-20788560 in the mouse abdominal irritant test ($n = 15$ mice per dose group). B, antagonist probe of morphine, SNC-80, or JNJ-20788560 induced antinociception in mouse abdominal irritant test ($n = 10–20$).

MAIT or that the δ-opioid receptor engaged by these compounds to produce antinociception in this test is largely naloxone and naltrindole insensitive.

Rat Spinal Nerve Ligation. In the rat SNL model of neuropathic pain, JNJ-20788560 exhibited its maximal antiallodynic effect at 1 h after oral dosing. The antiallodynic effect of the compound was dose-dependent, with an ED_{50} value of approximately 100 mg/kg p.o. (Table 2) and a maximal possible effect value of 72% at a dose of 300 mg/kg.

Repeated Dose Studies/Tolerance Evaluation

The potential for the development of tolerance to the efficacy of JNJ-20788560 was studied in two models, an inflammatory hyperalgesia and nociceptive model.

Rat CFA Radiant Heat Test. To evaluate the effect of repeated dosing of JNJ-20788560, the compound was dosed for 5 days. Response latencies shown in Fig. 5 were obtained before drug treatment on days 1 and 4, as well as after JNJ-20788560 treatment on day 5. Twenty-four hours after CFA administration, thermal response latencies were significantly decreased compared to the pretreatment latencies. Administration of JNJ-20788560 on day 5, following its administration for 4 days, significantly elevated response latencies, normalizing the response latency to predose levels. Thus, tolerance to the antihyperalgesic effect of JNJ-20788560 does not appear to rapidly develop in this model, with the compound continuing to fully reverse the CFA-induced hyperalgesia after 5 days of dosing.

Mouse Abdominal Irritant Test. To determine whether tolerance develops to the antinociceptive effect of JNJ-20788560 in the mouse abdominal irritant test, mice were administered vehicle or drug for 5 days, according to the scheme shown in Table 3. Under either dosing regimen, JNJ-20788560 significantly reduced responding to irritant administration. There was no significant difference in the extent of response attenuation by an ED_{50} dose of JNJ-20788560 after 1 or 5 days of dosing (see Chi square analysis summary in Table 4). Thus,
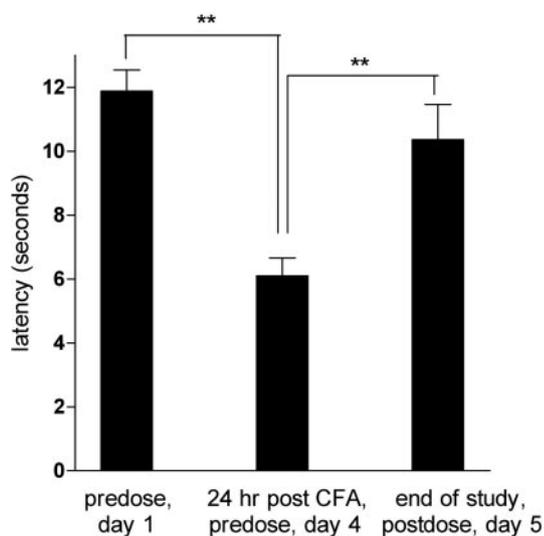

Fig. 5. Thermal response latencies during 5-day oral administration of 30 mg/kg JNJ-20788560 or vehicle to rats before and after the induction of inflammation by intraplantar CFA. Latencies were quantified immediately before drug administration on day 1 (predose) and on day 4 (24 h post-CFA). The "end of study" latency was the average of 30- and 60-min postdrug latencies on day 5. Shown are means ± S.E.M. n = 12 rats/group. **, $P < 0.001$.

administration of JNJ-20788560 for 5 days did not result in the development of tolerance to its antinociceptive effect in the mouse abdominal irritant test.

Side-Effect Evaluation

Potential for GI Erosion. Because some nonopioid NSAID analgesics have a propensity to produce GI irritation, ulceration, and bleeding, JNJ-20788560 was evaluated in a rat model of GI erosion, using ibuprofen as the positive control and celecoxib as a comparator. Lesion scores, a composite index including both the number and size of gastric lesions, showed that ibuprofen but not celecoxib produced a significant number of lesions. JNJ-20788560 produced no gastric lesions (Table 5).

Potential for Effect on GI Motility. *Mouse upper GI transit test.* Because marketed opioid analgesics produce constipation, JNJ-20788560 and morphine were evaluated in animal models of GI motility. JNJ-20788560 did not inhibit upper GI transit at the lowest dose tested (1 mg/kg) and inhibited upper GI transit only mildly to moderately (22% at 10 mg/ml and 33% at 100 mg/ml) at the higher doses tested (Fig. 6A). In contrast, morphine dose-dependently reduced upper GI transit, beginning at the lowest dose tested (1 mg/kg p.o.) and at the highest dose tested (100 mg/kg p.o.) reduced transit to a level that was only 11% of control. Thus, JNJ-20788560 dose-dependently reduced upper GI transit, but to an extent significantly less than that of morphine at each dose tested.

Fecal pellet output test. Evaluation of the effect of morphine and of JNJ-20788560 was also conducted in the fecal pellet output test in mice. Fecal output following dosing of JNJ-20788560 at 1, 10, and 100 mg/kg did not significantly differ from that of vehicle-treated animals, and no dose dependence in the response was observed (Fig. 6B). Fecal output following the dosing of morphine at 1, 10, and 100 mg/kg showed a trend toward dose-dependent reduction (Fig. 6B), but due to the variation in the control output, this dose dependence did not reach the level of significance. Therefore, JNJ-20788560 did not appear to attenuate colonic motility in mice, whereas morphine tended to exhibit a dose-dependent effect.

Potential for Respiratory Depression. When administered orally to rats, morphine but not JNJ-20788560 dose-dependently reduced blood pH and PO_2 and elevated PCO_2 (Fig. 7). For each index, the effects of morphine were statistically different from the corresponding vehicle measures at doses of 30 and 100 mg/kg, whereas these parameters in JNJ-20788560-treated rats did not differ from the corresponding values in vehicle-treated rats at any drug dose tested. Thus, under these conditions, JNJ-20788560 did not appear to alter respiratory indices.

Convulsions. Importantly, no convulsions have been observed in the many studies conducted (not all of which are described herein) on JNJ-20788560 administered intravenously, subcutaneously, or orally at pharmacologic doses or at doses in the toxicologic range to mouse, rat, guinea pig, dog, or primate. These studies, for example, included oral administration to rat of up to 1000 mg/kg JNJ-20788560 (producing drug plasma levels of 2200 ng/ml) and 20 mg/kg i.v. to dog (producing drug plasma levels of 24,000 ng/ml) (data not shown).

Other Opioid Side Effects. Evaluated orally in rat up to a dose of 40 mg/kg, JNJ-20788560 did not impair corneal or

TABLE 3

MAIT 5-day study

To probe the potential for tolerance development, mice were dosed for 1 or 5 days with JNJ-20788560, according to the scheme shown in this table. Thirty minutes after dosing on day 5, the abdominal constriction response of the mice to acetylcholine bromide was assessed. The percentage inhibition of this response in each treatment group is shown. $n = 20$ mice/treatment.

Group	Day 1	Day 2	Day 3	Day 4	Day 5	No. of MAIT Responders (Day 5)	MAIT Inhibition
							%
1	x	x	x	x	V	19 of 20	5
2	x	x	x	x	D	13 of 20	35
3	V	V	V	V	D	12 of 20	40
4	D	D	D	D	D	15 of 20	25

D, JNJ-20788560, dosed at $ED_{50} = 38.4$ μmol/kg p.o.; V, hydroxypropyl methocellulose, 10 ml/kg; x, not dosed.

TABLE 4

Chi square test results for MAIT 5-day study

To probe the potential for tolerance development, mice were dosed for 1 or 5 days with JNJ-20788560, according to the scheme shown in Table 3. Thirty minutes after dosing on day 5, the abdominal constriction response of the mice to acetylcholine bromide was assessed. The significance of differences between the groups (χ^2 analysis) is shown. $n = 20$ mice/treatment.

Comparison of Treatments	Significance at the 0.05 Level
1 vs. 2	Significant
1 vs. 3	Significant
3 vs. 4	Not significant
1 vs. 2-3-4	Significant

TABLE 5

Evaluation of the potential of ibuprofen, celecoxib, and JNJ-20788560 to produce gastric mucosal lesions in fasted rats

Shown are the mean ± S.E.M.

Treatment	Dose	n	Mean Lesion Score ± S.E.M.
	Mg/kg p.o.		
Vehicle	5 ml/kg	10	0 ± 0
JNJ-20788560	10	10	0 ± 0
JNJ-20788560	30	10	0 ± 0
JNJ-20788560	100	10	0 ± 0
Ibuprofen	200	10	2.2 ± 0.6*
Celecoxib	10	10	0.1 ± 0.1
Celecoxib	30	10	0 ± 0
Celecoxib	100	10	0 ± 0
Celecoxib	300	10	0.4 ± 0.2

* $P < 0.05$.

pinna reflexes, whereas morphine had ED_{50} values of 26 and 15 mg/kg p.o., respectively, at these end points.

Abuse Liability Evaluation

Precipitated Withdrawal. Behaviors precipitated by administration of an opioid antagonist to mice, previously administered the μ-opioid agonist morphine or the δ-opioid agonist JNJ-20788560, were evaluated. As shown in Table 6, morphine-treated mice exhibited circling and Straub tail, behaviors that persisted following the subsequent administration of vehicle or naltrindole. Dosing with naloxone precipitated characteristic withdrawal jumping. In contrast, mice administered JNJ-20788560 did not exhibit any of these behaviors or any other aberrant behaviors before or after dosing with the vehicle or either antagonist.

Rats were also administered increasing doses of morphine or JNJ-20788560 twice a day for 4 days. Administration of naloxone precipitated wet dog shakes, salivation, teeth chattering, and diarrhea in morphine-treated rats. However, none of these μ-opioid-associated behaviors or any other aberrant behaviors was observed following the administration of either naloxone or naltrindole to JNJ-20788560-treated rats (Table 7).
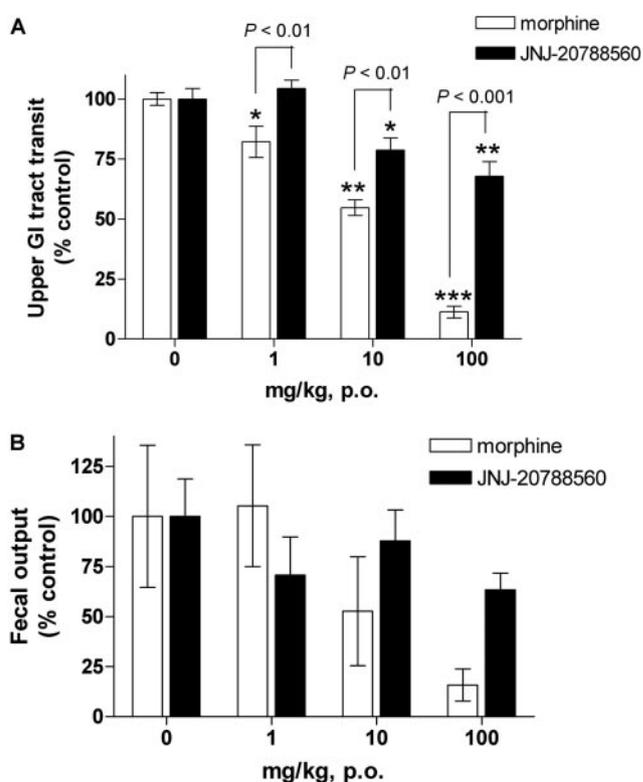

Fig. 6. GI effects of JNJ-20788560. A, effect of JNJ-20788560 and morphine on upper GI transit in mice. Data are means ± S.E.M., $n = 9$. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. B, effect of JNJ-20788560 and morphine on mouse fecal pellet output. No dose-dependent effect of JNJ-20788560 was observed, whereas a trend for increased morphine reduction of fecal output with dose was noted. $P = 0.0172$. Data are means ± S.E.M., $n = 10$.

Discussion

Although μ-opioid agonists, such as morphine, are considered the "gold standard" in the treatment of numerous painful conditions, their side effect liabilities, including tolerance development, constipation, respiratory depression, and abuse potential, limit their usefulness. Here, we report that JNJ-20788560, an orally bioavailable δ-opioid agonist, with 600-fold selectivity versus the μ-opioid receptor and 500-fold selectivity versus the κ-opioid receptor, is antihyperalgesic in several preclinical models of inflammatory hyperalgesia and exhibits an in vivo pharmacologic profile that clearly differentiates it from μ-opioid analgesics.

The analgesic characteristics of JNJ-20788560 are similar to those of several other nonpeptide DOR agonists, such as SNC-80 (Bilsky et al., 1995), TAN67 (Kamei et al., 1995, 1997), SB-235863, ARM390 (http://www.iddb3.com/iddb3/
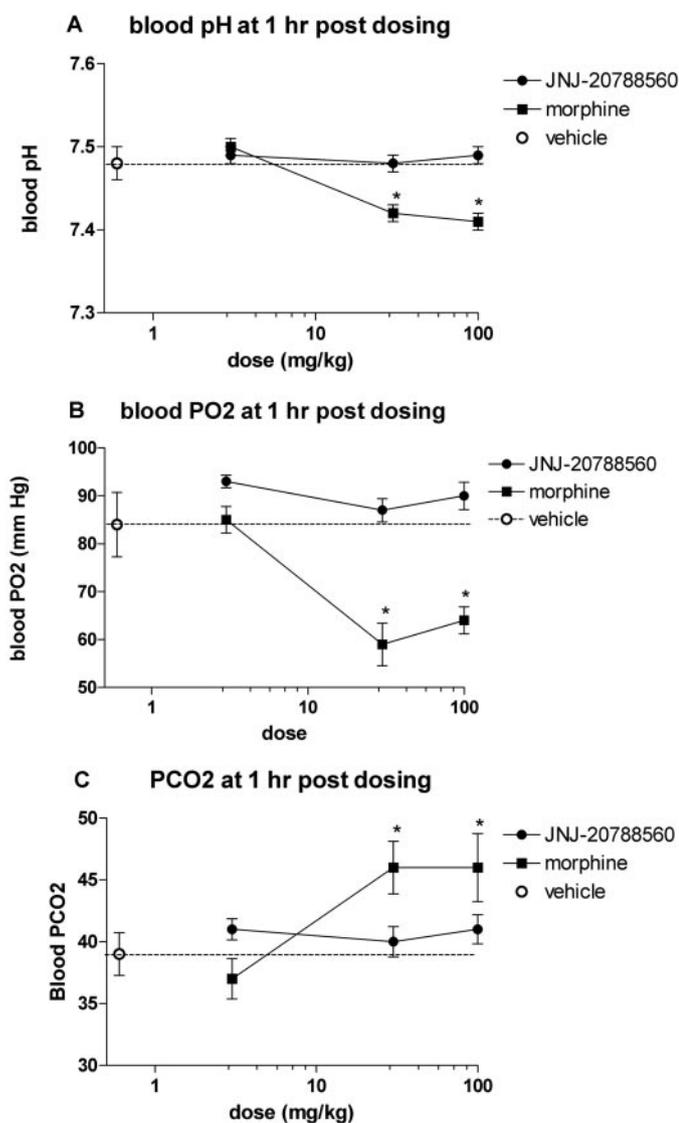
Fig. 7. Effect of JNJ-20788560 and morphine on respiratory parameters, evaluated by blood gas measurements in rat. A, blood pH. B, blood PO_2 levels. C, blood PCO_2 levels. Shown are the means ± S.E.M. ∗, $P < 0.05$. n = 6/group.

TABLE 6

Withdrawal study in mice

Opioid-like behavior and withdrawal study in mice

Agonist	Behavior	Percentage of Mice Exhibiting Behavior		
		Vehicle	Naltrindole	Naloxone
Morphine	Circling	100	80	0
	C-shaped tail	25	60	0
	Straub tail	25	40	0
	Jumping	0	0	60
JNJ-20788560	Any unusual[a]	None	None	None

[a] Includes μ -opioid-specific behaviors (itemized for morphine) and any other aberrant behavior.

iddb3_2/reports.display?id=498397&template=Meeting&i_query_id=10037933; http://www.iddb3.com/iddb3/iddb3_2/reports.display?id=25779&template=Drug&i_query_id=10038200), and ADL5859 (Le Bourdonnec et al., 2008), which are also potent at thermal or mechanical endpoints in models of inflammatory hyperalgesia. In contrast, JNJ-20788560 and other DOR agonists have little or no antinoci-

TABLE 7

Withdrawal study in rats

Behaviors during withdrawal study in rats.

Agonist	Behavior	Percentage of Rats Exhibiting Behavior	
		Naltrindole	Naloxone
Morphine	Wet dog shakes	NT[a]	50
	Salivation		33
	Teeth chattering		50
	Diarrhea		17
JNJ-20788560	Any unusual[b]	None	None

[a] Not tested.
[b] Includes μ -opioid-specific behaviors (itemized for morphine) and any other aberrant behavior.

ceptive effect in rodent models such as hot-plate, tail-flick, and noninflamed radiant heat. However, an exception has been achieved in some cases following central compound administration (i.e., intracerebroventricular or intrathecal) of SNC-80 (Bilsky et al., 1995), TAN67 (Tseng et al., 1997), or ARM390 (http://www.iddb3.com/iddb3/iddb3_2/reports.display?id=498397&template=Meeting&i_query_id=10037933; http://www.iddb3.com/iddb3/iddb3_2/reports.display?id=25779&template=Drug&i_query_id=10038200), raising the possibility that DOR agonists at high CNS exposures are antinociceptive. An alternative possibility is that DOR selectivity is compromised at the CNS drug levels achieved via these routes of administration.

A few studies have demonstrated limited efficacy of DOR agonists in preclinical neuropathic pain models. For example, SB-235863 was effective at a thermal but not at a tactile endpoint in the partial sciatic nerve ligation model. In addition, TAN67 exhibited greater analgesic potency in mice treated with streptozotocin, a diabetic model, than in normal mice (Kamei et al., 1995, 1997). Here, JNJ-20788560 proved effective at a tactile endpoint in the spinal nerve ligation model, albeit at higher doses than those found effective in inflammatory thermal and mechanical hyperalgesia models. Thus, whereas some DOR agonists have demonstrated the potential for treating certain types of neuropathic pain, more extensive investigations using a greater battery of compounds are required. Ultimately, whether or not a given DOR agonist comprises such potential may depend on its relative ability to engage one or more signaling mechanisms, as described below.

Perhaps because the proconvulsant effects of the DOR agonist SNC-80 have been so extensively reported (Bilsky et al., 1995; Jutkiewicz et al., 2005) and were found to be antagonized by naltrindole, this action has been presumed to be a class effect. However, the studies of other DOR agonists [SB-235863, ADL5859 (Le Bourdonnec et al., 2008), ARM390 (http://www.iddb3.com/iddb3/iddb3_2/reports.display?id=25779&template=Drug&i_query_id=10038200), earlier JNJ compounds (Carson et al., 2004; Coats et al., 2004)] as well as the present investigations with JNJ-20788560 have not revealed proconvulsant effects. Importantly, these several nonproconvulsant DOR agonists comprise diverse structural types, including morphinan, octahydroisoquinoline, tricyclic, and spiro and diarylmethylenepiperidine scaffolds. Although the etiology of the disparities in seizure profiles is not known, it is possible that these structurally variant DOR agonists may induce signaling via divergent cellular pathways, leading to their different pharmacologic phenotypes. Differential signaling pathways consequent to activation by

diverse agonists have been documented for other G protein-coupled receptors (for review, see Galandrin et al., 2007) and may arise through agonist-directed trafficking of DOR, differential rates of receptor return to the cell surface (Feschenko et al., 2006), or differential interactions with various DOR-containing receptors, including homo- and heterodimers, which may comprise δ1 and δ2 receptors (Bilsky et al., 1995; Kamei et al., 1997). Moreover, seizure induction may be dependent on the rate of compound administration and CNS penetration and/or the maximal concentration achieved at certain sites of action. In any case, the vast preponderance of available data suggests that DOR agonist-induced convulsions may be the exception rather than the rule.

Tolerance to the antihyperalgesic or antiallodynic effect of SB-235863 or ARM390 was not observed during 7 days of dosing in inflammatory or neuropathic pain models (http://www.iddb3.com/iddb3/iddb3_2/reports.display?id=25779&template=Drug&i_query_id=10038200), similar to findings following the multiday dosing of JNJ-20788560 in the CFA model of inflammatory hyperalgesia. Collectively, these studies suggest that, in contrast to MOR agonists, DOR agonists have a much lower propensity to produce pharmacological tolerance. A definitive conclusion to this effect may be precluded insofar as the dosing scheme used here may not have been optimized to produce tolerance, even though the same schedule is sufficient to demonstrate tolerance development to a MOR agonist, such as morphine. Moreover, interpretation of the lack of tolerance development in the mouse abdominal irritant test is mitigated by the fact that the analgesia produced by JNJ-20788560 in this model was not substantially antagonized by naltrindole. Nonetheless, the prospect that a DOR agonist analgesic would not demonstrate clinically meaningful tolerance that would require dose escalation or analgesic substitution warrants further study.

For the amelioration of pain involving tissue injury and inflammation, NSAIDs are a frequent clinical choice. Although often considered mild analgesics, NSAIDs can be superior to μ-opioid analgesics in some postoperative pain situations (Hardman and Limbird, 2001). Unfortunately, NSAIDs may have serious GI side effects, especially induction of ulceration and bleeding, sometimes resulting in death (Bystrianyk, 2002), and even the selective COX-2 agent celecoxib carries a black box warning for GI ulceration and bleeding. Because the δ-opioid agonist JNJ-20788560 offers an alternative mechanism of action to the NSAIDs for relief of pain of inflammatory origin, we evaluated the GI ulceration propensity of JNJ-20788560 alongside that of the traditional, nonselective COX inhibitor, ibuprofen. Importantly, JNJ-20788560, unlike ibuprofen, produced no GI lesions in a rat model used to predict clinical GI liability, suggesting a significantly improved clinical GI side effect profile. Moreover, marked slowing of GI transit was not exhibited by JNJ-20788560, ARM390 (http://www.iddb3.com/iddb3/iddb3_2/reports.display?id=498397&template=Meeting&i_query_id=10037933), or SB-235863. Lastly, JNJ-20788560 did not produce respiratory depression, as evidenced by blood gas and pH measurements. Taken together, these studies suggest that DOR agonists may exhibit a much improved safety and tolerability profile compared with NSAID and μ-opioid analgesics.

JNJ-20788560-treated rats and mice did not exhibit μ-opioid-associated behaviors or other unusual behaviors, nor did they exhibit withdrawal behaviors consequent to antagonist (naloxone or naltrindole) administration. Studied in parallel, however, morphine-treated mice and rats exhibited characteristic behaviors and withdrawal signs. Thus, JNJ-20788560 did not appear to induce the muscular rigidity that characterizes μ-opioid analgesics, nor did its administration engage a physiologic process that led to a withdrawal syndrome. In a related study, SNC-80 did not substitute for morphine in withdrawal studies in primates, nor did it exacerbate withdrawal from morphine (http://www.pharmacy.umaryland.edu/faculty/acoop/decfolder/an%20rep%20um&VCU/vcu%2099rpt.doc).

The evaluation of JNJ-20788560 (NIH-11211) in rhesus monkeys trained to self-administer alfentanil has been published (http://www.pharmacy.umaryland.edu/faculty/acoop/decfolder/an%20rep%20um&VCU/UMannrep2007-final%20(2).pdf). In these studies, increasing doses of alfentanil led to escalating rates of self-administration, the exact rate varying with the individual primate studied. Neither saline nor JNJ-20788560 maintained self-administration in any monkey studied; the response to JNJ-20788560 was similar to the response to saline or the lowest dose of alfentanil. Thus, JNJ-20788560 did not appear to have reinforcing effects under the conditions studied. Likewise, SNC-80 did not produce reinforcing effects in rhesus monkeys trained to self-administer heroin (Stevenson et al., 2005). Thus, the triad of pharmacological tolerance, physical dependence, and self-administration commonly associated with MOR agonists appears to be virtually absent with JNJ-20788560, at least to the extent and under the conditions evaluated here.

In summary, JNJ-20788560 is antihyperalgesic in inflammatory pain models, a profile characteristic of several other nonpeptide DOR agonists. Importantly, the compound was virtually devoid of side effects elicited by commonly utilized analgesics, such as respiratory depression, abuse potential, GI irritation, and slowing of motility, and lacked a propensity to produce convulsions. Collectively, these characteristics suggest the usefulness of δ-opioid agonists for the relief of certain types of pain, particularly those of inflammatory origin.

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