Lorcaserin, a Novel Selective Human 5-Hydroxytryptamine\textsubscript{2C} Agonist: in Vitro and in Vivo Pharmacological Characterization

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ABSTRACT

5-Hydroxytryptamine (5-HT\textsubscript{2C}) receptor agonists hold promise for the treatment of obesity. In this study, we describe the in vitro and in vivo pharmacological characterization of lorcaserin [(1R)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1H-3 benzazepine], a selective, high affinity 5-HT\textsubscript{2C} full agonist. Lorcaserin bound to human and rat 5-HT\textsubscript{2C} receptors with high affinity (K_i = 15 ± 1 nM, 29 ± 7 nM, respectively), and it was a full agonist for the human 5-HT\textsubscript{2C} receptor in a functional inositol phosphate accumulation assay, with 18- and 104-fold selectivity over 5-HT\textsubscript{2A} and 5-HT\textsubscript{2B} receptors, respectively. Lorcaserin was also highly selective for human 5-HT\textsubscript{2C} over other human 5-HT receptors (5-HT\textsubscript{1A}, 5-HT\textsubscript{2A}, 5-HT\textsubscript{2C}, 5-HT\textsubscript{5A}, 5-HT\textsubscript{6}, and 5-HT\textsubscript{7}), in addition to a panel of 67 other G protein-coupled receptors and ion channels. Lorcaserin did not compete for binding of ligands to serotonin, dopamine, and norepinephrine transporters, and it did not alter their function in vitro. Behavioral observations indicated that unlike the 5-HT\textsubscript{2A} agonist (±)-1-(2,5-dimethoxy-4-phenyl)-2-aminopropane, lorcaserin did not induce behavioral changes indicative of functional 5-HT\textsubscript{2A} agonist activity. Acutely, lorcaserin reduced food intake in rats, an effect that was reversed by pretreatment with the 5-HT\textsubscript{2C}-selective antagonist 6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yl)-pyridin-3-yl-carbamoyl]indoline (SB242,084) but not the 5-HT\textsubscript{2A} antagonist (R)-(+)α-(2,3-dimethoxyphenyl)-4-piperidine-methanol (MDL 100,907), demonstrating mediation by the 5-HT\textsubscript{2C} receptor. Chronic daily treatment with lorcaserin to rats maintained on a high fat diet produced dose-dependent reductions in food intake and body weight gain that were maintained during the 4-week study. Upon discontinuation, body weight returned to control levels. These data demonstrate lorcaserin to be a potent, selective, and efficacious agonist of the 5-HT\textsubscript{2C} receptor, with potential for the treatment of obesity.

Serotonin mediates its physiological effects through at least 14 different receptors. The serotonin 5-HT\textsubscript{2} receptor subfamily contains three distinct receptor subtypes, 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B}, and 5-HT\textsubscript{2C}, all of which share considerable sequence homology (>80% in transmembrane spanning regions) and activate common signaling pathways, including G\textsubscript{q}-mediated stimulation of phospholipase-C\textsubscript{β}, elevation of intracellular inositol phosphates, and elevation of intracellular calcium (Roth et al., 1998). Human 5-HT\textsubscript{2C} receptors are predominately expressed in the CNS, and they are highly enriched in choroid plexus, prefrontal cortex, hippocampus, basal ganglia, and other brain regions associated with the control of mood, cognition, and appetite (Roth et al., 1998). Thus, 5-HT\textsubscript{2C} receptors have been proposed as a therapeutic target for the treatment of CNS disorders, including epilepsy, obsessive compulsive disorder, Parkinson’s disease, schizophrenia, depression and anxiety, sleep disorders, and drug abuse (Tecott et al., 1995; Roth et al., 1998; Frank et al., 2002; Rocha et al., 2002; Meltzer et al., 2003; Isaac, 2005; Millan, 2005; Di Giovanni et al., 2006).

Received November 13, 2007; accepted January 29, 2008

Supplemental material to this article can be found at: http://jpet.aspetjournals.org/content/suppl/2008/02/13/jpet.107.133348.DC1

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The online version of this article (available at http://jpet.aspetjournals.org) contains supplemental material.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); CNS, central nervous system; Ro60-0175, (S)-(2R)-5-fluorindol-1-yl)-1-methyl-ethylamine; Y3248, (S)-(7-ethoxy-1H-indol-2-yl)-methyl; WAY-161503, 8,9-dichloro-2,3,4,5-tetrahydro-1-methyl-1H-3 benzazepine; WAY-163909, (7R,10R)-(2R)-[1,4]diazepino-[6,7,1hi]indole; VER2692, 1-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-4-(3-phenylpropyl)piperazine; ANOVA, analysis of variance; GPCR, G protein-coupled receptor; AUC, area under the curve.
A wealth of data also indicates that 5-HT<sub>2C</sub> agonists may provide an effective treatment for obesity, a disease that has reached epidemic proportions in many developed nations worldwide. 5-HT<sub>2C</sub> receptors are distributed in brain areas associated with regulation of food intake, including the nucleus of the solitary tract, dorsomedial hypothalamus, paraventricular hypothalamic nucleus, and the amygdala (Berthoud, 2002). 5-HT<sub>2C</sub> receptor knockout mice are hyperphagic, leading to obesity, partial leptin resistance, increased adipose deposition, insulin resistance, and impaired glucose tolerance (Tecott et al., 1995; Heisler et al., 1998; Nonogaki et al., 1998; Rosenzweig-Lipson et al., 2000; Tecott and Abdallah, 2003). Several studies have demonstrated that 1-(m-chlorophenyl)piperazine- and mCPP and Abdallah, 2003). Several studies have demonstrated that 1-(m-chlorophenyl)piperazine- and d-fenfluramine-induced weight loss in both rodents and humans is primarily due to activation of 5-HT<sub>2C</sub> receptors (Kettenn and Curzon, 1991; Sargent et al., 1997; Vickers et al., 2001, 2003), a finding consistent with the observation that the weight-lowering actions of both mCPP and d-fenfluramine are absent in 5-HT<sub>2C</sub> knockout mice (Tecott et al., 1995; Vickers et al., 1999). Likewise, other 5-HT<sub>2C</sub> agonists (Ro 60-0175, YM348, WAY-161503, WAY-163909, and VER2692), also reduce food intake in animals and enhance satiety (Clifton et al., 2000; Rosenzweig-Lipson et al., 2000; Bentley et al., 2004; Hayashi et al., 2004; Dunlop et al., 2005). Rats fed a high fat diet have lower levels of hypothalamic 5-HT<sub>2C</sub> mRNA (Schaffhauser et al., 2002), and recent reports suggest that certain atypical antipsychotic drugs such as risperidone may cause weight gain via blockade of the 5-HT<sub>2C</sub> receptor (Reynolds et al., 2005). Last, the psychoactive substance 3,4-methylenedioxymethamphetamine (ecstasy), promotes appetite suppression that is reversed by the 5-HT<sub>2C</sub>-selective antagonist RS102221 (Conductier et al., 2005). Several reviews regarding the utility of selective 5-HT<sub>2C</sub> agonists for the treatment of obesity have been published recently (Bickerdike, 2005; Miller, 2005; Halford, 2006; Nilsson, 2006; Smith et al., 2006).

Historically, a major obstacle limiting the development of 5-HT<sub>2C</sub> agonists has been a lack of selectivity over 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors. Activation of 5-HT<sub>2A</sub> receptors can induce hallucinations (Nichols, 2004). The activation of 5-HT<sub>2B</sub> receptors has been implicated in cardiac valvular insufficiency and possibly in pulmonary hypertension associated with dexfenfluramine use (Fitzgerald et al., 2000; Rothman et al., 2000; Launay et al., 2002; Roth, 2007). Thus, discovery of selective 5-HT<sub>2C</sub> receptor agonists could lead to the development of efficacious antiobesity drugs that have the possibility of avoiding side effects associated with older classes of nonselective serotoninergic modulators. In this communication, we describe the in vitro and in vivo characteristics of lorcaserin [(1R)-(-)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1H-3 benzazepine], a selective and potent 5-HT<sub>2C</sub> agonist.

**Materials and Methods**

**Drugs**

The chemical structure of lorcaserin (molecular mass 195.69 Da) is shown in Fig. 1. The synthesis has been described previously (Smith et al., 2005). The following reagents were purchased from commercial suppliers: HEK293 and COS7 cells (American Type Culture Collection, Manassas, VA); fetal calf serum, Dulbecco’s modified Eagle’s medium (DMEM), Opti-MEM I, and Lipofectamine (Invitrogen, Carlsbad, CA); myo-[<sup>1</sup>H]inositol, and [<sup>125</sup>I]DOI (PerkinElmer Life and Analytical Sciences, Boston, MA). Reference 5-HT<sub>2</sub> compounds were either purchased from Tocris Cookson Inc. (Ellisville, MO) or Sigma/RBI (Natick, MA).

**In Vitro Pharmacology Experiments**

**Cell Growth and Transient Transfection of Recombinant Human and Rat 5-HT<sub>2</sub> Receptors.** For development of functional human and rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> inositol accumulation assays, recombinant 5-HT<sub>2C</sub> receptors were transiently expressed in HEK293 cells using the Lipofectamine method. In brief, cells were incubated in 15-cm plates with DMEM, and they were grown to at least 60 to 80% confluence, rinsed with Opti-MEM I media, followed by addition of 2 ml of Opti-MEM I containing 60 μl of Lipofectamine and 16 μg of cDNA for human and rat 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors or 15 μl of Lipofectamine and 16 μg of cDNA for human and rat 5-HT<sub>2C</sub> receptors. Cells were then incubated for 5 h at 37°C in a 5% CO<sub>2</sub> incubator. Next, cells were aspirated, and 30 ml of DMEM was added. Cells were then used for assays 24 h after transfection. Under these conditions, cells expressing human 5-HT<sub>2C</sub> receptor displayed constitutive elevation of inositol phosphate accumulation that was reversed by the inverse agonist mianserin, whereas cells expressing human 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors display considerably less constitutive activity (data not shown).

For development of human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptor radioligand binding assays, recombinant receptors were stably expressed in HEK293 cells using standard protocols, and crude plasma membranes were prepared from these cells. For development of rat 5-HT<sub>2</sub> radioligand binding assays, rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors were transiently expressed in HEK293 cells using the Lipofectamine method as described above.

**Preparation of Plasma Membranes from Cells Expressing Recombinant 5-HT<sub>2</sub> Receptors.** HEK293 cells stably expressing recombinant human and rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors were collected, washed with ice-cold phosphate-buffered saline, pH 7.4, and then they were centrifuged at 48,000g for 20 min at 4°C. The cell pellet was then suspended in ice-cold wash buffer containing 20 mM HEPES, pH 7.4, and 0.1 mM EDTA, homogenized on ice using a Polytron (Brinkman Instruments, Westbury, NY), and centrifuged at 48,000g for 20 min at 4°C. The resultant pellet was then suspended in ice-cold 20 mM HEPES, pH 7.4, homogenized on ice, and centrifuged (48,000g for 20 min at 4°C). Crude membrane pellets were stored at −80°C until they were used for radioligand binding assays.

**[<sup>125</sup>I]DOI Binding to Recombinant Human and Rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> Receptors.** Radioligand binding assays for human and rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors were developed using the 5-HT<sub>2A</sub> agonist [<sup>125</sup>I]DOI as radioligand, and nonspecific radioligand binding was determined in the presence of 10 μM unlabeled DOI. Competition experiments consisted of addition of 95 μM assay buffer (20 mM HEPES, pH 7.4, and 10 mM MgCl<sub>2</sub>), 50 μl of membranes (5–25 μg of protein), 50 μl of [<sup>125</sup>I]DOI (0.5 nM final assay concentration), and 5 μl of test compound diluted in assay buffer (final concentrations ranging from 1 pM to 10 μM) to 96-well GFC microtiter plates (PerkinElmer Life and Analytical Sciences), and incubations were performed for 1 h at room temperature. Each lorcaserin radioligand competition study consisted of testing eight different concentrations in which triplicate determinations were per-
formed for each test compound concentration. Assay incubations were terminated by rapid filtration of microtiter plates under vacuum pressure using a cell harvester (Brandel Inc., Gaithersburg, MD), followed by washing filter plates several times with ice-cold wash buffer (50 mM Tris-HCl, pH 7.4). Plates were then dried at 45°C in an oven for a minimum of 2 h, 25 μl of BetaScint scintillation cocktail was added to each well, and microtiter plates were counted in a Packard TopCount scintillation counter (PerkinElmer Life and Analytical Sciences).

Radioassay and Binding Assays for Additional Human 5-HT Receptors and Neurotransmitter Transporters. Evaluations of lorcaserin competition for radioligand binding to human 5-HT7, 5-HT3A, 5-HT3B, 5-HT3C, and 5-HT7, receptors as well as serotonin, dopamine, and norepinephrine transporters were performed at Cerep, Inc. (Portiers, France) (see Supplemental Table 1 for general assay conditions) except for 5-HT3C, which was performed at Arena Pharmaceuticals using the same protocol described in Supplemental Table 1 except that assay buffer contained 50 mM Tris-HCl, pH 7.4, 2.5 mM MgCl2, and 1 mM EDTA. In particular, competition studies consisted of testing of lorcaserin at eight different concentrations in triplicate determinations were made at each concentration.

[3H]-Hydroxytryptamine, [3H]-Norepinephrine, and [3H]-Dopamine Uptake and Release from Rat Synaptosomes. These studies were performed at Cerep, Inc. (see Supplemental Table 2 for general assay conditions). Lorcaserin was tested at eight different concentrations in which duplicate determinations were made at each test concentration.

5-HT3 Inositol Phosphate Accumulation Assays. On the day before experiments, cells expressing receptors of interest were removed from cell culture plates by treatment with 2 ml of trypsin, additional growth medium was added to dilute the trypsin, and then cells were centrifuged at 3500 rpm at 4°C for 5 min. One hundred microliters of 1 mM calcium-free Dulbecco’s modified Eagle’s medium (titrated to pH 7.4 with sodium bicarbonate) containing 40 μCi of [3H]inositol were then added to each well, and plates were incubated overnight at 37°C with 5% CO2.

The next day, unincorporated [3H]inositol was carefully removed from wells by aspiration, and it was replaced with 90 μl of isotonol-free Dulbecco’s modified Eagle’s medium (titrated to pH 7.4 with sodium bicarbonate) also containing 10 mM LiCl and 10 μg pargyline followed by addition of 10 μl of test compound (1 μM–10 μM, triplicate determinations at each concentration) containing a final concentration of 0.5% dimethyl sulfoxide. Assay plates were then incubated for 2 h at 37°C with 5% CO2, and then the reaction mixture was removed by gentle aspiration. Then, 160 μl of stop solution containing 0.1 M formic acid was added to each well, and plates were frozen at –80°C overnight to promote cell lysis.

The following day, assay plates were thawed and [3H]inositol was separated from [3H]inositol phosphates by Dowex resin chromatography. Then, 200 μl of activated resin slurry was added to 96-well Whatman Unifilter plates (Whatman, Florhampark, NJ), cell lysate was added to plates, and plates were washed twice with 200 μl of water under vacuum pressure followed by elution of [3H]inositol phosphates with 180 μl of low-salt elution buffer. The eluant was then transferred to clean 96-well microtiter plate, and sealed plates were then incubated overnight in an oven at 45°C. Plates were then counted in a Packard TopCount scintillation counter after addition of 50 μl well of HiSafe3 scintillation cocktail. Dose-response evaluations involved testing lorcaserin at eight different concentrations in which triplicate determinations were made at each test concentration.

In Vitro Pharmacology Data Analysis. For radioligand binding experiments, IC50 values were obtained by fitting radioligand competition data to a sigmoidal function using a nonlinear least-squares program (Pasm; GraphPad Software Inc., San Diego, CA).

In all cases, data produced a better fit to a single-site model than a two-site model (data not shown). The same nonlinear curve fitting program was used to fit [3H]DOI saturation data for 5-HT7, receptors to a simple hyperbolic function for determination of Kd and Bmax values (data not shown). Kd values were calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973). For IP accumulation, EC50 values were also determined by fitting data to sigmoidal function using the same nonlinear least-squares curve-fitting program.

Pharmacokinetic Experiments

Oral Plasma and Brain Pharmacokinetics in the Rat. Male Sprague-Dawley rats (250–300 g) were obtained from Harlan (San Diego, CA). On the day of the experiment, rats received a single dose of lorcaserin in water via oral gavage. Dosing volume and dose were 5 ml/kg and 10 mg/kg, respectively. Lorcaserin was formulated in deionized water. The following sampling scheme was used (n = 3/time point): 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 40 h after dose. Rats were lightly anesthetized with isoflurane, blood was collected via cardiac puncture, and brains were removed from the cranium. Blood samples were dispensed into sodium-heparinized vials, and then vials were capped and stored at 4°C. Plasma was separated from formed elements in blood by centrifugation (10 min at 3000g), and then plasma was frozen. Brains were rinsed with ice-cold phosphate-buffered saline, blotted dry, weighed, and frozen. Plasma and brain samples were stored at −80°C before bioanalytical analysis.

Bioanalytical Method. Plasma samples were thawed on ice, and a 50-μl aliquot was transferred to a 1-ml plastic tube. An acetonitrile (110 μl) containing internal standard was added to the plasma, vortexed, and centrifuged (10 min at 3000g). A standard curve (1–2000 ng/ml) and quality control samples (3, 300, and 1500 ng/ml) were prepared in sodium-heparinized rat plasma in a similar manner. The resultant supernatants were analyzed for lorcaserin.

Brain samples were thawed on ice, placed into 50-ml plastic conical tubes, and 2 volumes of purified water per gram of brain was added. Brains were homogenized using a mechanical variable speed tissue homogenizer; 50 μl of brain homogenate was transferred to a 1-ml plastic tube. An acetonitrile (110 μl)-containing internal standard was added to brain homogenate, vortexed, and centrifuged (10 min at 3000g). A standard curve (1–2000 ng/ml) and quality control samples (3, 300, and 1500 ng/ml) were prepared in control brain homogenate in a similar manner. The resultant supernatants were analyzed for lorcaserin.

Processed plasma and brain samples were analyzed for lorcaserin and internal standard by liquid chromatography-tandem mass spectrometry. The LC separation used a binary gradient and C18 reverse phase column (Luna 3 μm C18 (2), 50 × 2 mm; Phenomenex, Torrance, CA) equipped with a C18 guard column (2 × 4 mm; Phenome- nex). The column was kept at a constant temperature of 35°C. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in acetonitrile. The flow rate was held constant at 0.5 ml/min. The following binary gradient was used for sample analysis: column equilibration for 1 min at 10% mobile phase B; after injection, mobile phase B was held constant for 0.3 min at 10%, increased linearly in 1.7 min to 90% B, and kept constant at 90% B for 1 min.

Detection of lorcaserin in internal standard was achieved using electrospray ionization (TurboIonSpray; Applied Biosystems, Foster City, CA) operating in positive ion mode. The source temperature was set to 350°C, with an ion spray voltage of 3400 V. Multiple reaction monitoring of the mass transition 196.1 atomic mass units to 176.1 atomic mass units was used for detection of lorcaserin. Lorcaserin and internal standard eluted within 1 min after injection.

The liquid chromatography-tandem mass spectrometric system consisted of an API4000 mass spectrometer (MDS Sciex, Concord, ON, Canada), a CTC HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland), two single-channel LC10AD VP high-performance liquid chromatography pumps, DGU-14A degasser, and a SCL10A VP high-performance liquid chromatography controller (Shimadzu, Kyoto, Japan).
a Cheminert Divert Valve (Valco Instruments Co., Houston, TX), and PEEK mixing tee (Alltech Associates, Deerfield, IL).

Pharmacokinetic Data Analysis. The lorcaserin noncompartmental plasma and brain pharmacokinetics were determined using the program WinNonlin version 4.0.1 (Pharsight, Mountain View, CA). The area under the curve plasma concentration-time profile was established by linear trapezoidal rule. The terminal phase half-life was calculated using at least three data points in the terminal phase excluding the Cmax. Standard deviations are represented by error bars on all pharmacokinetic figures.

In Vivo Pharmacological Experiments

Animals and Housing. Male Sprague-Dawley rats (Harlan), weighing 250 to 300 g, were used for acute food intake and behavioral studies. Upon arrival at the test facility, animals were housed in groups of two within a holding room controlled for temperature and light (lights off 10:30 AM–6:30 PM for food intake studies; lights off 6:30 PM–6:30 AM for behavioral observations). All food intake studies were conducted during the dark phase, whereas behavioral observations were conducted during the light phase. Rats were allowed ad libitum access to food and water unless stated otherwise.

For chronic studies, male obesity prone Sprague-Dawley rats in the weight range of 150 to 200 g were obtained from Charles River (Indianapolis, IN), triple housed in shoebox cages, and maintained in a holding room controlled for temperature and light on reversed phase lighting, with lights off for 7 h from 9:00 AM. Animals were handled and given free access to a pelleted high fat diet (D12226; Research Diets, New Brunswick, NJ; 31.6 kilocalorie percentage of fat) for 8 weeks before, and for the duration of the study. At study onset subjects weighed between 250 and 500 g.

Drug Administration. Lorcaserin and DOI were dissolved in sterile water, whereas MDL 100,907 and SB242,084 were finely suspended in a 1%Tween 80 aqueous solution. Lorcaserin was administered p.o. in a volume of 2 ml/kg. DOI was administered s.c. in a volume of 1 ml/kg. MDL 100,907 and SB242,084 were administered p.o. in a volume of 2 ml/kg. All drug doses are expressed as that of the salt.

Study 1. Effect of Lorcaserin on Food Intake in Rats. On the day of testing, animals were weighed, single housed in cages with grid floors at 8:30 AM, and they were allowed to acclimate to these cages for a 90-minute period, with free access to water and no food. At 10:00 AM, rats were administered vehicle or lorcaserin (3, 6, 12, or 24 mg/kg) via oral gavage. Thirty minutes after administration (10:30 AM), lights were turned off, and animals were allowed access to food. Food and water were then weighed at 2, 4, 6, and 22 h after food exposure. Animals were weighed again at 22 h after food presentation.

Study 2. Effects of 5-HT2C and 5-HT2A Antagonists on Lorcaserin-Induced Hypophagia. Procedures were the same as described above (study 1) except that antagonists (vehicle; 1 mg/kg i.p. SB242,084; or 0.5 mg/kg i.p. MDL 100,907) were injected 15 min before p.o. administration of lorcaserin or vehicle. Food intake was then measured after a 60-minute period after lights out (90 min after administration of lorcaserin).

Study 3. Chronic Lorcaserin Administration in Male Diet-Induced Obesity Rats: Effect on Food Intake, Body Weight, and Body Composition during Treatment and Withdrawal. At the start of the study, 56 male rats were weighed, single-housed, and allocated to one of seven weight-matched treatment groups, each containing eight animals. Rats then underwent a 1-week period of habituation to oral gavage dosing. Treatment groups were as follows: vehicle (p.o., b.i.d.) and lorcaserin (4.5, 9, and 18 mg/kg p.o., b.i.d.). An additional two groups of rats received either vehicle or the highest dose of lorcaserin according to the same treatment regimen. These rats were later used to assess body weight and food intake during withdrawal (see below). All treatments were administered daily just before lights off, and again before lights on. Body weight and food and water intake were measured every day before morning injections. After 28 days of dosing, all subjects except those in the withdrawal groups were sacrificed, and body composition was measured using dexascan analysis (see below). Food and water intake and body weight of the rats in the withdrawal groups were continuously monitored for a further period of 28 days subsequent to drug dosing.

Body Composition Analysis. Postmortem body fat and nonfat mass were measured by dual-energy X-ray absorptiometry with the Prodigy Advance scanner (Prodigy Advance, GE Healthcare, Madison, WI) using software designed for rats. The DEXA scanner was calibrated to quantify nonfat mass as lean tissue composed of 60% water, excluding bone mass.

Study 4. Behavioral Observations after Lorcaserin Administration: Comparison with the 5-HT2A Agonist DOI. For the measurement of behavioral signs, subjects were administered either lorcaserin (vehicle, 4.5, 9, or 18 mg/kg) via oral gavage, or DOI (vehicle or 1 mg/kg) via the subcutaneous route, and they were placed individually in Plexiglas observation chambers (15 × 22 × 36 cm), with a mirror placed behind the chamber to allow an all around view of behavior. Observations started immediately upon placement in the chamber. Over a 60-min period, the following behaviors were scored: penile grooming (number of distinct bouts of penile grooming accompanied by erection), wet dog shakes (total number), and back muscle contractions (total number). Scores of “active” or “resting/inactive” were also scored as present or absent within 5-min time bins, resulting in a total possible score of 12 for each. The experimenter was unaware of drug treatment at the time of testing.

In Vivo Data Analysis. Study 1. Cumulative food intake (grams) was subjected to separate one-way analyses of variance (ANOVA) s at each time point (2, 4, 6, and 22 h). Actual food intake data were subjected to two-way repeated measures ANOVA (dose and time as factors).

Study 2. Food intake over the 1-h consumption (grams) was subjected to two-way analysis of variance (lorcaserin and antagonist as factors). Separate ANOVAs were conducted for each antagonist.

Study 3. Food intake and body weight data for all rats over the 28-day dosing period were subjected to a two-way ANOVA (day and treatment factors). For rats that subsequently underwent withdrawal, food intake and body weight data were averaged for each week of withdrawal, and these data were subjected to a two-way ANOVA with week and treatment as factors.

Study 4. Data for which behaviors were counted (e.g., wet dog shakes) were subjected to a one-way ANOVA. Remaining data that were scored as present or absent within time bins (e.g., inactive) were subjected to square root transformation, and a one-way ANOVA. Because lorcaserin and DOI included their own vehicle controls, they were subjected to separate analyses. Where appropriate, significant main effects for all data were followed by post-hoc comparisons using the Newman-Keuls test.

In Vitro Pharmacology

Radioligand Binding and Functional Activity of Lorcaserin at 5-HT2 Receptors. The binding affinity of lorcaserin for recombinant human and rat 5-HT2 receptors is summarized in Table 1. Lorcaserin exhibited higher affinity for human 5-HT2C than to 5-HT2A (7.5-fold selective) and 5-HT2B (11.6-fold selective) as indicated by Kᵢ values of 15, 112, and 174 nM, respectively. Lorcaserin also bound to rat 5-HT2C, 5-HT2A, and 5-HT2B receptors, with Kᵢ values of 29, 159, and 190 nM. The S-isomer of lorcaserin had similar affinities as the (R)-isomer (data not shown). Furthermore, the major circulating metabolite of lorcaserin does not appreciably bind to human 5-HT2 receptors (data not shown). To establish the affinity of lorcaserin for 5-HT2C receptors in a tissue naturally expressing endogenous human 5-HT2C re-
formed using human choroid plexus membranes. A comparison with 10
Materials and Methods
all three human 5-HT2 receptors (Fig. 2), but the potency for
accumulation assays. Lorcaserin possesses agonist activity at
comparison, EC50 values for several different reference 5-HT2
compounds used in the same assay. The selectivity of lor-
caserin for 5-HT2C receptor relative to an additional 67
selectivity of serotonin, dopamine, and norepinephrine transport-
ers in rat synaptosomal preparations (Table 3; see Supple-
mental Material for assay conditions) in comparison with
values from radioligand competition studies with [3H]DOI were calculated from IC50 values as described under Materials and Methods. Efficacy (the % 5-HT response column) was determined by comparison with 10 μM 5-HT, which was assigned a value of 100%.

### Table 1: Radioligand binding, functional potency, and efficacy of lorcaserin for human and rat 5-HT receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Affinity Kᵢ</th>
<th>Potency EC5₀</th>
<th>Efficacy (% 5-HT Response)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human 5-HT₂C</td>
<td>15 ± 1 (28)</td>
<td>9 ± 0.5 (40)</td>
<td>100</td>
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<tr>
<td>Human 5-HT₂A</td>
<td>112 ± 7 (25)</td>
<td>168 ± 11 (34)</td>
<td>75</td>
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<tr>
<td>Human 5-HT₂B</td>
<td>174 ± 32 (18)</td>
<td>943 ± 90 (35)</td>
<td>100</td>
</tr>
<tr>
<td>Rat 5-HT₂C</td>
<td>29 ± 7 (7)</td>
<td>193 ± 12 (4)</td>
<td>100</td>
</tr>
<tr>
<td>Rat 5-HT₂A</td>
<td>159 ± 27 (14)</td>
<td>676 ± 129 (3)</td>
<td>100</td>
</tr>
<tr>
<td>Rat 5-HT₂B</td>
<td>190 ± 5 (3)</td>
<td>272 ± 87 (4)</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 2. Representative EC5₀ curves for 5-HT- and lorcaserin-mediated stimulation of inositol phosphate accumulation in HEK293 cells transiently expressing human 5-HT₂C (A), 5-HT₂A (B), and 5-HT₂B receptors. EC5₀ values of 8 nM (5-HT₂C), 157 nM (5-HT₂A), and 804 nM (5-HT₂B) were obtained for lorcaserin compared with EC5₀ values of 24 nM (5-HT₂C), 124 nM (5-HT₂A), and 32 nM (5-HT₂B) obtained for 5-HT.

1 μM (see Supplemental Tables 4 and 5 for assay conditions and results, respectively).

### Influence of Lorcaserin on Functional Activity of Human Monoamine Transporters

Lorcaserin was also tested to determine whether it alters the functional activity of serotonin, dopamine, and norepinephrine transporters in rat synaptosomal preparations (Table 3; see Supplemental Material for assay conditions) in comparison with reference compounds tested in the same assay. Lorcaserin did not stimulate [³H]norepinephrine, [³H]dopamine, or [³H]serotonin release from rat synaptosomes at submicro-
TABLE 3

Effect of lorcaserin on release and uptake of 5-HT, norepinephrine, and dopamine from rat brain synaptosomes.

Data represent EC\textsubscript{50} (neurotransmitter release) or IC\textsubscript{50} (neurotransmitter uptake) values derived from one determination in which eight concentrations of lorcaserin or reference compound were used. Duplicate determinations were made at each compound concentration.

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Stimulation of Release</th>
<th>Inhibition of Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lorcaserin EC\textsubscript{50}</td>
<td>Reference Compound (EC\textsubscript{50})</td>
</tr>
<tr>
<td>5-HT</td>
<td>49,000</td>
<td>Fenfluramine (470)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>&gt;100,000</td>
<td>Amitriptyline (35,000)</td>
</tr>
<tr>
<td>Dopamine</td>
<td>23,000</td>
<td>Amphetamine (1000)</td>
</tr>
</tbody>
</table>

molar concentrations. In particular, lorcaserin was approximately 100-fold less potent than fenfluramine at promoting serotonin release. Lorcaserin was also evaluated for its ability to inhibit neurotransmitter uptake into rat brain synaptosomes. Lorcaserin weakly inhibited norepinephrine (EC\textsubscript{50} = 2500 nM), serotonin (EC\textsubscript{50} = 1400 nM), and dopamine (EC\textsubscript{50} = 12,000 nM) uptake. In each case, lorcaserin was much less effective than the reference compound tested in the same assay, and it displayed activity only at concentrations 100 times the lorcaserin K\textsubscript{i} value for the 5-HT\textsubscript{2C} receptor.

Pharmacokinetics

Oral Plasma and Brain Pharmacokinetics in the Rat.

Lorcaserin brain and plasma pharmacokinetics were evaluated after a single oral administration of 10 mg/kg in male Sprague-Dawley rats (n = 3/time point) (Fig. 3; Table 4). Lorcaserin absorption from the gastrointestinal tract into the systemic circulation was rapid, resulting in a mean maximum concentration (C\textsubscript{max}) of 0.760 μg/ml at 0.25 h (the first time point collected). The time to brain maximal exposure was 1 h. The mean maximum brain concentrations were 8.0-fold greater than plasma (e.g., 6.1 μg/g for brain at 1 h and 0.760 μg/ml for plasma at 0.25 h). This ratio changed only slightly throughout the time course (after 0.25 h), where brain/plasma ratios at each time point ranged from 11.5 to 17.3. The ratio for brain-to-plasma exposure (AUC\textsubscript{INP}) was 13.3. The elimination half-life and mean residence time of lorcaserin in the brain (4.7 and 6.1 h, respectively) were similar to the values for lorcaserin in the systemic circulation (4.9 and 5.4 h, respectively).

In Vivo Pharmacology

Study 1: Effect of Lorcaserin on Acute Food Intake in Rats. Food intake was measured in rats after a single oral administration of lorcaserin (Fig. 4). Lorcaserin decreased cumulative food intake at 2, 4, 6, and 22 h [F(4,35) = 42.9, 13.2, 10.2, and 6.1, respectively; p < 0.01] (Fig. 4A), with a significant decrease continuing throughout the 22-h duration of the study at the highest dose (24 mg/kg) only. Using the cumulative food intake data, a single dose ED\textsubscript{50} of 18 mg/kg was derived at the 6-h time point (Fig. 4B). A two-way ANOVA of absolute (noncumulative) food intake over time demonstrated a significant effect of lorcaserin [F(4,35) = 6.06; p < 0.01], and a significant interaction of lorcaserin with time [F(12,105) = 9.1; p < 0.01] (data not shown). Post-hoc analysis at each time point indicated that the decreased cumulative food intake over the duration of the study was accounted for by a significant decrease during the first 2 h only; food intake over subsequent measurement times was not different from that of vehicle-treated rats, and the maintained effect on cumulative intake was due to a lack of compensatory hyperphagia.

Study 2: Selective Inhibition of Lorcaserin-Induced Hypophagia with a 5-HT\textsubscript{2C} Antagonist and Not a 5-HT\textsubscript{2A} Antagonist. To establish receptor subtypes mediating the hypophagic effect of lorcaserin, the selective 5-HT\textsubscript{2C} antagonist SB242,084 and the selective 5-HT\textsubscript{2A} antagonist MDL 100,907 were tested for their ability to inhibit lorcaserin-induced (18 mg/kg, oral administration) reductions in food intake (Fig. 5). ANOVA revealed a significant main effect of SB242,084 [F(1,28) = 64.8; p < 0.01] and a significant interaction with lorcaserin [F(1,28) = 12.0; p < 0.01], but no effect for MDL 100,907 [F(1,28) = 2.7; N.S.). For SB242,084, post-hoc analyses revealed a significant attenuation of the decrease in food intake induced by lorcaserin, in addition to a small but significant (p = 0.049) rise in baseline food intake with administration of SB242,084 alone. These data indicate that the reduction in food intake induced by lorcaserin is mediated by 5-HT\textsubscript{2C} and not 5-HT\textsubscript{2A} receptors.
Selective effects on fat mass revealed that decreases in body weight were attributable to the 9-mg/kg group. For subjects in the 4.5-mg/kg group, there was a trend toward reduction in body weight gain that failed for the 18-mg/kg dose from day 8 onward, and day 19 onward for the lorcaserin-treated group, resulting in recovery in body weight, such that body weights in the treated group did not differ from vehicle controls throughout the withdrawal period [F(1,14) = 0.1; N.S.]. Lorcaserin significantly increased the incidence of penile grooming [F(3,20) = 8.6; p < 0.01], an effect mediated by the 5-HT2C receptor, whereas DOI did not [F(3,20) = 0.1; N.S.]. In contrast, DOI produced large increases in both wet dog shakes [F(3,20) = 170.0; p < 0.01] and back muscle contractions [F(3,20) = 30.0; p < 0.01], behavioral outcomes mediated by activation of the 5-HT2A receptor, whereas lorcaserin had no effect on either measures [F(3,20) = 1.1 and 0.0; N.S.].

**Study 3: Chronic Lorcaserin Administration to Male Diet-Induced Obesity Rats.** A similar temporal pattern of effects on food intake and body weight gain emerged for lorcaserin-treated rats over time. Statistical analyses revealed significant reductions in both food intake [lorcaserin: F(3,43) = 29.3; p < 0.01] and body weight gain [lorcaserin: F(3,43) = 3.1; p < 0.05] over the 4 weeks of dosing. All doses of lorcaserin significantly reduced food intake over the first 13 days of dosing, after which time there was a progressive loss in food intake reduction, which was generally dose-dependent. For body weight gain, significant reductions relative to vehicle were apparent for the 18-mg/kg dose from day 8 onward, and day 19 onward for the 9-mg/kg group. For subjects in the 4.5-mg/kg group, there was a trend toward reduction in body weight gain that failed to reach statistical significance.

**TABLE 4** Pharmacokinetic parameters for lorcaserin after a single oral dose (10 mg/kg) in male Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>λZ (h⁻¹)</td>
<td>0.142</td>
<td>0.148</td>
</tr>
<tr>
<td>τ1/2 (h)</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td>AUClast (h · µg/ml or g)</td>
<td>3.202</td>
<td>44.010</td>
</tr>
<tr>
<td>AUCINF (h · µg/ml or g)</td>
<td>3.322</td>
<td>44.132</td>
</tr>
<tr>
<td>%AUCextrap</td>
<td>3.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>Cmax (µg/ml or µg/g)</td>
<td>0.780</td>
<td>6.110</td>
</tr>
<tr>
<td>AUMCzurt (h · h · µg/ml or g)</td>
<td>17.401</td>
<td>267.221</td>
</tr>
<tr>
<td>Mean residence time (h)</td>
<td>5.4</td>
<td>6.1</td>
</tr>
</tbody>
</table>

AUCzurt area under the moment curve of the last point.

Lorcaserin is a potent and selective 5-HT2C agonist with rapid oral absorption and efficacy to decrease food intake and body weight gain in the rat. Functional activity assessed by inositol phosphate accumulation demonstrates lorcaserin to be a high-affinity full agonist at human 5-HT2C receptors, with selectivity over both 5-HT2A (18-fold) and 5-HT2B (104-fold) receptors at which it is a partial and a full agonist, respectively. This functional selectivity may be critical in the context of potential side effects associated with activation of both 5-HT2A and 5-HT2B receptors. The differential 5-HT2A selectivity of lorcaserin is somewhat lower when evaluated in radioligand binding studies rather than by functional assays (EC50 values of 9, 168, and 943 nM obtained from functional assays compared with Kᵢ values of 15, 112, and 174 nM obtained from radioligand competition studies). Regardless, we believe that activity derived from functional rather than radioligand binding data are more relevant to the in vivo effects of lorcaserin.

Lorcaserin was also evaluated for its ability to interact with a number of other human GPCRs and neurotransmitter transporters. Lorcaserin bound only weakly to other human 5-HT receptors (5-HT1A, 5-HT3, 5-HT4C, 5-HT5A, 5-HT6, and 5-HT7 receptors), and it did not significantly compete for radioligand binding to serotonin, dopamine, or norepinephrine transporters until reaching micromolar concentrations. In addition, lorcaserin did not affect 5-HT, norepinephrine, or dopamine release from rat brain synaptosomes or synaptosomal neurotransmitter uptake until approaching micromolar concentrations. These data represent an important difference between lorcaserin and dexfenfluramine, which is a potent

**Discussion**

Lorcaserin was a Selective 5-HT2C Agonist for Obesity Treatment 583
serotonin reuptake inhibitor and releaser, suggesting that therapeutic doses of lorcaserin will not promote monoamine release. Finally, lorcaserin did not compete for radioligand binding to an additional panel of 67 other human GPCRs and ion channels when tested at a final concentration of 1 μM (see Supplemental Table 5). Taking these data together, our data
suggest that clinically important agonist activity of 5-HT2A occupancy may be further lessened by partial agonism at this human 5-HT2A and 5-HT2B receptors has been a major challenge for the discovery and development of 5-HT2C agonists. Agonist activity at the 5-HT2A receptor has been associated with hallucinations (Nichols, 2004). Although many compounds with potent 5-HT2A receptor agonist activity, such as d-lysergic acid diethylamide or psilocybin, are hallucinogenic, other potent 5-HT2A agonists such as lisuride, are not. Thus, it is currently difficult to predict whether a drug with 5-HT2A agonist activity will be hallucinogenic. Therefore, to explore this further, we evaluated the ability of lorcaserin to induce behaviors that are well known to be mediated by central 5-HT2A receptor activation. In this regard, doses of lorcaserin that potently reduced food intake and weight gain in rats did not induce wet dog shakes or back muscle contractions, but DOI, a potent 5-HT2A agonist, caused a robust stimulation of these responses (Fig. 9, C and D). These behaviors are indicative of 5-HT2A agonist activity, and importantly, they are induced by known hallucinogens such as DOI and d-lysergic acid diethylamide (Schreiber et al., 1995; Ouagazzal et al., 2001; Nichols, 2004) and also by nonselective serotonin modulators such as dexfenfluramine (Higgins et al., 2001). As such, the lack of induction of these behaviors by lorcaserin suggests that the functional consequences of 5-HT2A activation are minimal or absent, and they demonstrate that CNS exposures of the compound that result in potent reductions in food intake and weight gain do not induce behavioral effects that are typical of most potent 5-HT2A agonists. Thus, our preclinical pharmacology data suggest that clinically important agonist activity of 5-HT2A receptors at therapeutic doses of lorcaserin should be minimal or absent, and the impact of any such 5-HT2A receptor occupancy may be further lessened by partial agonism at this receptor.

Other potential liabilities of agonist activity at human 5-HT2A or 5-HT2B receptors include cardiac valvular disease (Connolly et al., 1997; Devereux, 1998; Fitzgerald et al., 2000; Rothman et al., 2000; Horowski et al., 2004; Roth, 2007) and possibly pulmonary hypertension (Abenhaim et al., 1996; Launay et al., 2002). These receptors are both expressed on cardiac valvular interstitial cells and smooth cells, and agonist activity at these receptors has been implicated in causing cardiac valvular insufficiency and possibly pulmonary hypertension. Although activation of both receptors has been suggested as a mechanism, a substantial body of evidence indicates that activation of the 5-HT2B Receptor and not the 5-HT2A receptor is responsible (Fitzgerald et al., 2000; Rothman et al., 2000; Roth, 2007). With respect to pulmonary hypertension, the fenfluramines, through their primary metabolites, the norfenfluramines, have potent 5-HT2B activity with only modest 5-HT2A activity (Roth, 2007), and mice are protected from the development of hypoxia-induced disease either by genetic or pharmacological restriction of 5-HT2B activity (Esteve et al., 2007; Hauso et al., 2007).

As reported herein, lorcaserin has human functional receptor selectivity for 5-HT2C versus 5-HT2B and 5-HT2A. Also reported herein, lorcaserin exposure in rodents was 13.3-fold higher in the brain versus peripheral blood. Using these facts and assuming that the rodent CNS-to-plasma ratio holds in humans, the predicted blood concentration required to activate peripheral 5-HT2B and 5-HT2A receptors are approximately 1400-fold (5-HT2B/5-HT2C ratio of 105, CNS/plasma ratio of 13.3) and 250-fold (5-HT2A/5-HT2C ratio of 19, CNS/plasma ratio of 13.3), respectively, above the required blood concentrations to stimulate CNS 5-HT2C receptors. Thus, these data suggest that the theoretical risk of cardiac valvulopathy with lorcaserin administration in humans is very low.

Lorcaserin exhibits rapid absorption and significant exposure in rat brain. Acutely, lorcaserin decreased food intake in rats in a dose-dependent manner, with efficacy observed at 3 mg/kg and above. Mediation by 5-HT2C receptors was confirmed by studies in which lorcaserin-induced decreases in food intake were reversed by the 5-HT2C-selective antagonist SB242,084, but not the 5-HT2A antagonist MDL 100,907. In the diet-induced obese Levin rat, efficacy to decrease food intake was maintained over repeated daily doses, although there was a dose-dependent tendency for food intake to return to control levels over time. However, despite apparent tolerance to the hypophagic effects of lorcaserin, there was no rebound hyperphagia, and decreased body weight gain compared with vehicle was maintained throughout the 28 days of dosing. It is noteworthy that this reduction in body weight gain reflected selective effects on fat rather than lean mass. Cessation of treatment in rats that had been dosed for 28 days with lorcaserin led to a rebound hyperphagia and a gradual return of body weight to control levels. This rebound hyperphagia coupled with maintenance of food intake at or below control levels during dosing suggests there are additional mechanisms of food intake suppression that require further elucidation. In addition to the study in diet-induced obese Levin rats, we have recently reported that chronic lorcaserin administration reduces food intake and weight gain in Sprague-Dawley rats fed a normal chow diet, demonstrating that lorcaserin has consistent efficacy across different strains and diets (Smith et al., 2008).

In conclusion, the present study demonstrates lorcaserin to be a potent and selective 5-HT2C agonist, with a 18- and 104-fold functional selectivity over 5-HT2A and 5-HT2B receptors, respectively. Lorcaserin reduced food intake acutely in
the rat, an effect that was reversed by preadministration of a selective 5-HT₂C antagonist. Administration of lorcaserin to the rat for 28 days resulted in transiently reduced food intake and sustained reductions in body weight gain compared with vehicle. These data show lorcaserin to have potential as a safe and efficacious treatment for obesity. Lorcaserin is currently undergoing evaluation in phase 3 clinical trials for the treatment of obesity.

References


Dimethoxy-4-iodophenyl)-2-aminopropane)-induced head twitches in the rat are mediated by 5-hydroxytryptamine (5-HT2A) receptors: modulation by novel 5-HT2A receptor antagonists, D1 antagonists, and 5-HT1A agonists. J Pharmcol Exp Ther 273:101–112.


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