3-[5-(3,4-Dichloro-phenyl)-1-(4-methoxy-phenyl)-1H-pyrazol-3-yl]-2-m-tolyl-propionate (JNJ-17156516), a Novel, Potent, and Selective Cholecystokinin 1 Receptor Antagonist: In Vitro and in Vivo Pharmacological Comparison with Dexloxiglumide

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ABSTRACT

3-[5-(3,4-Dichloro-phenyl)-1-(4-methoxy-phenyl)-1H-pyrazol-3-yl]-2-m-tolyl-propionate (JNJ-17156516) is a novel, potent, and selective cholecystokinin (CCK)1-receptor antagonist. In this study, the pharmacology of JNJ-17156516 was investigated both in vitro and in vivo, and the pharmacokinetic profile was evaluated in rats. JNJ-17156516 expressed high-affinity at the cloned human (pKᵢ = 7.96 ± 0.11), rat (pKᵢ = 8.02 ± 0.11), and canine (pKᵢ = 7.98 ± 0.04) CCK1 receptors, and it was also highly selective for the CCK1 receptor compared with the CCK2 receptor across the same species (~160-, ~230-, and ~75-fold, respectively). The high affinity of JNJ-17156516 at CCK1 receptors in vitro was confirmed in radioligand binding studies on fresh human gallbladder tissue (pKᵢ = 8.22 ± 0.05). In a functional in vitro assay of guinea pig gallbladder contraction, JNJ-17156516 behaved as a competitive antagonist, with a pKᵢ value of 8.00 ± 0.07. In vivo, JNJ-17156516 produced a parallel, rightward shift in the CCK-8S-evoked contraction of the guinea pig gallbladder. The dose required to shift the CCK-8S dose-response curve was 240 nmol kg⁻¹ i.v. In the anesthetized rat, JNJ-17156516 produced a dose-related decrease in the number of duodenal contractions evoked by infusion of CCK-8S, with an ED₅₀ = 484 nmol kg⁻¹. Pharmacokinetic analysis of JNJ-17156516 in rats, revealed that JNJ-17156516 had a half-life of 3.0 ± 0.5 h and a very high bioavailability (108 ± 10%) in this species. Overall, we have demonstrated that JNJ-17156516 is a high-affinity selective human CCK1 receptor antagonist with good pharmacokinetic properties in rats.

Cholecystokinin (CCK) was originally identified in the gastrointestinal tract where it was shown to mediate contraction of the gallbladder (Ivy and Oldberg, 1928). Subsequent purification and peptide sequencing of a cholecystokinin-containing extract from the hog intestinal mucosa (Jorpes and Mutt, 1966) revealed that CCK was a 33-amino acid peptide, identical to the hormone implicated in pancreatic enzyme secretion (pancreozymin). In addition to these functions, CCK has been shown, in humans, to mediate stomach motility (Liddle et al., 1986; Meyer et al., 1989; Straathof et al., 1998), stimulate colonic motility (Snape et al., 1978; Renny et al., 1983), and increase the occurrence of transient lower esophageal sphincter relaxations (Boulant et al., 1997). CCK mediates its effects through two receptor subtypes, the CCK1 and CCK2 receptors (formerly CCK-A and CCK-B). These receptors are guanine nucleotide-coupled receptors, and they share approximately 48% amino acid homology (Noble et al., 1999). The CCK1 and CCK2 receptors were first discriminated pharmacologically based on differences in the affinity of the hormones gastrin and CCK (Innis and Snyder, 1980; Sankaran et al., 1980). Subsequently, it has been shown that the CCK1 receptor mediates many of the peripheral functions of CCK, including gastrointestinal motility

ABBREVIATIONS: CCK, cholecystokinin; IBS, irritable bowel syndrome; JNJ-17156516, 3-[5-(3,4-dichloro-phenyl)-1-(4-methoxy-phenyl)-1H-pyrazol-3-yl]-2-m-tolyl-propionate; CHO, Chinese hamster ovary; HEK, human embryonic kidney; BH-CCK-8S, Bolton Hunter-sulfated cholecystokinin octapeptide; E/[A], concentration-effect; HAC, high-amplitude contraction; YF476, (R)-1-[2,3-dihydro-2-oxo-1-pivaloylmethyl-5-(2'-pyridyl)-1H-1,4-benzodiazepin-3-yl]-3-(3-methyl-phenyl)urea; L-364-718, 3S(-)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide.
and pancreatic enzyme secretion, whereas the CCK2 receptor is a key regulator of gastric acid secretion (Noble et al., 1999). Due to the physiological actions of CCK and the potential therapeutic value of modulating its activity, a number of CCK receptor-selective antagonists have been developed (Herranz, 2003). Of these, loxiglumide and its single enantiomer (S)-3,4-dichloro-benzoylamo-4-[(3-methoxy-propyl)-pentyl-carbamoyl]-butyric acid (dexloxiglumide) have demonstrated efficacy in humans for the treatment of irritable bowel syndrome (IBS), pancreatitis, and functional dyspepsia (Herranz, 2003). However, in two additional placebo-controlled phase III clinical studies of 12-week treatment duration, involving more than 1400 women with constipation-predominant IBS, dexloxiglumide had no statistically significant effect, although a trend toward improvement was noted. It may be that the relatively short half-life of dexloxiglumide (2.3 h; Webber et al., 2003), complex pharmacokinetics (Persiani et al., 2002), and low affinity for the CCK1 receptor (human gallbladder pKᵢ = 6.95 ± 0.11; Maselli et al., 2001) did not result in sufficient CCK1 receptor blockade to be therapeutically useful (Persiani et al., 2002).

Here, we describe the primary pharmacological characterization of a novel CCK1 receptor antagonist, JNJ-17156516, which was arrived at by medicinal chemistry optimization of a novel CCK1 receptor antagonist, JNJ-17156516, involving more than 1400 women with constipation-predominant IBS, dexloxiglumide had no statistically significant effect, although a trend toward improvement was noted. It may be that the relatively short half-life of dexloxiglumide (2.3 h; Webber et al., 2003), complex pharmacokinetics (Persiani et al., 2002), and low affinity for the CCK1 receptor (human gallbladder pKᵢ = 6.95 ± 0.11; Maselli et al., 2001) did not result in sufficient CCK1 receptor blockade to be therapeutically useful (Persiani et al., 2002).

Inhibition of CCK-8S-Induced Guinea Pig Gallbladder Contraction Using JNJ-17156516. Male Hartley guinea pigs (300–650 g) were sacrificed by asphyxiation using a rising concentration of CO₂. A midline incision was made into the abdominal cavity, and the gallbladder was removed and cleared of any adherent liver and connective tissue. Tissue strips were dissected from the central band of the gallbladder in a circular orientation (1 × 4 mm; mucosa intact), and they were mounted in 20-ml organ baths containing Krebs-Henseleit solution (118 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.0 mM Na₂HPO₄, 25 mM NaHCO₃, and 10 mM d-glucose; Sigma Chemical, Poole, Dorset, UK) at 37°C and continuously gassed with O₂-CO₂ (95:5). A resting tension of 1 g was applied to the tissue strips, and they were then equilibrated for 1 h, during which time the buffer was replaced at 30-min intervals. CCK-8S concentration-effect (E/[A]) curves were obtained by cumulative dosing in each tissue, and once a maximal response was obtained, the preparations were washed by replacing the buffer at 10-min intervals until the response tension returned to baseline. Antagonists JNJ-17156516 (25, 63, 160, 400, and 1000 nM) and dexloxiglumide (0.1, 2.5, 6.3, and 16 μM) were equilibrated for 90 min before another CCK-8S E/[A] curve was obtained (repeat-curve design). This study design allowed the effect of the antagonist to be directly compared with the vehicle control on the same tissue preparation; therefore, it reduced any effect of tissue-to-tissue variation, which can occur in vitro bioassays of guinea pig gallbladder. In an initial study, the response to CCK-8S before and after 90-min equilibration with vehicle control was evaluated. This study revealed that there was no effect of 90-min incubation time on the midpoint location of paired CCK-8S E/[A] curves (first CCK-8S curve pᵦ₀₅₀ = 8.15 ± 0.21, n = 8; second CCK-8S curve pᵦ₀₅₀ = 8.02 ± 0.19, n = 8; p > 0.05). Contractile tissue responses were measured using isometric transducers, and they were displayed on a dual-channel flat-bed recorder (Kipp and Zonen, Deift, The Netherlands).

Materials and Methods

All procedures and experiments were performed according to the internationally accepted guidelines for the care and use of laboratory animals in research, and they were approved by the local Institutional Animal Care and Use Committee.

Cell Culture. Chinese hamster ovary-K cells that had undergone stable transfection with the human, rat, or canine CCK1 receptor or the rat and canine CCK2 receptor were maintained in Dulbecco's modified Eagle's medium (Ham's F-12) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 U ml⁻¹ penicillin, 50 μg ml⁻¹ streptomycin, and 0.6 mg ml⁻¹ Geneticin (G-418) for continuous selection (all materials from Invitrogen, Carlsbad, CA). For radioligand binding studies, the cells were harvested by cell scraping, and resulting pellets were immediately frozen at −80°C (approximately 50 × 10⁶ cells/pellet).

Radioligand Binding Studies. Frozen pellets of CHO cells, stably transfected with the CCK1 or CCK2 receptor of interest, were used. For the human CCK2 receptor assay, HEK cells that had undergone stable transfection with the human, rat, or canine CCK1 receptor or the rat and canine CCK2 receptor were maintained in Dulbecco's modified Eagle's medium (Ham's F-12) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 U ml⁻¹ penicillin, 50 μg ml⁻¹ streptomycin, and 0.6 mg ml⁻¹ Geneticin (G-418) for continuous selection (all materials from Invitrogen, Carlsbad, CA). For radioligand binding studies, the cells were harvested by cell scraping, and resulting pellets were immediately frozen at −80°C (approximately 50 × 10⁶ cells/pellet).

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Inhibition of CCK-8S-Induced Guinea Pig Gallbladder Contraction Using JNJ-17156516. Male Hartley guinea pigs (300–650 g) were sacrificed by asphyxiation using a rising concentration of CO₂. A midline incision was made into the abdominal cavity, and the gallbladder was removed and cleared of any adherent liver and connective tissue. Tissue strips were dissected from the central band of the gallbladder in a circular orientation (1 × 4 mm; mucosa intact), and they were mounted in 20-ml organ baths containing Krebs-Henseleit solution (118 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.0 mM Na₂HPO₄, 25 mM NaHCO₃, and 10 mM d-glucose; Sigma Chemical, Poole, Dorset, UK) at 37°C and continuously gassed with O₂-CO₂ (95:5). A resting tension of 1 g was applied to the tissue strips, and they were then equilibrated for 1 h, during which time the buffer was replaced at 30-min intervals. CCK-8S concentration-effect (E/[A]) curves were obtained by cumulative dosing in each tissue, and once a maximal response was obtained, the preparations were washed by replacing the buffer at 10-min intervals until the response tension returned to baseline. Antagonists JNJ-17156516 (25, 63, 160, 400, and 1000 nM) and dexloxiglumide (0.1, 2.5, 6.3, and 16 μM) were equilibrated for 90 min before another CCK-8S E/[A] curve was obtained (repeat-curve design). This study design allowed the effect of the antagonist to be directly compared with the vehicle control on the same tissue preparation; therefore, it reduced any effect of tissue-to-tissue variation, which can occur in vitro bioassays of guinea pig gallbladder. In an initial study, the response to CCK-8S before and after 90-min equilibration with vehicle control was evaluated. This study revealed that there was no effect of 90-min incubation time on the midpoint location of paired CCK-8S E/[A] curves (first CCK-8S curve pᵦ₀₅₀ = 8.15 ± 0.21, n = 8; second CCK-8S curve pᵦ₀₅₀ = 8.02 ± 0.19, n = 8; p > 0.05). Contractile tissue responses were measured using isometric transducers, and they were displayed on a dual-channel flat-bed recorder (Kipp and Zonen, Deift, The Netherlands).

Pharmacokinetic Properties in the Rat. The pharmacokinetic profiles of JNJ-17156516 and dexloxiglumide were assessed in male Sprague-Dawley rats (230–350 g) after oral and intravenous administration. The 2 μmol kg⁻¹ JNJ-17156516 or 20 μmol kg⁻¹ dexloxiglumide was administrated by oral gavage to animals that had been fasted for 18 h. An additional group of rats were given 2 μmol kg⁻¹ JNJ-17156516 i.v. or 20 μmol kg⁻¹ dexloxiglumide via the tail vein. All animals were allowed water ad libitum, and those fasted were refed 4 h following oral dosing. Blood samples (250 μl) were obtained from the tail vein at various times after dosing (six time points for the oral dosing study and seven time points for the iv. study). Samples were stored on melting ice (<30 min) until such time that the plasma could be collected by centrifugation. Plasma was collected and frozen at −20°C pending analysis of JNJ-17156516 or dexloxiglumide using high-pressure liquid chromatography/tandem mass
spectrometry in the electrospray-positive mode by selected reaction monitoring (ACE C18 column, 2 × 50 mm, 3- or 5-μm particle size; Phenomenex, Torrance, CA). The lower limit of quantification was 0.03 μM.

Pharmacokinetic-Pharmacodynamic Relationship in Anesthetized Rats: In Vivo Measurement of Duodenal Contractility. The potency of JNJ-17156516 and dexloxiglumide was assessed in anesthetized rats using a newly developed model (Freedman et al., 2006). In brief, male Sprague-Dawley rats (170–340 g) were anesthetized with 1 to 3% isoflurane in room air, and the jugular vein and carotid artery were cannulated to allow intravenous infusion of CCK-8S and measurement of blood pressure, respectively. The femoral vein was cannulated and used to administer the test compounds. A mid-line abdominal incision was made, and the stomach was exposed. Then, an incision was made in the stomach. A specially made balloon/catheter (−8 mm in diameter) was inserted into the duodenal bulb through the incision, across the pyloric sphincter. The catheter was attached to a pressure transducer, and the balloon was distended with saline to give a resting pressure of 5 mm Hg. The stomach and the peritoneal cavity were sutured closed and covered in saline-soaked gauze.

The contractile response of the duodenum to continuous infusion of CCK-8S exhibited tachyphylaxis; therefore, a protocol was developed in which a 1-nmol kg⁻¹ dose of CCK-8S was administered as a 1-min infusion through the jugular vein at 10-min intervals (Freedman et al., 2006). This resulted in a stable response to CCK-8S, and it was quantified by measuring the number of high-amplitude contractions (HACs) defined as an increase >5 mm Hg over baseline. To determine the effect of JNJ-17156516 and dexloxiglumide on the CCK-8S-induced contraction, the number of HACs was counted 30 min after the administration of antagonist. The effect of each treatment was expressed as the percentage of decrease of the number of HACs after antagonist treatment.

Pharmacokinetic-Pharmacodynamic Relationship in Anesthetized Guinea Pigs: In Vivo Measurement of Gallbladder Contraction. Male Hartley guinea pigs (350–550 g) were anesthetized with 1 to 3% isoflurane vaporized in clean, dry air. The right jugular vein and left carotid artery were cannulated for injection of drugs and measurement of blood pressure, respectively. The trachea was isolated, and a tracheostomy was performed for the administration of the anesthesia. The peritoneal cavity was opened along the rib line of the right side, and then the gallbladder was exposed and drained of its contents using a syringe. A saline-filled balloon was inserted into the lumen of the organ through a small incision. The gallbladder was then closed around the balloon using a 6-0 prolene suture (3-0 silk). The balloon was distended with saline to give a resting pressure of ~5 mm Hg, resulting in a final balloon diameter of ~5 to 10 mm. The pressure developed in the gallbladder was measured using a pressure transducer coupled to a digital recording system (PowerLab; ADInstruments Pty Ltd., Castle Hill, Australia). Dose-response curves to CCK-8S were constructed after intravenous administration of a range of CCK-8S doses (3 pmol–3 nmol kg⁻¹), and the peak developed pressure was taken as a measure of CCK1 receptor activation. The doses were administered when the previous response had returned to baseline (between 5 and 20 min). A single intravenous bolus dose of 2 μmol kg⁻¹ JNJ-17156516 or 20 μmol kg⁻¹ dexloxiglumide was administered after the first dose-response curve to CCK-8S. A second dose-response curve to CCK-8S was then constructed immediately after dosing, and a third curve 2 h later. Time matched controls were also included in these studies to assess whether the location of the CCK-8S dose-response curve shifted with consecutive dose-response curves.

Drugs. Dexloxiglumide, JNJ-17156516, 2-NAP, and YF476 were synthesized by Johnson & Johnson Pharmaceutical Research & Development L.L.C (La Jolla, CA). CCK-8S was obtained from Sigma-Aldrich (St. Louis, MO), and 125I-BH-CCK-8S was from PerkinElmer Life and Analytical Sciences. All general buffer reagents were from Sigma-Aldrich. For in vitro studies, all compounds were dissolved in dimethyl sulfoxide to give stock concentrations of 1 mM, and further dilutions were made in 50 mM Tris-HCl buffer. For in vivo studies, dexloxiglumide and JNJ-17156516 were administered via the intravenous route in a 20% hydroxypropyl-β-cyclodextrin solution, whereas for oral administration, a solution of water plus 1 equivalent of NaOH was used.

Data Analysis and Statistics. Values are represented as the mean ± S.E.M., n = 3 to 9. Statistical significance was determined using one-way analysis of variance (p < 0.05) followed by a Tukey test for multiple comparisons.

For the isolated in vitro gallbladder assay, individual E/[A] curve data were fitted to the following Hill equation (eq. 1), to provide estimates of midpoint location (IA50), maximal asymptote (a), and Hill slope (nH) parameters, where [A] is the agonist concentration, and E is the measured effect expressed in grams:

$$ E = \frac{a \times [A]^{nH}}{[A]^{nH} + [A]^{nH}} $$

Analysis of competitive antagonism, expressed as pK_H values was performed by direct model-fitting to the Gaddum-Schild equation as described by Black et al. (1985). For in vivo experiments, ED50 refers to the dose of the compound that produced a half-maximal effect. For radioligand competition-inhibition curve data and for in vivo analysis of the inhibition of duodenal and gallbladder contraction, concentration-response curve data were fitted to the following four-parameter general logistic function (eq. 2):

$$ B = \frac{a_{min} + (a_{max} - a_{min}) \times [1 + 10^{(logECC50 - log[IC50])}]}{1 + 10^{(logECC50 - log[IC50])}} $$

Results are presented as mean values ± S.E.M. Data between treatment groups were compared using Student’s two-tailed t tests with p < 0.05 being considered significant. All data were analyzed using the software package GraphPad Prism, version 3.01 (GraphPad Software Inc., San Diego, CA).

Noncompartmental analysis of JNJ-17156516 pharmacokinetics was performed using WinNonlin Professional, version 4.0.1 (Pharsight, Mountain View, CA). Individual plasma concentrations and sample times for each animal were used in the analysis. For intravenous administration of JNJ-17156516, terminal elimination half-life (t1/2), total area under the plasma concentration-time curve extrapolated to infinity, systemic plasma clearance, and steady-state volume of distribution were calculated by standard methods. After oral administration of JNJ-17156516, the peak plasma concentration (Cmax) and the time to Cmax (Tmax) were taken directly from individual profiles. The areas under the curve were calculated by standard methods as for the i.v. dosing, and the oral bioavailability was determined.

Results

Affinity and Selectivity of JNJ-17156516 and Dexloxiglumide at Human, Rat, and Canine CCK Receptors. JNJ-17156516 (see Fig. 1 for structure) and dexloxiglumide both produced a concentration-dependent displacement
of bound $^{125}$I-BH-CCK-8S from all CCK1 receptors expressed in cell lines (Fig. 2). There were no species-dependent differences in the affinity of JNJ-17156516 or dexloxiglumide at the cloned human, rat, and canine CCK1 receptors. Furthermore, in all these assays, the affinity of JNJ-17156516 (average $pK_A = 7.99 \pm 0.02$) was approximately 3-fold higher than dexloxiglumide (average $pK_A = 7.56 \pm 0.03$; see Table 1 for individual values). In human gallbladder membranes, this difference in affinity was increased to greater than 10-fold (JNJ-17156516, $pK_A = 8.22$ and dexloxiglumide $pK_A = 7.10$). JNJ-17156516 also expressed a higher affinity than dexloxiglumide at the human, rat, and canine CCK2 receptors (Table 1). Consequently, the degree of CCK1 receptor selectivity for JNJ-17156516 and dexloxiglumide was similar for the rat and canine receptors, with both being ~250-fold rat CCK1-selective and ~80-fold canine CCK1-selective. However, the fold selectivity for dexloxiglumide at the human CCK1 could not be accurately determined, because the $pK_I$ for this compound was below the limit of quantification at the human CCK2 receptors. Notwithstanding this, the most conservative estimate for human CCK1 versus CCK2 receptor selectivity for dexloxiglumide is ~340-fold (assuming a $pK_I = 5$) and for JNJ-17156516 the selectivity would be between 150 and 290 depending on which CCK1 radioligand binding assay was used (cloned expression system or human gallbladder binding).

Affinity of JNJ-17156516 and Dexloxiglumide in a Functional in Vitro Bioassay of CCK-8S-Induced Gallbladder Contraction. The nonselective CCK-receptor agonist CCK-8S produced concentration-dependent E/\([A]\) curves in guinea pig gallbladder tissue (Fig. 3; mean ± S.E.M.; midpoint location: $pA_{50} = 7.83 \pm 0.21$; Hill slope parameter: $n_H = 0.81 \pm 0.06$; maximal asymptote: $\alpha = 949 \pm 127$ mg). Preincubation with JNJ-17156516 or dexloxiglumide produced rightward shifts of the CCK-8S E/\([A]\) curve (Fig. 3, A and B). No change in slope was observed between treatment groups, although in some of the individual paired curve experiments significant changes in maxima were observed for both JNJ-17156516 and dexloxiglumide (not observed in paired control curves). Notwithstanding this, Schild analysis was performed on the data for both antagonists (Fig. 3, C and D). The Schild plot slope parameter was not significantly different from unity (1.17 ± 0.16) for JNJ-17156516, and, when the slope was subsequently constrained to unity, an estimate of $pK_B = 8.00 \pm 0.07$ was obtained. The Schild plot slope for dexloxiglumide was significantly less than 1 (0.70 ± 0.13). From inspection of the corresponding Schild plot, it is evident that the two highest concentrations of antagonist (6.3 and 16 $\mu$M) failed to produce as large a dose ratio as expected. When the data from these two concentrations were omitted, the slope was not significantly different from unity (1.17 ± 0.21), and when the slope was constrained to unity, an estimate of $pK_B = 6.85 \pm 0.09$ was obtained.

Pharmacokinetic Properties in Rats. After intravenous administration, JNJ-17156516 (2 $\mu$mol kg$^{-1}$) had a relatively small volume of distribution (0.21 ± 0.04 $\text{kg}^{-1}$) and a low clearance (48 $\text{ml kg}^{-1} \text{h}^{-1}$), resulting in a half-life of 3.0 ± 0.5 h in this species. After oral administration of the same dose, JNJ-17156516 was found to have a very high oral bioavailability (108 ± 10%). Conversely, dexloxiglumide (20 $\mu$mol kg$^{-1}$ i.v. and p.o.) had an oral bioavailability of 51 ± 14% and a terminal half-life of 0.76 ± 0.05 h.

Pharmacological Characterization of CCK-8S Evoked Responses in the Rat Duodenum. Infusion of 1 nmol kg$^{-1}$ CCK-8S over 1 min produced a reproducible contraction of the duodenum that could be repeated every 10 min to assess the pharmacodynamic effect of CCK1 receptor antagonists (Fig. 4). The number of HACs occurring after each challenge dose of CCK-8S was counted, and it was used as the response metamer. Dexloxiglumide and JNJ-17156516 reduced the number of HACs occurring in response to CCK-8S infusion in a dose- and concentration-related manner (Fig. 5, A and B). For JNJ-17156516, the $ED_{50}$ for inhibition of HACs was 484 nmol kg$^{-1}$ ($pED_{50} = 6.31 \pm 0.11$). The plasma concentration associated with this effect was 1.1 $\mu$M (negative log10 of the plasma concentration that produced 50% effect = 5.94 ± 0.14). In this preparation, JNJ-17156516 and dexloxiglumide were equipotent (dexloxiglumide $pED_{50} = 6.09 \pm 0.11$ and negative log10 of the plasma concentration that produced 50% effect = 6.27 ± 0.17).

Pharmacokinetic-Pharmacodynamic Relationship in Anesthetized Guinea Pigs. Intravenous administration of CCK-8S caused a dose-related and time-dependent increase in the developed pressure in the guinea pig gallbladder (Fig. 6A), such that multiple dose-response curves to CCK-8S could be constructed in a single animal. In vehicle-treated animals, the location of the CCK-8S dose-response curve was not significantly different for successive curves in the same animal (Table 2). JNJ-17156516 and dexloxiglumide caused dose-dependent, parallel rightward shifts of the CCK-8S E/\([A]\) curve (Fig. 6, B and C). From these data, the difference between the control CCK-8S curve and the CCK-8S curve in the presence of the antagonist was estimated, and dose ratios were given as a measure of potency (Table 2). From the dose ratios determined immediately after dosing, the receptor affinity normalized dose ratios (equivalent to a $pA_2$, termed $pA_{2\text{dose}}$) were calculated to be 6.62 ± 0.08 and 6.12 ± 0.11 for JNJ-17156516 and dexloxiglumide, respectively. Thus, a dose of 240 nmol kg$^{-1}$ of JNJ-17156516: A Novel CCK1 Receptor Antagonist 565
171756516 produced a 2-fold, rightward shift of the CCK-8S dose-response curve. From these data, JNJ-17156516 is 3-fold more potent than dexloxiglumide in terms of intravenous dose in this species. The plasma concentration of JNJ-17156516, 1 and 2 h after the 2 μmol kg⁻¹ i.v. dose, was 0.78 ± 0.28 and 0.23 ± 0.03 μM, respectively. Calculation of the analogous affinity estimates (pA₂plasma concentration) for JNJ-17156516, from the mean plasma concentration data at these two time points, yields values of −7.04 and −7.77. There was insufficient plasma concentration data for dexloxiglumide to make an estimate of pA₂plasma concentration.

Analysis of the duration of the pharmacodynamic response showed that the effect of JNJ-17156516 was well maintained in the guinea pig, whereas the response produced by dexloxiglumide was reduced by half, over the course of the 2-h experiment (Fig. 6, A–C; Table 2). This finding is consistent with the shorter half-life of dexloxiglumide compared with JNJ-17156516 in this and other species.

### Discussion

CCK1 receptor antagonists are potential candidates for the treatment of a number of disorders, including pancreatitis and irritable bowel syndrome. Indeed, promising clinical data for the CCK1 receptor antagonist loxiglumide and the enantiomerically pure form dexloxiglumide have been published in relation to these disorders. However, although this compound is still reported as being investigated in functional dyspepsia (clinical trials identifier NCT00303264; www.clinicaltrials.gov), it is no longer being developed for IBS,

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**TABLE 1**

Summary of all affinity estimates generated for JNJ-17156516 and dexloxiglumide in radioligand binding and functional in vitro tissue bioassays. All experiments were performed a minimum of three times in triplicate with the exception of dexloxiglumide in the human gallbladder assay, which was performed once in sextuplet. The estimated Hill slope parameters were not significantly different from unity in all cases.

<table>
<thead>
<tr>
<th>Receptor (Expression System)</th>
<th>Radioligand or Agonist</th>
<th>JNJ-17156516 Affinity ± S.E.M.</th>
<th>Dexloxiglumide Affinity ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human CCK1 (CHO)</td>
<td>125I-CCK-8S</td>
<td>pkᵢ = 7.96 ± 0.11</td>
<td>pkᵢ = 7.53 ± 0.07</td>
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<td>Rat CCK1 (CHO)</td>
<td>125I-CCK-8S</td>
<td>pkᵢ = 8.02 ± 0.11</td>
<td>pkᵢ = 7.63 ± 0.09</td>
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<tr>
<td>Canine CCK1 (CHO)</td>
<td>125I-CCK-8S</td>
<td>pkᵢ = 7.98 ± 0.04</td>
<td>pkᵢ = 7.53 ± 0.11</td>
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<tr>
<td>Human CCK1 (gallbladder)</td>
<td>[³H]-364,718</td>
<td>pkᵢ = 8.22 ± 0.05</td>
<td>pkᵢ = 7.1</td>
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<tr>
<td>Guinea pig CCK1 (gallbladder)</td>
<td>CCK-8S</td>
<td>pkᵢ = 8.00 ± 0.07</td>
<td>pkᵢ = 6.85 ± 0.09</td>
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<tr>
<td>Human CCK2 (HEKZFP)</td>
<td>125I-CCK-8S</td>
<td>pkᵢ = 5.76 ± 0.02</td>
<td>pkᵢ &lt; 5</td>
</tr>
<tr>
<td>Rat CCK2 (CHO)</td>
<td>125I-CCK-8S</td>
<td>pkᵢ = 5.65 ± 0.07</td>
<td>pkᵢ = 5.23 ± 0.04</td>
</tr>
<tr>
<td>Canine CCK2 (CHO)</td>
<td>125I-CCK-8S</td>
<td>pkᵢ = 6.10 ± 0.14</td>
<td>pkᵢ = 5.56 ± 0.05</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Inhibition of CCK-8S induced contraction of the isolated, in vitro guinea pig gallbladder bioassay using JNJ-17156516 (A) and dexloxiglumide (B). Representative CCK-8S E/[A] control curves (closed symbols) and CCK-8S E/[A] curves in the presence of a single concentration of antagonist (1 μM JNJ-17156516 shown with open squares and 1 μM dexloxiglumide with open circles) are shown. Corresponding Schild plots derived from the dose ratio produced with five concentrations of antagonist, in repeat curve experiments, are also shown (C and D).

**Fig. 4.** CCK-8S induced contraction of rat duodenum in vivo and suppression by JNJ-17156516 and dexloxiglumide. The characteristic contractile response of the duodenum evoked by infusion of a 1-nmol kg⁻¹ dose of CCK8S over 1 min was suppressed by intravenous administration of JNJ-17156516 and dexloxiglumide.
because the beneficial effects, reported in early trials, failed
to reach significance in phase III clinical trials. Therefore, we
aimed to identify an improved CCK1 receptor antagonist in
terms of primary affinity, pharmacokinetics, and, subse-
quently, pharmacodynamics. This medicinal chemistry pro-
gram resulted in the identification of JNJ-17156516 (Mc-
Clure et al., 2006; Sehon et al., 2006). This study describes
the pharmacological characterization of this compound.

The affinity of JNJ-17156516 or dexloxiglumide at the
CCK1 receptor, expressed in cell lines, did not seem to be
species-dependent. Therefore, the affinity of JNJ-17156516
for the human, rat, and canine CCK1 receptor was approxi-
mately 3-fold greater than dexloxiglumide. In the human
gallbladder radioligand binding studies and the guinea pig
gallbladder in vitro bioassay, this difference in affinity was
even greater, such that JNJ-17156516 expressed
\[ \sim 13 \text{- to } 14 \text{-fold increased affinity over dexloxiglumide.} \]
Although, dexloxiglumide was only tested once in the human gallbladder assay, the value obtained here was not significantly different from the \( pK_B \) reported previously from a functional
assay of human gallbladder contraction (\( pK_B = 6.95 \pm 0.11; \)
Maselli et al., 2001). JNJ-17156516 was also highly selective
over the CCK2 receptors (\( \sim 150 \text{-fold at cloned receptor sys-
tems and } \sim 280 \text{-fold using human gallbladder CCK1 receptor
affinity value).} \)

Dexloxiglumide and JNJ-17156516 produced a rightward
shift of the CCK-8S curve in the guinea pig in vitro bioassay.
However, pharmacological complexity was observed for both
compounds. For JNJ-17156516 and dexloxiglumide, an
increase in the CCK-8S maximal response was observed in the
second concentration-effect curve; however, this was not seen
with the corresponding paired control curves. In addition, the
Schild slope value for dexloxiglumide was significantly flat
(\( n_{S} = 0.70 \pm 0.13 \)). This may indicate that dexloxiglumide is
interacting with two receptor subtypes in the guinea pig
gallbladder or that the assay was conducted under nonequi-
librium conditions. CCK receptor heterogeneity in the guinea
pig gallbladder has been suggested previously based on the
finding that the E/[A] curves to CCK-8S extend over a con-
centration range of 6 log units (Maubach, 1991). However, it
was reported by Bishop et al. (1992) that the use of smaller
pieces of guinea pig gallbladder tissue resulted in an increase
in the potency of CCK-8S E/[A] curves (\( \sim 10 \text{-fold} \) and a
change in the Hill slope value from \( \sim 0.5 \) to 1. Previous
experiments with dexloxiglumide in the rat pancreas did not
demonstrate pharmacological complexity (\( n_{H} = 0.90 \pm 0.36; \)
Varga et al., 1997); in addition, the affinity value estimated
in those studies was consistent with that described here
(\( pA_2 = 6.41 \pm 0.38 \)). Overall, in the in vitro functional assay
of CCK1 receptors, JNJ-17156516 expressed an \( \sim 14 \text{-fold}
higher affinity than dexloxiglumide.

In addition to primary affinity, we aimed to identify a
compound with a good pharmacokinetic profile. In the rat,
JNJ-17156516 displayed \( \sim 100\% \) bioavailability and approxi-
mately a 4-fold longer half-life than dexloxiglumide. The

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Fig. 5. Dose (A) and plasma (B) concentration-effect curves for JNJ-
17156516 (open squares) and dexloxiglumide (closed circles) in the in vivo
model of CCK-8S-induced rat duodenal contractility.

Fig. 6. CCK-8S induced contraction of guinea pig gallbladder
in vivo (A) was inhibited in a concentration-dependent
manner by \( 2 \mu \text{mol kg}^{-1} \) i.v. JNJ-17156516 (B) and \( 20 \mu \text{mol}
kg\(^{-1}\) i.v. dexloxiglumide (C). CCK-8S dose-response curves
were conducted both immediately (closed symbols) and 2 h
after administration (open symbols) of the CCK-receptor
antagonists JNJ-17156516 and dexloxiglumide.
previously reported complex pharmacokinetic properties of dexloxioglumide likely contribute to its marginal efficacy in clinical trials (Persiani et al., 2002). Therefore, if the parameters observed here translate into humans, JNJ-17156516 may demonstrate a higher bioavailability and half-life, and, consequently, a greater, sustained pharmacodynamic effect.

The in vivo potency of JNJ-17156516 and dexloxioglumide were also compared in animal models of CCK1 receptor function. In a guinea pig model of gallbladder contraction, both were also compared in animal models of CCK1 receptor function. In a guinea pig model of gallbladder contraction, both compounds produced parallel, rightward shifts of the CCK-8S dose-response curve. A 240-nmol kg$^{-1}$ dose of JNJ-17156516 produced a 2-fold shift in the CCK-8S dose-response curve, whereas for dexloxioglumide, a dose of 630 nmol kg$^{-1}$ was required, demonstrating that JNJ-17156516 is ~3-fold more potent than dexloxioglumide in this species. This finding is consistent with the in vitro affinity estimates for these compounds. An in vivo affinity estimate for JNJ-17156516 was also made using the plasma concentration determined 1 and 2 h after administration (pK$_\text{a}_{2,\text{plasma concentration}}$). This value was between 7.04 and 7.77, and this is in good agreement with the pK$_\text{a}$ determined using the guinea pig gallbladder in vitro bioassay (pK$_\text{a}$ = 8.00 ± 0.07). Furthermore, the robust nature of this assay made it possible to assess the duration of action of both compounds. Thus, JNJ-17156516 had no loss in effect 2 h after administration, whereas the effect of dexloxioglumide was reduced by approximately 2-fold. This study demonstrated that the improved pharmacokinetics of JNJ-17156516, shown in the rat, translated into a functional effect in the guinea pig.

To generate in vivo potency estimates for JNJ-17156516 in the rat, the recently developed duodenal contraction assay was used (Freedman et al., 2006). No difference in the potency was observed between JNJ-17156516 and dexloxioglumide, either in terms of the dose or the plasma concentration for effect. It may be that the 3-fold difference in affinity observed in the in vitro assay could not be observed in the in vivo setting due to species-dependent variation tissue distribution of the compound.

Overall, this comparative study demonstrated that JNJ-17156516 expressed between 3- and 14-fold greater affinity than dexloxioglumide in vitro, approximately 3-fold increased potency in the guinea pig gallbladder assay in vivo, and no difference in potency in the rat model of duodenal contraction. In addition, the pharmacokinetic properties of JNJ-17156516 were improved, and this translated into improved pharmacodynamic activity in vivo using the gallbladder contraction assay. The data indicate that JNJ-17156516 would provide a good pharmacological tool for the investigation of this class of compounds in the clinic.

### References


Snape WJ Jr, Matarazzo SA, and Cohen S (1978) Effect of eating and gastrointest-

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**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (i.v. Bolus)</th>
<th>pK$_{a}$</th>
<th>Log Dose Ratio</th>
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<tr>
<td>Vehicle</td>
<td>Predrug</td>
<td>10.06 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>10.06 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vehicle + 2 h</td>
<td>9.91 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>JNJ-17156516</td>
<td>Predrug</td>
<td>10.08 ± 0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 μmol kg$^{-1}$</td>
<td>9.15 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 μmol kg$^{-1}$ + 2 h</td>
<td>8.95 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Dexloxioglumide</td>
<td>Predrug</td>
<td>9.98 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 μmol kg$^{-1}$</td>
<td>8.56 ± 0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 μmol kg$^{-1}$ + 2 h</td>
<td>9.38 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

Summary of previously published data on the pharmacological properties of JNJ-17156516 and dexloxioglumide. The data indicate that JNJ-17156516 would provide a good pharmacological tool for the investigation of this class of compounds in the clinic.


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