LLY-2707, A Novel Nonsteroidal Glucocorticoid Antagonist That Reduces Atypical Antipsychotic–Associated Weight Gain in Rats

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ABSTRACT

Weight gain and diabetes have been reported during treatment with atypical antipsychotic drugs (AAPDs). Patients treated with the glucocorticoid receptor antagonist (GRA) mifepristone [estr-a-4,9-dien-3-one, 11-[4-(dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)-(11β,17β)-(8CI)] experienced significant reduction in the weight gain observed when patients were treated with olanzapine or risperidone. To understand the pharmacology responsible for this finding, we discovered LLY-2707 [N-(5-[(tert-butyl)-3-(2-fluoro-5-methylpyridin-4-yl)-2-methyl-1H-indol-7-yl]methanesulfonamide], a novel and selective GRA, and evaluated its utility in preclinical models of AAPD-associated weight gain and diabetes. In vitro, LLY-2707 was a highly selective and potent GRA. GR occupancy in vivo was assessed using ex vivo binding where LLY-2707 inhibited [H]dexamethasone binding to the liver. Modest but statistically significant decreases in brain ex vivo binding were observed with high doses of CORT-108297 [(R)-4α-(ethoxymethyl)-1-(4-fluorophenyl)-6-[(4-(trifluoromethyl)phenyl)sulfonyl]-4,4a,5,6,7,8-hexahydro-1H-pyrazolo[3,4-g]isoquinoline] and LLY-2707, but mifepristone inhibited at all doses. Central activity of the GRAs was confirmed by their ability to suppress amphetamine-induced increases in locomotor activity. The increases in the body weight of female rats treated with olanzapine (2 mg/kg PO) over 14 days were reduced in a dose-dependent manner by coadministration of LLY-2707. Similar decreases, although less robust, in body weight were seen with mifepristone and CORT-108297. In addition, sGRAs prevented the glucose excursion after intragastric olanzapine infusions consistent with a direct effect on the hyperglycemia observed during treatment with AAPDs. At doses effectively preventing weight gain, LLY-2707 did not substantially interfere with the dopamine D2 receptor occupancy by olanzapine. Therefore, GRA coadministration may provide a novel treatment modality to prevent the weight gain and diabetes observed during treatment with AAPDs.

Introduction

Glucocorticoids are important mediators of the actions of insulin and adipose tissue. Endogenous or exogenous glucocorticoid excess cause a number of clinical symptoms, including diabetes and obesity (for review, see Mazzotti et al., 2011). In particular, diabetes is a common complication of glucocorticoid excess and, along with hypertension, is important in the morbidity and mortality in Cushing’s disease (Etxabe and Vazquez, 1994). Patients treated long-term with glucocorticoids have a 2-fold increase in the incidence of diabetes (Henriksen et al., 1997; Darmon et al., 2006; Donih et al., 2006). In addition, there was a correlation with the duration and dosage of glucocorticoids. Even low-dose glucocorticoids impair glucose tolerance when administered long-term (van Raalte et al., 2011). Glucocorticoid receptors (GRs) are expressed in the pancreatic β cell where they impair function and insulin secretion (van Raalte et al., 2009). Through genomic actions, glucocorticoids slow glucose uptake and metabolism by pancreatic β cells, leading to reduced efficacy of Ca2+ in mediating the exocytotic process of insulin containing secretory vesicles (Seino et al., 2010). In a clinical study, short-term exposure to glucocorticoids reduced the insulinotropic effects of GLP-1 (Hansen et al., 2010). In addition, glucocorticoids decrease glucose uptake in skeletal muscle, liver, and adipose by inhibiting glucose transporter translocation to the cell surface (Mazzotti et al., 2011). Glucocorticoids also influence insulin sensitivity indirectly by increasing lipolysis, producing elevations of plasma free fatty acids that impair glucose uptake and disposal (Rondinone, 2006). In addition, glucocorticoid excess stimulates proteolysis, resulting in increased amino acid concentrations that impair insulin signaling (Pivonello et al., 2010). The expression and activity of substances known to modulate insulin sensitivity such as adiponectin, leptin, and apelin are modulated by glucocorticoids (Rondinone, 2006; Castan-Laurell

Supplemental material to this article can be found at: http://jpet.aspetjournals.org/content/suppl/2013/10/25/jpet.113.205864.DC1
et al., 2011). Therefore, glucocorticoids assert an important role in the etiology of obesity and insulin resistance.

Atypical antipsychotic drugs (AAPDs) are effective in treating a range of psychiatric symptoms, and fewer extrapyramidal adverse events are reported with them than with older antipsychotic medications (Stahl, 1999). However, patients treated with AAPDs have reported clinically significant weight gain, dyslipidemia, and diabetes (Bergman and Ader, 2005). In addition to antagonizing the D2 dopamine receptor, olanzapine interacts with a number of additional neurotransmitter receptors (Bymaster et al., 1999, 2001). To date, the pharmacologic mechanism potentially related to the metabolic changes remains unknown. Because hyperactivity of the glucocorticoid system is associated with obesity and insulin resistance (Prpić-Križevac et al., 2012), it is a compelling target for intervention. In preclinical studies, estra-4,9-dien-3-one, 11-[4-(dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)-(11b,17b)-(9Cl), a GR antagonist (GRA) and progesterone receptor antagonist (PRA) medication (Ulmann et al., 1990), reduced the weight gain seen in young obese fa/fa Zucker rats (Langley and York, 1990) and decreased the elevated insulin levels and weight gain observed in olanzapine-treated rats (Beebe et al., 2006). Patients treated with mifepristone experienced a reduction in weight gain during treatment with AAPDs, which included risperidone (Gross et al., 2010) and olanzapine (Gross et al., 2009). Statistically significant reductions in weight, body mass index (BMI), and waist circumference were observed over 14-day treatment in drug-naïve patients treated with mifepristone and olanzapine versus patients treated with olanzapine alone (Gross et al., 2009). When combined with risperidone for 28 days, patients treated with mifepristone experienced reduced weight gain compared with patients treated with risperidone alone (Gross et al., 2010). In addition, improvements were noted in the insulin tolerance test, plasma triglycerides, plasma insulin, and the homeostasis model assessment of insulin resistance (HOMAIR). A second-generation compound, CORT-108297 [(R)-4a-(ethoxymethyl)-1-(4-fluorophenyl)-6-[(4(trifluoromethyl)phenyl)sulfonyl)-4,4a,5,6,7,8-hexahydro-1H-pyrazolo[3,4-g]isoquinoline], has been reported to have a high affinity for GRs with no demonstrated activity at PRs (Ulmann et al., 2005). In addition to antagonizing the D2 dopamine receptor, CORT-108297 for efficacy in preclinical models of olanzapine treatment-emergent weight gain and diabetes. Because weight gain itself is associated with a reduction in glycemic control, we pursued the hypothesis that selective GRAs have a direct effect on the loss of glycemic control seen with short-term olanzapine administration in rats via the intragastric route.

**Materials and Methods**

**In Vitro Binding.** Mifepristone, CORT-208197, and LLY-2707 (Fig. 1) were all synthesized at Eli Lilly and Company (Indianapolis, IN). Steroid receptor competition binding assays were run in a buffer containing 20 mM HEPES buffer (pH = 7.6), 0.2 mM EDTA, 75 mM NaCl, 1.5 mM MgCl2, 20% glycerol, 20 mM sodium molybdate, 0.2 mM DTT, 20 μg/mL aprotinin, and 20 μg/mL leupeptin (assay buffer). Radiolabeled ligands were used to detect binding to cells expressing receptors including 0.25 nM [3H]aldosterone for mineralocorticoid receptor (MR) binding, 0.3 nM [3H]dexamethasone for GR binding, 0.36 nM [3H]methyltrienolone for aldosterone receptor (AR) binding, and 0.29 nM [3H]methyltrienolone for PR binding (PerkinElmer Life and Analytical Sciences, Waltham, MA). Receptors were recombinantly expressed in human embryonic kidney 293 (HEK-293) cells, and 20 μg of 293-MR lysate, 20 μg of 293-GR lysate, 22 μg of 293-AR lysate, or 40 μg of 293-PR lysate were added per well. Competing test compounds were added at various concentrations from 0.01 nM to 10 μM. Nonspecific binding was determined in the presence of 500 nM aldosterone for MR binding, 500 nM dexamethasone for GR binding, or 500 nM methyltrienolone for AR and PR binding. The binding reactions (140 μl) were incubated overnight at 4°C, then 70 μl of cold charcoal-dextran buffer (containing per 50 ml of assay buffer, 0.75 g of charcoal, and 0.25 g of dextran) was added to each reaction. Plates were then centrifuged at 3000 rpm at 4°C for 10 minutes. A 120-μl aliquot of the binding reaction mixture was then transferred to another 96-well plate, and 175 μl of Wallac Optiphase Hisafe 3 scintillation fluid was added to each well. The plates were sealed and shaken vigorously using an orbital shaker. After a 2-hour incubation, the plates were counted using a Wallac MicroBeta counter (PerkinElmer Life and Analytical Sciences).

These data were used to calculate an IC50, and the percentage inhibition was determined at 10 μM. The IC50 values for compounds were converted to Kd using the Cheng-Prusoff equation. The Kd for [3H]aldosterone for MR binding, [3H]dexamethasone for GR binding, [3H]methyltrienolone for AR binding, or [3H]methyltrienolone for PR binding was determined by saturation binding.

![Fig. 1. Chemical structures of glucocorticoid receptor antagonist compounds used in the studies.](image-url)
Additional selectivity studies were performed by submitting LLY-2707 to a panel of frequent cross-reactive drug targets where the compound was evaluated at concentrations of and 10 μM. This panel included serotonin 5-hydroxytryptamine_{1b}, acetylcholinesterase, adenosine A_{1}, B_{1} adrenergic receptor, B_{2} adrenergic receptor, dopamine D_{1}, dopamine D_{2a}, histamine H_{3}, muscarinic M_{1}, muscarinic M_{3}, norepinephrine transporter, opioid μ, α_{1} adrenergic receptor, α_{2} adrenergic receptor, calcium channels (dihydropyridine site), calcium channels (diltiazem site), calcium channels (verapamil site), chloride channels, and central benzodiazepine receptors. A reduction in binding/activity at both a 1 and 10 μM concentration of LLY-2707 would trigger a K_{i} determination. These studies were conducted under contract by Cerep, Inc. (Redmond, WA; http://www.cerep.fr).

**Glucocorticoid Receptor Translocation Assay.** This assay served as an indicator of binding of test compounds to GR and the subsequent translocation of the complex into the cell nucleus. The PathHunter Nuclear Translocation Assay (93-0002; DiscoveRx, Fremont, CA) was used to analyze translocation of GR upon ligand stimulation. In brief, hamster hamster ovary K1 (CHO-K1) cells, stably expressing GR that was linked to a β-galactosidase fragment and coexpressing a complementary β-galactosidase fragment linked to a nuclear localization sequence (Patel et al., 2009), were grown in T150-cm² flasks in F-12 medium with 10% fetal bovine serum (FBS). The cells were trypsinized, plated at a density of 15,000 cells per well in 384 well dishes in Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 Nutrient Mixture with 5% CS-FBS, and incubated overnight at 37°C under 5% CO₂. The cells were then exposed to various concentrations of test compound ranging from 0.09 nM to 25 μM and incubated for an additional 6 hours at 37°C under 5% CO₂. After incubation with compounds, the cells were lysed and β-galactosidase activity measured using the PathHunter Detection Kit (DiscoveRx 93-0001). Data were fit to a four-parameter-fit logistics curve to determine the EC_{50} values. The percentage efficacy was determined by comparing the compound stimulation with the maximum stimulation obtained using 100 nM dexamethasone as the agonist.

**Tyrosine Aminotransferase Enzyme Activity Assay.** This assay was used to determine whether these compounds act as a GR agonists or GR antagonists. The full GR agonist, dexamethasone, induced GR transactivation, resulting in the synthesis of tyrosine aminotransferase in hepatoma tissue culture (HTC) cells. As an indicator of functional activity of test compound on the GR, the tyrosine aminotransferase enzyme (TAT) activity assay was performed in agonist and antagonist modes. In brief, rat hepatoma cells H4IIEC3 (CRL-1600; American Type Culture Collection [ATCC], Manassas, VA) were grown in T150-cm² flasks in DMEM with 10% FBS. The cells were trypsinized and replated in 96-well plates at 100,000 cells per well and incubated overnight at 37°C under 5% CO₂. After this incubation, the media were replaced with DMEM/F-12 containing 5% CS-FBS and incubated for another 24 hours at 37°C under 5% CO₂. Test for agonist activity, cells were exposed to various concentrations of test compounds ranging from about 0.09 nM to 25 μM and incubated for an additional 24 hours at 37°C under 5% CO₂. To test for antagonist activity, cells were treated with various concentrations of test compounds ranging from 0.09 nM to 25 μM for 1 hour before the addition of 1 nM dexamethasone and incubated for an additional 24 hours at 37°C under 5% CO₂. After incubation with test compounds, the cells were washed in phosphate-buffered saline, lysed with solubilization buffer at pH 7.6 containing 125 mM potassium phosphate, 1 mM EDTA, 1 mM DTT, and 0.5% Nonidet P-40 and frozen at −80°C. Upon thawing the plates, TAT substrate solution at pH 7.6 containing 125 mM potassium phosphate, 4.74 mM L-tyrosine, 13.3 mM L-ketoglutarate, and 0.07 mM pyrocatechol phosphate was added to the lysates. The reaction was allowed to develop for 2 hours at 37°C under 5% CO₂. After 2 hours, the p-hydroxyphenylpyruvate produced in the reaction was converted to p-hydroxybenzaldehyde with the addition of 10 N potassium hydroxide and incubation for 30 minutes. Absorbance at 340 nM was read for each well using a spectrophotometer. The p-hydroxybenzaldehyde concentration of samples was calculated based on a standard curve, and the resulting data were fit to a four-parameter-fit logistics to determine EC_{50} values for agonist activity and IC_{50} values for antagonist activity.

**Ex Vivo Binding of [³H]Dexamethasone to Rat Liver and Brain after Oral Administration.** All animal experimentation was performed with approval from the Eli Lilly Institutional Animal Care and Use Committee in accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals. Ex vivo binding was conducted using a previously described protocol with slight modifications (Luzzani et al., 1983). Male Sprague-Dawley rats (approximately 240 g; Harlan, Indianapolis, IN) were dosed in a random fashion with LLY-2707 or vehicle alone (1% carboxymethylcellulose [w/v]; Sigma-Aldrich, St. Louis, MO), with five rats per group. One hour after administration, the rats were decapitated, and the left lateral lobe of the liver was dissected, weighed, and frozen using dry ice. Whole brains were removed and placed in tubes on wet ice with assay buffer (10 mM Tris-HCl, 1.5 mM EDTA, 25 mM sodium molybdate, 7.4, with 1 mM diithiothreitol and 9% glycerol [v/v]) added fresh the day of the assay) until the live phase of the assay was complete.

To prepare the brain tissues for the assay, both hemispheres of the cerebral cortex were dissected, weighed, and placed in tubes on ice. Five volumes of cold assay buffer were added to the cortex, and five volumes of room temperature assay buffer were added to the frozen liver. Tissues were homogenized with a Polytron homogenizer (Kinematics AG, Lucerne, Switzerland) and spun in an ultracentrifuge at 105,000g. The clear supernatant (cytosol) was extracted and frozen at −80°C until time of the binding assay.

For [³H]dexamethasone binding, aliquots of assay reagents were added to incubation tubes for final assay concentrations of cytosol (0.7 mg/ml protein), [³H]dexamethasone (3.3 nM; PerkinElmer Life and Analytical Sciences), and either dexamethasone (10 μM in ethanol, to assess nonspecific binding) (Sigma-Aldrich) or assay buffer. Tubes were incubated at 4°C for 24 hours. The incubations were terminated by adding aliquots of 5% Dextran Coated Charcoal (Sigma-Aldrich) (33% of assay volume), mixed well and incubated at 4°C for 10 minutes to adsorb the unbound [³H]dexamethasone. Tubes were then centrifuged at 2000g for 20 minutes. Aliquots of the supernatant were combined with Filtron-X (National Diagnostics, Atlanta, GA) and counted using a 1-H liquid scintillation counter. Specific binding was calculated using Microsoft Excel 2007 by subtracting the nonspecific disintegrations per minute from the total binding disintegrations per minute. Results were expressed as the percentage of inhibition compared with the vehicle-treated group. Statistical analysis using one-way analysis of variance (ANOVA) and Dunnett’s post hoc test were performed using GraphPad Prism 4 (GraphPad Software, San Diego, CA).

**Reversal of Amphetamine-Induced Locomotor Activity.** Amphetamine is a psychostimulant that increases locomotion in rodents via an interaction with the striatal dopaminergic system. Glucocorticoids have been shown to be involved in modulation of sensitized amphetamine-induced hyperactivity (De Vries et al., 1996). In this assay, reversal of amphetamine-induced locomotion was used as a measure of central activity of GRAs. Locomotor activity (LMA) in mice was measured using the MotorMonitor software and SmartFrame Cage rack system from Kinder Scientific (Poway, CA) using a 7 × 15 photo beam grid to quantitate horizontal movement.

Male C57Bl/6 mice (20–22 g; Harlan) were housed in standard rodent cages with ad libitum access to house water and Teklad 2014 rodent Chow (Harlan). To induce amphetamine sensitivity, the mice were dosed via intraperitoneal injection with amphetamine (Amph, 0.9-amphetamine sulfate, 3 mg/kg in saline, 10 ml/kg; Sigma-Aldrich) on experimental days 1 and 3. On experiment day 5, the mice were moved from their home cages to the behavioral testing room and were allowed to acclimate for 1 hour. Study groups (n = 8) were formed to...
Effect of GRAs on Plasma Glucose, Insulin, and Corticosterone after Intragastric Infusion of Olanzapine. This model was based on a recently published method (Girault et al., 2012) showing increases in plasma glucose after intragastric infusion of olanzapine. The live phase of the intragastric infusion studies was conducted at Bioanalytical Systems, Inc. (BASI, West Lafayette, IN). Male Wistar rats (300–350 g, eight per group; Harlan) were administered a single intragastric bolus dose of glucocorticoid antagonist or vehicle (pre-treatment, 1% lactic acid, 1 mg/kg) at a dose of 0.5 mg/kg (LY-2707), 6 mg/kg (mifepristone), or 15 mg/kg (CORT-108279) on days −3, −2, −1, and 1. The doses of the sGRAs were based on the threshold doses in the treatment-emergent weight gain model (Fig. 4; Supplemental Figs. 5 and 6). Approximately 2 hours after the pretreatment dose on day 1, the animals were then administered olanzapine or vehicle by intragastric infusion for 160 minutes at a rate of 3 mg/kg per hour equivalent to a volume of 2 ml/kg per hour. Control animals received an equal volume of vehicle over the infusion period. Entry into the animal rooms was minimized and limited to dosing and checking the sample collection status.

Whole-blood samples were collected from the arterial catheter using the Culex automated blood sampling system (BASI). Blood samples were collected approximately 30 minutes before pretreatment dosing (T = 0) and at 150 (immediately before the treatment dose), 180, 210, 240, 270, 300, and 330 minutes after the T = 0 sample (eight samples per animal). Samples were processed to plasma and frozen at −80°C until analyzed. Plasma glucose values were determined with a Roche Cobas c311 blood chemistry analyzer (Roche, Basel, Switzerland). Plasma corticosterone was measured by radio-immunoassay (MP Biomedicals, Orangeburg, NY). Plasma insulin was determined using a MSD mouse/rat insulin assay kit (Meso Scale Discovery, Gaithersburg, MD). Data were analyzed using a two-way ANOVA with Bonferroni post hoc testing.

Evaluation of D2 Receptor Occupancy by Olanzapine. Functional antagonism of the dopamine D2 receptor is a common pharmacologic feature of all current antipsychotic medications. The purpose of this assay was to assess if the D2 receptor occupancy (RO) of olanzapine was changed when coadministered with LLY-2707.

Male Sprague-Dawley rats (225–250 g; Harlan) were dosed orally with increasing doses of olanzapine (in a vehicle containing 0.1% lactic acid, 1 ml/kg), and a second set of rats were administered the combination of olanzapine and LLY-2707 at 10 mg/kg. One hour later, the animals were administered a tracer dose of the D2 antagonist raclapride (3 µg/kg i.v. in dilute lactic acid; Sigma-Aldrich) (Barth et al., 2000). Fifteen minutes later, the animals were sacrificed by decapitation. Brains were removed from the skulls, and the striatum and cerebellum were dissected. Tissue and plasma were frozen at −70°C until analysis.

Tissue concentrations of the tracer were measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS, API-4000; Applied Biosystems). Tissues were thawed on wet ice and then homogenized with a 4 × volume of acetonitrile containing 0.1% formic acid using a sonic probe dismembrator (Thermo Fisher Scientific). Samples were then centrifuged at 14,000 rpm for 16 minutes. Supernatants were removed and diluted 1:9 with sterile water. For tracer analysis, standard curves were generated for a range of 0.3 to 100 ng/g tissue. Reduction of the tracer concentrations by LLY-2707 were then transformed into percentage of receptor occupancy. Data were analyzed by two-way ANOVA, and comparison between dose groups was determined using an F-test.

Results

To evaluate the potency and selectivity of GRAs (Fig. 1), binding assays to the various steroid receptors were used. In these assays, LLY-2707 exhibited a Kᵢ of 2.13 nM at GR with substantially lower potency at the other steroid receptors (Table 1). In contrast, mifepristone has high affinity at both

Consist of vehicle (veh)/VEH, veh/Amp, test compound/Amp groups. Mice were then dosed via oral gavage with various doses of test compound or vehicle (veh, 1% carboxymethylcellulose in water) and placed in a plastic 8 × 16 rodent cage containing 1 inch of wood-chip bedding within the SmartFrame Cage rack system. The LMA was recorded for a 60-minute habituation period. The mice were then injected with amphetamine (3 mg/kg i.p.) or vehicle (VEH, saline) and replaced in the locomotor chamber; then the LMA was recorded for an additional 60 minutes. The sum of the x and y horizontal distance traveled (in centimeters) recorded by the MotorMonitor software was combined in 60-minute bins representing the habituation and amphetamine-induced LMA periods. Statistical analysis using one-way ANOVA and Dunnett’s post-hoc test were performed using GraphPad Prism 4 (GraphPad Software).

Food Intake and Body Weight Evaluations. This study determined whether the selective GRIA LLY-2707 could prevent atypical antipsychotic treatment-emergent weight gain in female Sprague-Dawley rats. For comparison, similar studies were performed evaluating mifepristone and CORT-018297. This assay was used to determine the efficacious dose of the antagonists, and the efficacy dose (threshold dose) was defined as the dose that brought the body weight to equal that of the vehicle dose group. These studies were performed under contract by Covance (Greenfield, IN).

Because previous studies have documented that olanzapine is associated with body weight increases in female rats but not male rats (Pouzet et al., 2003), females were used for this study. Lean (approximately 235 g) adult female Sprague-Dawley rats (Harlan) were housed individually with normal chow (Teklad 2014; Harlan) and water available ad libitum for a 1-week acclimation period with lights off at 10:00 AM. The animals were then acclimated to vehicle dosing for 1 week.

After acclimation, the body composition analysis was performed by quantitative nuclear magnetic resonance (QNMR) using EchoMRI-700 Whole Body Composition Analyzer (EchoMRI, Houston, TX) for fat mass. The fat-free mass was calculated as body weight − fat mass. The animals were block randomized on body weights to seven treatment groups. From days 1 to 14, vehicle (0.1% lactic acid, n = 8), olanzapine (Olz) (2 mg/kg; n = 8), olanzapine (2 mg/kg) + LLY-2707 (0.3, 1.0, 3.0, or 10.0 mg/kg, n = 8/dose group), or test compound only (10 mg/kg, n = 8) was administered orally once daily in a volume of 1 ml/kg. Studies using mifepristone and CORT-108279 as the primary test compounds were conducted using identical vehicle and volume. A positive control group was also included, which consisted of olanzapine (2 mg/kg) + CORT-108279 (30 mg/kg, n = 8) as part of the LLY-2707 study. A second body-composition analysis was performed on day 15 to obtain the fat mass and fat-free mass. Data were analyzed by one-way ANOVA followed by Dunnett’s t test with comparisons against vehicle or olanzapine separately. The significance levels were set at 0.025, so that overall significance threshold is 0.05.

After the weight loss study, a fifteenth dose of LLY-2707 was administered the next day. Plasma samples were obtained by tail clip with the following procedure: First, three animals from each dose group were sampled at 0, 30 minutes, 2 hours, and 6 hours. Second, three animals from each dose group were sampled for the time points 15 minutes, 1 hour, 4 hours, and 24 hours. The last two animals from each dose group were used for plasma samples at 2 hours, Samples were processed to plasma and were frozen at −80°C until analyzed. Plasma concentrations of LLY-2707 were measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS, Sciex API-4000; Applied Biosystems, Foster City, CA). To compare these values, additional pharmacokinetic studies were performed using short-term administration of 1 mg/kg LLY-2707 to male and female Sprague-Dawley rats (fed or fasted overnight) that were maintained on stock rat chow (Harlan Teklad 2014). Blood was collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing. Pharmacokinetic parameters for both studies were determined using Watson LIMS (Thermo Fisher Scientific, Waltham, MA). The studies and analytical work were performed under contract at Covance and Advion (Indianapolis, IN), respectively.

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GR and PR while having modestly lower affinity at AR and much lower affinity at MR. Similar to LLY-2707, CORT-108297 had only high affinity at GR with a $K_i$ of 0.45 nM. The ability of the compounds to stimulate or antagonize GR was evaluated in a TAT enzyme activity assay using rat hepatoma cells. The selective GR compounds were potent antagonists with minimal agonist activity (Table 2) while mifepristone exhibited no agonist activity. In the glucocorticoid receptor translocation assay, LLY-2707 displayed an EC$_{50}$ of 9.12 nM (data not shown), thus demonstrating that the compound not only bound to GR, but the complex is subsequently translocated into the nucleus, indicating that it was an active antagonist of GR. Taken as a whole, these results were consistent with these compounds predominantly exhibiting antagonist activity in vitro. In a panel of frequently cross-reactive drug targets, LLY-2707 did not inhibit binding-functional readouts greater than 50% at both the 1 and 10 μM screening concentrations.

Having established the in vitro potency and selectivity of compounds, we then evaluated these compounds in $[^{1}H]$dexamethasone binding ex vivo to determine their potency at GR in liver and brain (Fig. 2) after short-term oral administration. In these studies, LLY-2707 produced significant inhibition of binding at all doses in liver and the brain. However, the inhibition in the cerebral cortex was only partial. By comparison, mifepristone produced almost complete inhibition in liver and brain at doses of 3 mg/kg or greater (Supplemental Fig. 1). CORT-108297 produced a dose-dependent decrease at 3, 10, and 30 mg/kg in liver while a small but statistically significant inhibition of binding was observed in brain at the highest dose of 30 mg/kg (Supplemental Fig. 2).

The functional antagonism of GRA in the brain by these compounds was evaluated using an amphetamine sensitization paradigm where the subsequent increases in LMA were reported to be glucocorticoid dependent (De Vries et al., 1996). LMA was evaluated after a third dose of 3 mg/kg amphetamine. The raw data and summarized data from these experiments are shown in Fig. 3. In this paradigm, LLY-2707 had no significant activity of baseline LMA, but decreased amphetamine-evoked LMA in a dose-dependent manner with a calculated ED$_{50}$ of 40.5 mg/kg for the 1-hour period after administration. For comparison, mifepristone also had no significant effect on baseline LMA but inhibited amphetamine-evoked LMA in a dose-dependent manner with a calculated ED$_{50}$ of 10.5 mg/kg (Supplemental Fig. 3), consistent with its higher potency in ex vivo binding. On the other hand, CORT-108297 did produce a reduction in baseline LMA (Supplemental Fig. 4), complicating the interpretation of the amphetamine-induced LMA. Nevertheless, a reduction in the amphetamine-evoked LMA was observed with an ED$_{50}$ of 23.3 mg/kg. In this assay, all three compounds produced statistically significant decreases in evoked LMA, demonstrating that the compounds did produce a physiologically relevant inhibition of brain GR in vivo.

Having established the effective doses of these antagonists using ex vivo binding, we evaluated the ability of these compounds to prevent the treatment-emergent weight gain during olanzapine administration to female Sprague-Dawley rats. As seen in Fig. 4, the olanzapine-treated group experienced a treatment-emergent body weight gain of 11.8 g versus vehicle. The compound LLY-2707, when coadministered at doses of 0.1, 0.3, 1.0, and 3.0 mg/kg with olanzapine, reduced the cumulative body weight gain in a dose-dependent manner relative to olanzapine treatment alone. The olanzapine treatment group experienced a treatment-emergent body weight gain of 41 g versus vehicle (Supplemental Table 1). Coadministration of LLY-2707 with olanzapine reduced the cumulative food intake in a dose-dependent manner relative to olanzapine treatment alone. The olanzapine reduced the cumulative food intake increase of 41 g versus vehicle. The compound LLY-2707, when coadministered with olanzapine, significantly reduced the fat mass at the 1 and 3 mg/kg doses versus olanzapine alone. Fat-free mass was increased 8.2 g by olanzapine alone versus vehicle and was reduced by LLY-2707 relative to olanzapine treatment alone. A calculated threshold effective dose for LLY-2707 of 0.44 mg/kg in reversal of the olanzapine treatment-emergent weight gain (Table 3). Similar studies

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performed using mifepristone and CORT-108297 resulted in calculated threshold effective doses of 5.33 and 11.13 mg/kg, respectively (Table 3; Supplemental Figs. 5 and 6). No overt effects of GRA treatment with or without olanzapine administration were noted in the rats.

The effects of the GRA compounds on glycemic regulation were evaluated using intragastric infusion of olanzapine. Previous studies have demonstrated that this method can increase plasma corticosterone and glucose without affecting plasma insulin concentrations in a time-dependent manner (Girault et al., 2012). We tested the hypothesis that pretreatment with GRAs can prevent this glucose excursion. As summarized in Fig. 5, pretreatment each of the three compounds did not produce an appreciable effect on plasma glucose after vehicle infusion, but consistent and time-dependent increases in plasma glucose were observed after olanzapine.

![Graph](image1)

**Fig. 2.** Displacement of [3H]dexamethasone from liver and brain cytosol by LLY-2707 ex vivo. Rats were administered 3, 10, and 30 mg/kg LLY-2707 orally and sacrificed 1 hour later. Liver and brain cytosols were then subjected to [3H]dexamethasone binding in vitro. Compound contained within the cytosol preparations competed for the binding of [3H]dexamethasone. While nearly complete inhibition was seen in the liver, modest but statistically significant decreases were seen in the brain. ***P < 0.001, *P < 0.05, n = 5.

![Graph](image2)

**Fig. 3.** LLY-2707 decreased the locomotor response to amphetamine in sensitized mice. After a sensitization procedure, mice were administered vehicle (veh) or various doses of LLY-2707 as indicated. One hour later, the animals received vehicle or 3 mg/kg amphetamine (amph). (A) Time dependence of locomotor activity. (B) Cumulative locomotor activity after amphetamine administration. N = 8, *P < 0.05.
infusion, which reached statistical significance at 120 and 150 minutes after the beginning of the infusion. Pretreatment with all three GRAs reduced the glucose excursion to levels that were not significantly different from intragastric infusion of the vehicle alone.

The plasma samples were also evaluated for the concentrations of insulin and corticosterone. A change in plasma insulin levels was not observed after the olanzapine infusions, despite the increases in plasma glucose observed. With all three GRAs, there were no statistically significant changes in insulin with or without olanzapine infusion (Supplemental Fig. 7). After the olanzapine infusion, a rapid increase in the plasma concentrations of corticosterone that were observed from the 30-minute sample onward in the mifepristone and CORT-108297 experiments (Supplemental Fig. 8), and the 60-minute sample onward in the LLY-2707 experiment. Pretreatment with mifepristone appeared to shift the time dependence of this change, with the 120-minute time point reaching statistical significance. Pretreatment with CORT-108297 reduced the corticosterone to levels that were not significantly different from the control levels at all time-point studies. Finally, LLY-2707 reduced the corticosterone levels and only reached statistical significance at the final 150-minute time point. Collectively, these compounds reduced both the corticosterone and glucose excursions after intragastric infusions of olanzapine without significantly affecting insulin concentrations, consistent with an improvement in insulin sensitivity.

To evaluate whether coadministration of LLY-2707 would affect the efficacy of olanzapine in vivo, we evaluated the potential effects on D2 receptor occupancy using raclopride. These data are summarized in Table 4. Increasing doses of olanzapine resulted in a displacement of raclopride binding to the striatal D2 receptor in vivo. The addition of 10 mg/kg LLY-2707 did not change the D2 displacement appreciably.

Because the rat model of weight gain only works in female rats, we also investigated the pharmacokinetics of LLY-2707 in both male and female rats after short-term administration (Supplemental Table 2). Female rats exhibited a significantly higher plasma area under the curve (AUC) when compared with male rats and a somewhat longer elimination half-life ($T_{1/2}$), though this did not reach statistical significance. The difference between male and female fats was observed in both the fed and fasted states. A significant difference between the $C_{\text{max}}$ observed in the fed female and male groups was also noted.

Compared with fasted animals, the fed animals exhibited a delay in the time for peak plasma levels ($T_{\text{max}}$), but this was statistically significant only the in male group. After completion of the feeding study outlined in Fig. 4, those animals were administered a fifteenth dose 24 hours after body weight assessment, and the pharmacokinetics of LLY-2707 were assessed. These data are summarized in Supplemental Table 3. At a dose of 1 mg/kg, the area under the curve for LLY-2707

TABLE 3
Effect of GR antagonists on olanzapine treatment-emergent weight gain

<table>
<thead>
<tr>
<th>Compound</th>
<th>Threshold Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLY-2707</td>
<td>0.44</td>
</tr>
<tr>
<td>Mifepristone</td>
<td>5.33</td>
</tr>
<tr>
<td>CORT-108297</td>
<td>11.13</td>
</tr>
</tbody>
</table>

Fig. 4. The effect of 14 days of LLY-2707 (LLY) administration on body weight in female Sprague-Dawley rats with and without concurrent administration of 2 mg/kg olanzapine (OLZ). In these studies, olanzapine produced a treatment-emergent significant increase in body weight that was prevented by coadministration of LLY-2707. When administered without olanzapine, 3 mg/kg LLY-2707 decreased body weight relative to vehicle-treated controls. For reference, 30 mg/kg CORT-108297 (CORT) was administered with olanzapine, producing a reduction in body weight compared with both olanzapine and vehicle administration. The 14-day summary results are found in Supplemental Table 1.
was similar to that observed in the short-term studies (Supplemental Table 2), and the $T_{\text{max}}$ was later (6 vs. 1.7 hours) than was observed in the short-term studies. The late $T_{\text{max}}$ seen in this group did not allow for an accurate calculation of the $T_{1/2}$ at this dose. The instrument sensitivity settings did not allow for accurate pharmacokinetic calculations at the doses of LLY-2707 less than 1 mg/kg. At the dose of 3 mg/kg LLY-2707, no substantial differences were seen between the groups dosed with or without olanzapine. These studies were not conducted in a manner that allowed statistical comparison of these parameters between dosing groups or the short-term study summarized in Supplemental Table 2.

Discussion

Body weight gain and metabolic alterations have been reported during treatment with atypical antipsychotic medications. Clinically significant weight gain of 0.9 kg/month resulting in a 6- to 10-kg body weight gain was reported after 1 year of treatment with olanzapine (Nemeroff, 1997). In the Clinical Antipsychotic Trials of Interventions (CATIE) study, 30% of olanzapine-treated schizophrenics gained more than 7% of their initial body weight (Meyer et al., 2005). Because the weight gain occurred in a population that is already susceptible to diabetes mellitus and coronary artery disease (Henderson et al., 2005), this issue is of particular importance. Given the complex pharmacology of olanzapine (Bymaster et al., 1999; Bymaster et al., 2001), little is known about the drug targets that contribute to the weight gain. It has been proposed that blockade of the 5-hydroxytryptamine$_{2c}$ and histamine H1 receptors may contribute to food craving and binge eating (Stahl, 1998), but this has not been proven.

A number of pharmacologic strategies were pursued in the clinic to mitigate the treatment-emergent weight gain seen with AAPDs but with limited efficacy (Maayan et al., 2010). In a proof-of-concept clinical trial, mifepristone prevented the weight gain observed during treatment with olanzapine in healthy Indian men (Gross et al., 2009). In a similar trial, mifepristone also prevented the weight gain seen with risperidone (Gross et al., 2010). One of the challenges of finding novel clinical approaches has been identifying a suitable preclinical model to test a broad range of pharmacotherapies. In recent years, the weight gain could be modeled in female Sprague-Dawley rats (Albaugh et al., 2006). In this model, concomitant administration of mifepristone with olanzapine reduced the weight gain and body fat observed during treatment with olanzapine administration. Because mifepristone has a similar affinity for both PR and GR as well as substantial affinity for AR, a more selective agent would be desirable for clinical development. As a result, the antagonist CORT-108297 was discovered by scientists at Corcept (Menlo Park, CA) (Clark, 2008). Though no clinical data are available to date, it has been evaluated in the rat weight gain model. In these studies, CORT-108297 was able to reverse or prevent the weight gain observed during treatment with olanzapine administration (Clark, 2008) indicating that GR antagonism was the primary pharmacology responsible for the findings.

In the present study, we sought to identify a selective GRA to pursue this hypothesis clinically. The result of these efforts is the highly selective GRA, LLY-2707. In functional assays, LLY-2707 is a potent antagonist with slight agonist activity similar to what was observed with CORT-108297. In vivo, LLY-2707 produced maximal reduction in $[^{3}H]$dexamethasone binding to rat liver ex vivo at all doses tested. In the same

<table>
<thead>
<tr>
<th>Olanzapine</th>
<th>Percentage D2 Receptor Occupancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>53.8 ± 7.3 mg/kg</td>
</tr>
<tr>
<td>LLY-2707 (10 mg/kg)</td>
<td>52.4 ± 5.4 mg/kg</td>
</tr>
<tr>
<td>LLY-2707 (5 mg/kg)</td>
<td>74.6 ± 4.8 mg/kg</td>
</tr>
<tr>
<td>LLY-2707 (0.5 mg/kg)</td>
<td>92.7 ± 0.5 mg/kg</td>
</tr>
<tr>
<td>LLY-2707 (0.05 mg/kg)</td>
<td>65.9 ± 4.4 mg/kg</td>
</tr>
<tr>
<td>LLY-2707 (0.01 mg/kg)</td>
<td>90.7 ± 0.7 mg/kg</td>
</tr>
</tbody>
</table>
associated weight gain without interfering with olanzapine and olanzapine administration did not alter the pharmacokinetics of the compound on GRs. However, the possibility that off-target activities in the selectivity panel, this is unlikely because of the ability to antagonize D2 receptors in rats. Although olanzapine does have a number of additional pharmacologies that may contribute to its antipsychotic efficacy, we did not assess the effects of LLY-2707 on those activities.

The mechanism by which GRAs mitigate weight gain during treatment with AAPDs is not clear. Elevated plasma concentrations of cortisol are associated with schizophrenia, diabetes, and obesity. Even without treatment, patients with schizophrenia are at risk for obesity and diabetes (Bergman and Ader, 2005). Cushing's disease is defined by inappropriate high levels of ACTH secretion, with the consequent increased circulating cortisol as the root cause of the disease. By direct and indirect mechanisms, this produces both deposition of adipose and diabetes (for review, see van Raalte et al., 2009) and can be treated successfully with mifepristone. However, patients treated long term with olanzapine or quetiapine experienced a reduction in both plasma ACTH and cortisol concentrations of healthy volunteers, and this activity may be an important component of their efficacy (Cohrs et al., 2006).

Interestingly, circulating levels of cortisol may not accurately reflect the role of this hormone in obesity. The enzyme 11B-hydroxysteroid dehydrogenase (11B-HSD) is responsible for converting the less active metabolite of cortisol cortisone back to cortisol. In the livers of obese patients, 11B-HSD appears to be impaired while its activity was markedly higher in adipose tissue (Sandep et al., 2005). The activity of 11B-HSD has been reported to be increased in rats treated with the AAPD clozapine (Tulipano et al., 2007). In addition to weight gain, a decrease in insulin sensitivity has been reported during treatment with AAPDs (Sacher et al., 2008). Because excess glucocorticoids are implicated in both weight gain and decreased insulin sensitivity, GRAs are a logical target to try to mitigate these issues.

Because weight gain is associated with a reduction in insulin sensitivity, it is difficult to evaluate the direct effect of agents on glycemias with long-term administration. Therefore, we used an short-term intragastric infusion of olanzapine (Girault et al., 2012) to understand the potential direct effects of sGRAs on the hyperglycemia. By use of a similar methodology with an oral bolus administration, it was shown that AAPDs that increase body weight cause an increase in plasma glucose and corticosterone, whereas the APDs that do not increase body weight do not increase these parameters (Assie et al., 2008). The advantage of using intragastric administration is that these changes occur without behavioral compromise, primarily sedation that occurs with bolus administration. All three GRAs prevented the hyperglycemia observed after intragastric infusion of olanzapine without affecting plasma insulin concentrations. In general, pretreatment with sGRAs also prevented the increase in corticosterone though one time point was still significantly increased in two of the studies. These data would suggest that the acute increase in plasma glucose is associated with hypercortisolism, but this will require further study to make a definitive association.

Mifepristone was first used clinically in 1982 in combination with a prostaglandin as an abortifacient, although a number of additional clinical uses have been proposed (Ullman et al., 1990; Kettel, 1995). Until recently, mifepristone was only approved for short-term or single-dose administration. On the basis of its high affinity for GR, mifepristone was approved recently for the treatment of Cushing syndrome. Because excess glucocorticoids are implicated in both weight gain and decreased insulin sensitivity, GRAs are a logical target to try to mitigate these issues.

Because weight gain is associated with a reduction in insulin sensitivity, it is difficult to evaluate the direct effect of agents on glycemias with long-term administration. Therefore, we used an short-term intragastric infusion of olanzapine (Girault et al., 2012) to understand the potential direct effects of sGRAs on the hyperglycemia. By use of a similar methodology with an oral bolus administration, it was shown that AAPDs that increase body weight cause an increase in plasma glucose and corticosterone, whereas the APDs that do not increase body weight do not increase these parameters (Assie et al., 2008). The advantage of using intragastric administration is that these changes occur without behavioral compromise, primarily sedation that occurs with bolus administration. All three GRAs prevented the hyperglycemia observed after intragastric infusion of olanzapine without affecting plasma insulin concentrations. In general, pretreatment with sGRAs also prevented the increase in corticosterone though one time point was still significantly increased in two of the studies. These data would suggest that the acute increase in plasma glucose is associated with hypercortisolism, but this will require further study to make a definitive association.

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Acknowledgments

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Authorship Contributions

Participated in research design: Sindelar, Morin, Shaw, Gehlert.

Conducted experiments: Morin, Shaw, Barr, Need, Alexander-Chacko.

Contributed new reagents or analytic tools: Carson, Coghlan.

Performed data analysis: Sindelar, Morin, Shaw, Barr, Need, Alexander-Chacko, Gehlert.

Wrote or contributed to the writing of the manuscript: Sindelar, Morin, Shaw, Barr, Need, Alexander-Chacko, Gehlert.

References


Address correspondence to: Dr. Donald R. Gehlert, Mail Code 0510, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285. E-mail: gehlert_donald_r@lilly.com.
Supplemental Figure 1. Rats were administered vehicle or vehicle containing 3, 10 or 30 mg/kg mifepristone (RU486). Binding of $^3$H-dexamethasone to cytosols from liver and cerebral cortex were determined ex vivo. Inhibition of binding by compound contained in the cytosol reflects occupancy of the glucocorticoid receptor at the respective dose. ***p<0.001, n=5.
Supplemental Figure 2. Rats were administered vehicle or vehicle containing 3, 10 or 30 mg/kg CORT-108297. Binding of $^3$H-dexamethasone to cytosols from liver and cerebral cortex were determined ex vivo. Inhibition of binding by compound contained in the cytosol reflects occupancy of the glucocorticoid receptor at the respective dose. ***p<0.001, *p<0.05, n=5.
Supplemental Figure 3. Mifepristone decreased the locomotor response to amphetamine in sensitized mice. After a sensitization procedure, mice were administered vehicle (veh) or various doses of Mifepristone as indicated in the Figure. One hour later, animals received veh or 3mg/kg amphetamine (amph).  
A. Time dependence of locomotor activity.  
B. Cumulative locomotor activity following amph administration.  
n=8, *p<0.05
Supplemental Figure 4. CORT-108297 (CORT) decreases the locomotor response to amphetamine in sensitized mice. After a sensitization procedure, mice were administered vehicle (veh) or various doses of CORT-108297 as indicated in the Figure. One hour later, animals received veh or 3 mg/kg amphetamine (amph). 

A. Time dependence of locomotor activity.
B. Cumulative locomotor activity following amph administration.
C. Locomotor activity during habituation phase (0 to 60 minutes). n=7-8, *p<0.05
Supplemental Figure 5. The effect of 14 days of mifepristone (Mif) administration on body weights of female Sprague-Dawley rats with and without concurrent administration of 2 mg/kg olanzapine (OLZ). In these studies, an OLZ treatment-emergent increase in body weight was observed and prevented by co-administration of mifepristone.
Supplemental Figure 6. The effect of 14 days of CORT-108297 (CORT) administration on body weights of female Sprague-Dawley rats with and without concurrent administration of 2 mg/kg olanzapine (OLZ). In these studies, an OLZ treatment-emergent increase in body weight was observed and was prevented by co-administration of CORT-108297.
Supplemental Figure 7. Effect of GRAs on plasma insulin following intragastric infusion of 3 mg/kg/hr olanzapine in rats. Animals were surgically prepared with indwelling jugular and intragastric cannulae and blood samples obtained using the Culex system. Blood samples were drawn at time zero and twenty minutes thereafter the animals were treated with either Mifepristone (Mfi, 6 mg/kg) (A), CORT-108297 (CORT, 15 mg/kg) (B) or LLY-2707 (LLY, 0.5 mg/kg) (C) via the intragastric cannula. Two hours later, olanzapine (olz) was infused for 150 minutes at a rate of 3 mg/kg/hr and blood samples obtained at the times indicated in the Figure. Subsequent plasma samples were assayed for insulin. As seen in the graph, no significant changes in plasma insulin concentrations were observed following olz infusion or GRA pretreatments. *p<0.05 compared to vehicle infusion, ANOVA followed by Bonferroni post-hoc test.
Supplemental Figure 8. Effect of GRAs on the increase in plasma corticosterone following intragastric infusion of 3 mg/kg/hr olanzapine (olz) in rats. Animals were surgically prepared with indwelling jugular and intragastric cannulae and blood samples obtained using the Culex system. Blood samples were drawn at time zero and twenty minutes thereafter the animals were treated with either Mifepristone (Mif, 6 mg/kg) (A), CORT-108297 (CORT, 15 mg/kg) (B) or LLY-2707 (LLY, 0.5 mg/kg) (C) via the intragastric cannula. Two hours later, olz was infused for 150 minutes at a rate of 3 mg/kg/hr and blood samples obtained at the times indicated in the Figure. Subsequent plasma samples were assayed for corticosterone. As seen in the graph, a time-dependent increase in plasma corticosterone was observed following olz infusion. Pretreatment with CORT-108297 (B) eliminated the corticosterone excursion to a level that was not statistically different from vehicle infusion. Pretreatment with mifepristone (A) or LLY-2707 (C) reduced this increase in plasma corticosterone those significant increases were seen at later time points. No significant effect was seen with GRA pretreatment followed by intragastric infusion of vehicle. *p<0.05 compared to vehicle infusion, #p<0.05 compared to olz infusion. ANOVA followed by Bonferroni post-hoc test.

Supplemental Table 1. Food Intake and Body Composition Following 14 days of Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>14 day Food Intake (g)</th>
<th>Final 14 Day Body Weights (g)</th>
<th>Fat Mass (Change from Baseline, g)</th>
<th>Fat Free Mass (Change from Baseline, g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>206.0±4.3</td>
<td>1.2 ± 1.0</td>
<td>9.5 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>OLZ</td>
<td>247.0±5.0</td>
<td>7.6 ± 1.3</td>
<td>17.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>OLZ + 0.1 mg/kg LLY2707</td>
<td>242.1±7.2</td>
<td>8.2 ± 1.2</td>
<td>12.2 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>OLZ + 0.3 mg/kg LLY2707</td>
<td>233.4 ± 3.0</td>
<td>10.4 ± 1.0</td>
<td>8.6 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>OLZ + 1 mg/kg LLY2707</td>
<td>212.3 ± 10.9</td>
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</tr>
<tr>
<td>OLZ + 3 mg/kg LLY2707</td>
<td>186.5 ± 4.3</td>
<td>-2.8 ± 1.4</td>
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<tr>
<td>3 mg/kg LLY2707</td>
<td>157.4 ± 4.3</td>
<td>-7.5 ± 1.1</td>
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<tr>
<td>30 mg/kg CORT-108297</td>
<td>210.3±7.4</td>
<td>2.8 ± 1.3</td>
<td>-5.0 ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

Supplemental Table 2. Plasma Pharmacokinetic Parameters for LLY-2707 After Acute Dosing to Fasted and Fed Male and Female Sprague-Dawley Rats.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Feeding Status</th>
<th>LLY-2707 (mg/kg)</th>
<th>Olanzapine (mg/kg)</th>
<th>AUC (0-24hrs)</th>
<th>Cmax (ng/ml)</th>
<th>T1/2 (hrs.)</th>
<th>Tmax (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Fasted</td>
<td>1</td>
<td>0</td>
<td>308</td>
<td>59.3</td>
<td>2.4</td>
<td>0.67</td>
</tr>
<tr>
<td>Male</td>
<td>Fed</td>
<td>1</td>
<td>0</td>
<td>371</td>
<td>49.1</td>
<td>2.6</td>
<td>2.0@</td>
</tr>
<tr>
<td>Female</td>
<td>Fasted</td>
<td>1</td>
<td>0</td>
<td>749*</td>
<td>91.3</td>
<td>3.9</td>
<td>0.88</td>
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<tr>
<td>Female</td>
<td>Fed</td>
<td>1</td>
<td>0</td>
<td>838*</td>
<td>103*</td>
<td>3.7</td>
<td>1.7</td>
</tr>
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</table>

N=3 for each group, *p<0.05 when compared to the corresponding male group. @p<0.05 compared to the male fasted group.

Supplemental Table 3. Plasma Pharmacokinetic Parameters for LLY-2707 Following 15 Day Co-treatment of Female Rats with and without Olanzapine on a High Fat Diet.

<table>
<thead>
<tr>
<th>Sex (days)</th>
<th>FeedingStatus</th>
<th>LLY-2707 (mg/kg)</th>
<th>Olanzapine (mg/kg)</th>
<th>AUC (0-24hrs)</th>
<th>Cmax (ng/ml)</th>
<th>T1/2 (hrs.)</th>
<th>Tmax (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (15)</td>
<td>Fed</td>
<td>1</td>
<td>2</td>
<td>702</td>
<td>45</td>
<td>(NC)</td>
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<tr>
<td>Female (15)</td>
<td>Fed</td>
<td>3</td>
<td>0</td>
<td>3900</td>
<td>261</td>
<td>7.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Female (15)</td>
<td>Fed</td>
<td>3</td>
<td>2</td>
<td>4610</td>
<td>372</td>
<td>5.6</td>
<td>4.0</td>
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