

Acute Effects of Atypical Antipsychotics on Whole-Body Insulin Resistance in Rats: Implications for Adverse Metabolic Effects

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Although it is generally accepted that atypical antipsychotics differ in their risk for diabetic side effects, the underlying pharmacological mechanisms are unknown. Studies on the mechanisms of antipsychotic-induced hyperglycemia or insulin resistance are often confounded by the concomitant weight gain and dyslipidemia, known diabetic risk factors. To investigate whether antipsychotics can acutely cause metabolic effects before any change in body composition, we studied the effects of four atypical antipsychotics on whole-body insulin resistance. Using the hyperinsulinemic, euglycemic clamp technique in conscious rats, insulin and somatostatin were infused at a constant rate to provide constant hyperinsulinemia and to suppress pancreatic insulin secretion. Glucose was infused at a variable rate, adjusted to maintain euglycemia. At steady state, animals were administered vehicle (V) or antipsychotic and the glucose infusion rate was monitored as an index of insulin sensitivity. Clamp experiments using radiotracers and studies on glucose uptake into isolated skeletal muscle were conducted to differentiate between effects on hepatic glucose production (HGP) and on peripheral glucose uptake. Olanzapine (OLAN) and clozapine (CLOZ) acutely impaired whole-body insulin sensitivity in a dose-dependent manner ($P < 0.001$ vs V), whereas ziprasidone and risperidone had no effect. CLOZ also induced profound insulin resistance after dosing 10 mg/kg/day for 5 days ($P < 0.05$ vs V). Tracer studies indicated that acute changes mainly reflect increased HGP, consistent with the lack of effect on glucose uptake. OLAN and CLOZ can thus rapidly induce marked insulin resistance, which could contribute to the hyperglycemia and ketoacidosis reported for patients receiving those therapies.

Neuropsychopharmacology (2007) 32, 289–297. doi:10.1038/sj.npp.1301209; published online 11 October 2006

Keywords: schizophrenia; insulin resistance; olanzapine; clozapine; ziprasidone; risperidone

INTRODUCTION

Numerous studies have consistently found that metabolic syndrome is highly prevalent in schizophrenic patients in comparison with the general population (Meyer *et al*, 2005; McEvoy *et al*, 2005; De Hert *et al*, 2006). As the majority of the subjects in these studies have received antipsychotic

treatment, it is likely that the risk of developing metabolic side effects is exacerbated by treatment with those atypical antipsychotics that have been associated with the development of adverse metabolic events (American Diabetes Association *et al*, 2004; Bergman and Ader, 2005; Newcomer, 2005; Lieberman *et al*, 2005). These side effects include weight gain, dyslipidemia, and in particular insulin resistance and hyperglycemia, reflecting impaired glucose metabolism. For instance, a recent study found that insulin resistance is highly prevalent (30%) in patients on antipsychotics and often goes undiagnosed (Sernyak *et al*, 2005). As weight gain and dyslipidemia are well-known diabetic risk factors, it is often assumed that the increased incidence of hyperglycemia and diabetes in the schizophrenic patient population is simply a consequence of treatment with those antipsychotics that cause significant increases in body mass and dyslipidemia. However, rapid induction of hyperglycemia, sometimes accompanied by ketoacidosis, has been reported in patients on clozapine (CLOZ) and olanzapine (OLAN) without weight gain. In several of these cases, the hyperglycemia could be linked

A portion of the data from this study was presented in poster form at the Annual Meetings of the European and American Colleges of Neuropsychopharmacology (Amsterdam, 2005 and Hawaii, 2005) and the European Association for the Study of Diabetes (Athens, Greece, 2005).

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Received 27 March 2006; revised 11 July 2006; accepted 11 August 2006

Online publication: 22 August 2006 at <http://www.acnp.org/citations/Npp082206060197/default.pdf>

to a direct action of the drug, as discontinuation of the antipsychotic medication led to restoration of normal glucose control (Koller *et al*, 2001; Koller and Doraiswamy, 2002).

These epidemiological data suggested that antipsychotic-induced metabolic side effects could occur without weight gain, which was confirmed by two clinical studies that were specifically designed to address this issue. By matching patients for adiposity or for body mass index, it was demonstrated that antipsychotic treatment could cause impaired glucose regulation independent of adiposity (Newcomer *et al*, 2002) and that non-obese patients on CLOZ and OLAN still display significant insulin resistance (Henderson *et al*, 2005). Moreover, these epidemiological data and clinical findings are in good agreement with the results of a recent pre-clinical study in dogs, showing that sub-chronic OLAN treatment caused impaired β -cell function and insulin resistance that was independent of weight gain (Ader *et al*, 2005).

Although these studies have convincingly demonstrated that drug-induced disturbances in glucose metabolism can occur independently of weight gain, other changes caused by chronic drug treatment, for example in fat distribution and lipid profile, would certainly contribute to additional drug effects. In attempting to unravel the cause of these direct drug-induced metabolic abnormalities, it is critical to utilize experimental protocols that can separate drug effects that acutely destabilize glucose homeostasis from those that are a secondary consequence of changes in body composition. Therefore, our studies used acute *in vitro* and *in vivo* models to determine which pharmacological effects contribute to the impairment of glucose regulation by comparing antipsychotic drugs that differ in their metabolic side-effect liabilities. Recently, a consensus development conference (American Diabetes Association *et al*, 2004), reviews (Bergman and Ader, 2005; Newcomer, 2005), and the results of the CATIE trial (Lieberman *et al*, 2005) provided more evidence that antipsychotics differ in their propensity to cause metabolic disturbances. Based on these reports, we used CLOZ and OLAN as two compounds with the highest risk for hyperglycemia or diabetes, risperidone (RISP) as a compound with intermediate diabetic risk, and ziprasidone (ZIPR) as an antipsychotic with negligible diabetic risk.

Given the limited information about the pharmacological mechanisms involved, experimental models that assess components of drug-induced glucose destabilization, such as insulin sensitivity, are needed to advance our understanding of antipsychotic-induced hyperglycemia and insulin resistance. The hyperinsulinemic, euglycemic clamp technique provides an ideal and highly sensitive *in vivo* means for examining acute drug effects on whole-body insulin sensitivity in rodents and humans (Bergman *et al*, 1989). We used this technique in rats to examine changes in insulin sensitivity following acute and sub-chronic treatment with the selected atypical antipsychotics. Radioactive tracer studies were conducted in conjunction with the clamp experiments to detect which tissues play a significant role in the disruption of glucose homeostasis. In addition, the effects of OLAN and CLOZ on 2-deoxyglucose uptake were measured in isolated rat muscle to identify possible inhibitory effects on glucose transport. Data generated in these studies could indicate whether atypical antipsychotic drugs with high metabolic liability such as OLAN and

CLOZ, affect whole-body insulin sensitivity, independent of changes in body composition.

METHODS

Animals

Study protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Pfizer Global Research and Development, Groton, CT. For glucose clamp studies, male Wistar Han rats (280–350 g) were fitted with indwelling jugular vein and carotid artery catheters by the vendor (Taconic Farms Inc., Germantown, NY, USA) 1 day before shipping. Following shipment, animals were allowed to acclimate (5–7 days) before study assignment. For *ex vivo* glucose transport assays, female CD/Sprague-Dawley rats (~120 g; Charles River Breeding Laboratories Inc., Wilmington, MA, USA) were used. Animals were singly housed and allowed *ad libitum* access to chow (Purina 5001, PMI Nutritional International, St Louis, MO, USA) and water.

Pharmaceutical Agents

Pharmaceutical agents were either purchased (CLOZ; #C-6305, RISP, #R-118, Sigma Aldrich, St Louis, MO, USA) or synthesized by Pfizer (OLAN, ZIPR). Dosing solutions were prepared fresh daily. OLAN and CLOZ were formulated in a vehicle (V) composed of 1 N HCl and 50 mM HEPES/saline. The pH of the solution was adjusted to final pH = 6.0 using 1 N NaOH. ZIPR was formulated in 40% 2-hydroxy- β -cyclodextrin (CTD Inc., High Springs, FL, USA). All drugs and Vs were administered in a volume of 2 ml/kg body weight.

Doses of the test compounds were selected based on doses used in numerous pre-clinical behavioral and neurochemical studies (Kapur *et al*, 2003; Johnson *et al*, 2005a) and ranged from 0.32–32.0 mg/kg subcutaneously (s.c.). In rats, this dose range results in receptor occupancies that correspond with clinically relevant D₂ receptor occupancies, as shown for CLOZ, OLAN, and RISP (Kapur *et al*, 2003).

Glucose Transport into Isolated Skeletal Muscle and 3T3-L1 Adipocytes

Glucose uptake in isolated epitrochlearis muscle was assessed as described (Etgen *et al*, 1997). Animals were injected with OLAN, CLOZ, or V 60 min before muscle dissection. In rats treated with OLAN or CLOZ, all subsequent *ex vivo* incubations were supplemented with OLAN or CLOZ at 0.1–10 μ M final concentrations. Potential *in vitro* effects were tested by incubating OLAN (0.1–10 μ M) with muscle isolated from untreated animals. Hexose uptake into mature 3T3-L1 adipocytes (8–12 days following initiation of differentiation) was determined as described previously (Frost and Lane, 1985). On the day of the experiments, adipocytes were incubated in serum-free Dulbecco's modified Eagle's medium for 2 h at 37°C before 50 min incubation with either OLAN (0.1–100 μ M) or V (0.1% DMSO). Insulin-stimulated hexose uptake was then assessed following a 10 min incubation with insulin (10 nM) as described (Frost and Lane, 1985).

Glucose Clamp Procedures

The hyperinsulinemic, euglycemic clamp technique was performed in conscious, unrestrained rats fitted with indwelling jugular and carotid catheters as described previously (Rossetti *et al*, 1987, 1993). Food was removed for 5 h before initiation of the experiments. Saline was infused during a basal equilibration period, followed by a primed continuous infusion of insulin (3 mU/kg min). In order to remove confounding effects of endogenous insulin secretion from the pancreas, somatostatin (3 µg/kg min) was continuously infused to inhibit endogenous insulin secretion. Plasma insulin concentrations were quantified during the clamp to ensure that treatment effects were not due to differences in plasma insulin concentrations (data not shown). During the basal (no insulin infusion) period, plasma insulin concentrations averaged 0.43 ± 0.1 ng/ml, and were elevated to a mean of 2.2 ± 0.07 ng/ml during the hyperinsulinemic clamp. Mean insulin concentrations were not influenced by drug treatment ($P > 0.05$). Glucose (25% solution) was infused at a variable rate to maintain euglycemia (approximately 120 mg/dl). Blood samples were collected every 5–10 min from the carotid artery catheter to determine plasma glucose concentrations so that the glucose infusion rate (GIR) could be adjusted as needed to achieve or maintain euglycemia. Once steady-state glucose concentrations were achieved (three consecutive blood glucose concentrations in the range of 110–130 mg/dl with no change in GIR), animals were administered drug or V (s.c.) and the GIR was monitored for an additional 120 min. The GIR is an index of whole-body insulin sensitivity; insulin resistant states have lower GIR compared to insulin-sensitive states.

The glucose clamp technique was also used in combination with radiotracers to allow determination of hepatic glucose production (HGP) and glucose uptake into insulin-sensitive tissues (skeletal muscle, liver, and adipose tissue) in animals treated with V, OLAN, CLOZ, or ZIPR (RISP was not included in this portion of the study owing to lack of effect on GIR, and significant costs associated with radiotracer studies). The basic glucose clamp protocol was modified to include a continuous infusion of [$3\text{-}^3\text{H}$] glucose (NEN Life Science Products; 20 µCi bolus, 0.6 µCi/min) initiated during the basal equilibration period and maintained throughout the course of the experiment. Additionally, a bolus of 40 µCi of [$U\text{-}^{14}\text{C}$] 2-deoxyglucose (Perkin-Elmer Life Sciences Inc., Boston, MA, USA) was infused 40 min before the end of study. Plasma samples for determination of [$3\text{-}^3\text{H}$] glucose concentrations were obtained at 10-min intervals throughout the experiment. Steady-state conditions for plasma glucose concentration and specific activity were achieved within 60 min. At the culmination of the tracer clamp procedure, rats were killed and tissues were rapidly collected and frozen in liquid N_2 . All tissue samples were stored at -80°C for subsequent analysis.

Analytical Procedures

During the glucose clamps, plasma glucose was rapidly quantified by the glucose oxidase method (Analox Instruments USA, Lunenburg, MA, USA). Plasma insulin concentrations were determined using an ultra-sensitive rat

enzyme immunoassay (Alpco Diagnostics, Windham, NH, USA); inter- and intra-assay coefficients of variation averaged $< 10\%$. For radiotracer studies, plasma [$3\text{-}^3\text{H}$] glucose radioactivity was measured in the supernatants of $\text{Ba}(\text{OH})_2$ and ZnSO_4 precipitates (Somogyi, 1945) of plasma samples (50 µl) after evaporation to dryness to eliminate tritiated water. Glucose concentrations in precipitates were determined in duplicate using the glucose hexokinase method (Roche Diagnostics, Indianapolis, IN, USA). Tissue concentrations of [$U\text{-}^{14}\text{C}$]-2 deoxyglucose (2-DG) phosphate were determined as described previously (Kraegen *et al*, 1985).

Calculations and Statistical Analyses

Calculations for indices of *in vivo* glucose metabolism were conducted as described (Bergman *et al*, 1989). Briefly, under steady-state conditions, the rate of plasma glucose disappearance (R_d) equals the rate of plasma glucose appearance (R_a). The latter was calculated as the ratio of the rate of infusion of [$3\text{-}^3\text{H}$] glucose (d.p.m./min) and the steady-state plasma [$3\text{-}^3\text{H}$] glucose-specific activity (d.p.m./mg). When exogenous glucose was administered, the rate of endogenous glucose production was calculated as the difference between R_a and the exogenous GIR (mg/kg min). The ^{14}C -deoxy-glucose-phosphate accumulation in tissue is calculated from the difference in specific activity of total counts and specific activity of supernatant counts.

Data were analyzed by analysis of variance using SAS (Statistical Analysis System, StatView software, SAS Institute Inc., Cary, NC, USA). Differences among treatment groups were tested using Fisher's LSD test when the main effect of treatment was significant ($P < 0.05$).

RESULTS

Single doses of CLOZ or OLAN, but not ZIPR or RISP, markedly reduce the GIR in a dose-dependent manner. Figure 1 illustrates the effects of four atypical antipsychotic drugs on the rate of glucose infusion (GIR) to maintain euglycemia, a sensitive measure of whole-body insulin sensitivity. CLOZ and OLAN (3.2 and 10 mg/kg) caused a large, dose-dependent reduction in GIR within 40–60 min following a single dose (Figure 1a and b), consistent with profound insulin resistance. In contrast, there was no effect of ZIPR or RISP on GIR (Figure 1c and d), even at a very high dose of ZIPR (32 mg/kg; Figure 1c).

Single Doses of CLOZ or OLAN, but not ZIPR, Markedly Increase HGP

To further examine the effects of the drugs on whole-body insulin action, and to identify the insulin-sensitive tissue(s) contributing to the insulin resistance observed with CLOZ and OLAN treatment, we conducted glucose clamps that incorporated the use of radiotracers. Specifically, by infusing [$3\text{-}^3\text{H}$] glucose during the clamp, we were able to quantify rates of whole-body glucose disposal and determine drug effects on HGP (Figure 2). As observed in our first set of experiments (Figure 1), CLOZ and OLAN, but not ZIPR caused significant insulin resistance as indicated by a large fall in GIR (65.2 and 43%, respectively) following a

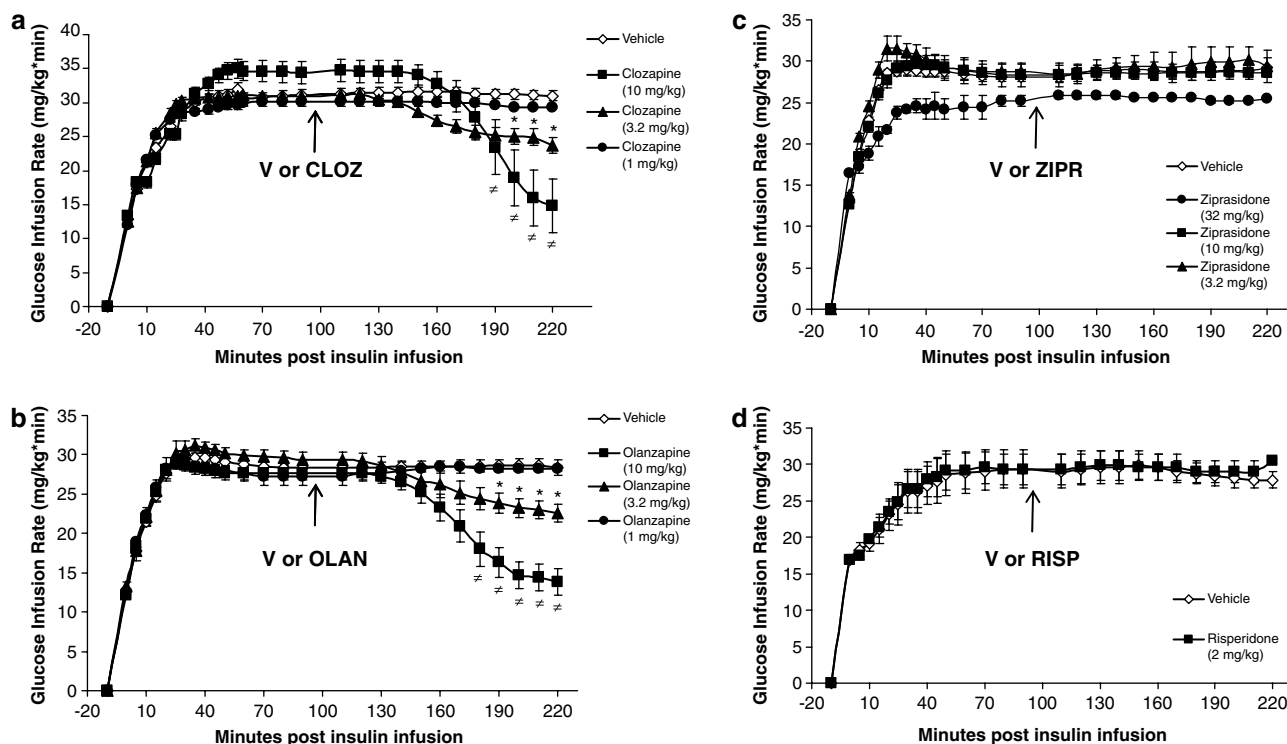


Figure 1 Dose-dependent reduction in GIR with CLOZ and OLAN but not ZIPR or RISP treatment in normal rats. Hyperinsulinemic, euglycemic clamp experiments were conducted using male Wistar rats as describe in Methods. Once animals reached steady-state clamp conditions, they were administered a single dose of V or CLOZ (1, 3.2, or 10 mg/kg (a)), OLZN (1, 3.2, or 10 mg/kg (b)), ZIPR (3.2, 10, or 30 mg/kg), or RISP (2 mg/kg) and GIR was monitored to assess whole-body insulin sensitivity. * $P < 0.01$ vs V; $^{\#}P < 0.0001$ vs V; $n = 2-8$ per treatment group.

single dose (Figure 2a). This conclusion was confirmed when the rate of disappearance (R_d) of plasma [^3H] glucose was quantified in the same experiment (Figure 2b). R_d was similar across treatment groups, both during the basal (no insulin) and insulin only portions of the clamp, before any drug treatment. However, a single injection of CLOZ or OLAN (10 mg/kg) caused a significant reduction in R_d (OLAN, $17.0 \pm 5.1\%$ reduction; $P < 0.03$) with CLOZ having the greatest effect compared to V or ZIPR treatment groups ($34.5 \pm 4.4\%$ reduction; $P < 0.001$).

To determine whether the effects on whole-body insulin action were in part due to hepatic effects, we examined the ability of insulin to inhibit HGP following drug treatment (Figure 2c). As expected, rates of HGP were high during the basal state, as these animals were fasted before the onset of the study. Furthermore, insulin treatment significantly suppressed HGP during the insulin-only period, consistent with the function of insulin to suppress HGP under normal conditions. Following single-dose treatment with ZIPR, there was no change in HGP. In contrast, a single dose of OLAN and CLOZ (10 mg/kg) significantly impaired the ability of insulin to inhibit HGP (18.5- and 22.7-fold increase in HGP vs insulin only, respectively), consistent with severe hepatic insulin resistance ($P < 0.001$).

Effect of CLOZ on Suppression of GIR is Sustained Following Repeated Dosing

To determine whether the acute insulin resistance induced by a single dose of CLOZ could be sustained following repeated dosing, we conducted a sub-chronic study and

dosed animals with CLOZ (10 mg/kg) once daily for 4 days before conducting the glucose clamp. On day 5, 24 h following the 4th CLOZ dose, the glucose clamp was initiated during which a 5th dose of CLOZ was administered. As illustrated in Figure 3, GIR during the insulin-only portion of the clamp were similar between V and CLOZ treatments (25.9 ± 1.8 and 29.0 ± 1.0 mg/kg min, respectively; $P < 0.15$) as were plasma insulin concentrations ($V = 2.09 \pm 0.3$ ng/ml; CLOZ = 2.27 ± 0.13 ng/ml; $P < 0.81$). However, during the drug treatment period, the significant inhibition of GIR ($48.1 \pm 8.9\%$) and R_d ($28.0 \pm 5.4\%$) was replicated after 5 days of dosing (Figure 3a and b), as was the severe hepatic insulin resistance (9.4-fold increase in HGP; Figure 3c). These data show that sub-chronic dosing does not cause tolerance to the CLOZ-induced hepatic insulin resistance. In addition, after 5 days dosing, basal GIR values were not significantly different from those in naïve rats, indicating that the effect of single CLOZ dose is reversible following sub-chronic dosing. Finally, although drug-induced behavioral changes could not be quantifiably assessed during the clamp experiments, gross behavioral observations showed that all test compounds caused similar dose-dependent reductions in spontaneous locomotor activity. Thus, the whole-body insulin resistance induced by CLOZ and OLAN was not due to behavioral sedative effects.

Effects of Atypical Antipsychotics on Insulin-Stimulated Glucose Uptake

To determine whether treatment effects on whole-body glucose metabolism were due to changes in insulin-

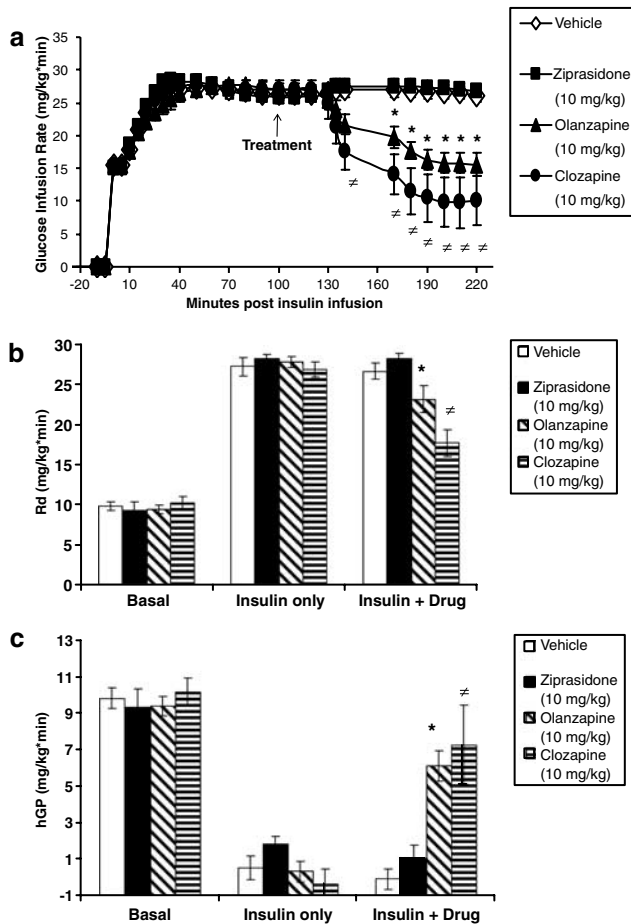


Figure 2 CLOZ and OLAN but not ZIPR cause acute whole-body and hepatic insulin resistance. Hyperinsulinemic, euglycemic clamp experiments were conducted using male Wistar rats as described in Methods. At steady state, animals were administered a single dose of V or CLOZ (10 mg/kg), OLAN (10 mg/kg), or ZIPR (10 mg/kg) and GIR (a) was monitored to assess whole-body insulin sensitivity. The rates of whole-body glucose disposal (R_d (b)) and HGP (c) were assessed as described in Methods. * $P < 0.05$ vs V; $\neq P < 0.01$ vs V; $n = 4-6$ per treatment group.

stimulated glucose uptake into peripheral tissues, we examined uptake of [$U-^{14}C$] 2-DG into skeletal muscle, adipose tissue, and liver at the culmination of the glucose clamp studies (Table 1). The [$U-^{14}C$] 2-DG analog is not further metabolized in these tissues; therefore, tissue concentrations reflect treatment effects on insulin-stimulated glucose uptake. Concentrations of 2-DG in liver, adipose tissue, and skeletal muscles were unchanged by acute OLAN or ZIPR treatment. Interestingly, acute CLOZ but not OLAN or ZIPR treatment caused an increase in 2-DG uptake into adipose tissue and liver and a reduction in 2-DG uptake into skeletal muscle. This result was unexpected, and not consistent with a whole-body insulin resistance phenotype. The underlying mechanisms for this CLOZ effect are unknown at this time; nevertheless improved insulin sensitivity in adipose tissue of CLOZ treated animals was not sufficient to overcome the profound hepatic insulin resistance. Furthermore, acute effects of CLOZ on tissue glucose uptake were not observed following 5 days of CLOZ treatment (Table 1), suggesting that these effects are transient in nature.

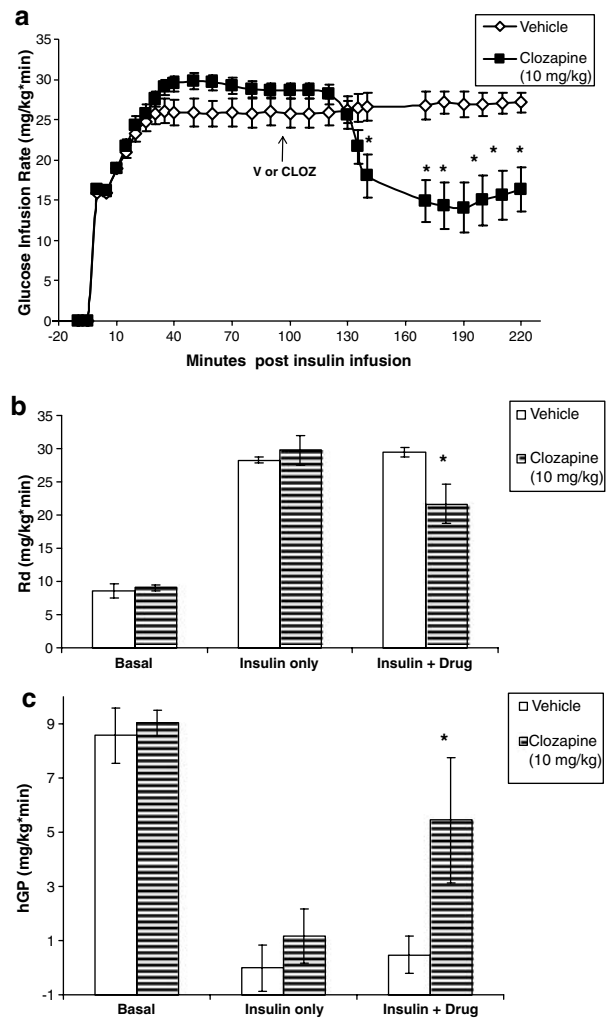


Figure 3 CLOZ induced whole-body and hepatic insulin resistance is maintained following 5 days of dosing. Hyperinsulinemic, euglycemic clamp experiments were conducted in male Wistar rats following 4 days of treatment with V or CLOZ (10 mg/kg) as described in Methods. On day 5, at steady state, animals were administered a single dose of V or CLOZ (10 mg/kg) and GIR (a) was monitored to assess whole-body insulin sensitivity. The rates of whole-body glucose disposal (R_d (b)) and HGP (c) were determined as described in Methods. * $P < 0.05$ vs V; $n = 4$ per treatment group.

To further examine the acute effect of OLAN and CLOZ on insulin-stimulated glucose uptake, we determined effects of treatment *in vitro* and *in vivo* on insulin-stimulated glucose uptake into isolated epitrochlearis skeletal muscles (Figure 4). OLAN treatment *in vitro* had no effect on basal or insulin-stimulated glucose uptake (Figure 4a). Furthermore, in isolated muscle from rats treated with OLAN (10 mg/kg; yields 7 μ M exposure in muscle *in vivo*), and subsequently incubated with OLAN (0.1–10 μ M) *in vitro*, basal and insulin-stimulated glucose uptake was unchanged (Figure 4b). CLOZ (10 mg/kg) was also without effect in this experimental paradigm (Figure 4c).

Finally, we examined the acute effects of OLAN on glucose uptake into adipocytes *in vitro*. 3T3-L1 adipocytes were cultured with 0.1–100 μ M OLAN for 60 min before assessment of 2-DG uptake in the absence or presence of 10 nM insulin. There was no significant effect of OLAN

Table 1 ^{14}C -Deoxyglucose-Phosphate (2-DGP) Accumulation in Tissues from Animals Receiving a Single Dose (Acute) or Multiple Doses (Chronic; 5 days) of Atypical Antipsychotic Medications^a

Tissue ^b	V	Acute			Chronic	
		CLOZ	OLAN	ZIPR	V	CLOZ
Liver	57.8 ± 4.6	88.2* ± 6.72	63.5 ± 3.1	66.19 ± 5.9	58.5 ± 3.7	67.6 ± 10.9
Soleus	123.5 ± 17.1	42.0* ± 9.2	96.5 ± 15.6	129.8 ± 28.8	115.5 ± 7.0	149.1 ± 39.8
Quadriceps	95.3 ± 6.8	51.8* ± 9.9	78.1 ± 15.3	107.7 ± 7.1	126.9 ± 8.8	107.8 ± 37.7
Adipose tissue ^c	9.2 ± 1.8	30.7 ± 9.9	9.8 ± 3.1	13.9 ± 3.6	10.9 ± 1.0	24.0* ± 2.7

^aClozapine (CLOZ, 10 mg/kg), olanzapine (OLAN, 10 mg/kg), ziprasidone (ZIPR, 10 mg/kg), and corresponding vehicle (V) were administered as described in Methods.

^bUnits are pmol/g*40 min.

^cEpididymal adipose tissue depot.

* $P < 0.01$ vs V; $n = 4$ –13 per treatment group.

treatment on insulin-stimulated glucose uptake into adipocytes, at any of the drug concentrations tested. The only significant effect of OLAN treatment on 2-DG uptake was a 60% increase in glucose uptake into cells treated with the 100 μM OLAN dose in the absence of insulin (data not shown).

DISCUSSION

The present study describes the first *in vivo* evidence that atypical antipsychotics known to have metabolic liability in human subjects, cause severe and acute insulin resistance following a single dose in healthy animals. We utilized the highly sensitive glucose clamp technique (Bergman *et al*, 1989) to unequivocally show acute effects of OLAN and CLOZ, but not of ZIPR or RISP, on whole-body insulin sensitivity that are independent of drug-induced changes in body composition. By employing radiotracers in the clamp studies, we also provided convincing evidence that the primary target tissue for the drug-induced insulin resistance is the liver, as demonstrated by a large increase in HGP following single doses of OLAN or CLOZ.

Hyperglycemia associated with type 2 diabetes mellitus results from impaired ability of insulin to stimulate glucose uptake into peripheral tissues such as skeletal muscle and adipose tissue, reduced ability of insulin to suppress glucose production by the liver coupled with inappropriate insulin secretion by the pancreas to overcome the insulin resistance (DeFronzo, 2004). Furthermore, the function of these target tissues to mediate whole-body glucose metabolism can be regulated directly at the tissue or via the central nervous system (Iguchi *et al*, 1988; Nonogaki and Iguchi, 1997). Therefore, the metabolic side effects observed with certain atypical antipsychotic drugs (American Diabetes Association *et al*, 2004; Bergman and Ader, 2005; Newcomer, 2005; Lieberman *et al*, 2005) could be exacerbated by, or due to, drug effects on one or more of these target tissues.

Schizophrenia *per se* is known to impact the pathogenesis of diabetes (Meyer *et al*, 2005; McEvoy *et al*, 2005; De Hert *et al*, 2006), which is 1.5–2 times more prevalent in schizophrenic patients than in the general population (American Diabetes association *et al*, 2004). Although genetic predisposition and environmental factors, such as

smoking and poor diet, contribute to the increased diabetes risk, there is accumulating evidence of antipsychotic-induced weight gain, dyslipidemia, and diabetes (Holt *et al*, 2004). As weight gain and dyslipidemia are known diabetic risk factors, antipsychotic-induced hyperglycemia and diabetes could merely be a secondary consequence of these antipsychotic side effects. However, earlier epidemiological data have indicated that weight-gain-independent hyperglycemia occurs and can be reversed by switching to another antipsychotic (Koller *et al*, 2001; Koller and Doraiswamy, 2002). These findings are supported by clinical reports, in which patients were matched for adiposity or for body mass index. Results from these studies demonstrated antipsychotic-induced impairment of glucose regulation independent of adiposity (Newcomer *et al*, 2002) and insulin resistance in non-obese patients who were treated with CLOZ or OLAN (Henderson *et al*, 2005). To further address this issue in a non-schizophrenic animal model, Ader *et al* (2005) treated dogs with OLAN or RISP for 4–6 weeks and assessed parameters related to weight gain, adiposity, insulin resistance, and pancreatic β -cell function. They found that treatment with OLAN, but not with RISP, caused marked hepatic insulin resistance and abolished the compensatory β -cell response to obesity-induced insulin resistance. In addition, chronic OLAN dramatically increased visceral and s.c. adiposity with minimal effect on body weight. Taken together, these clinical and pre-clinical data strongly suggest that antipsychotic-induced insulin resistance is not dependent on weight gain. The possibility remains that other changes in body composition, such as fat distribution, could have contributed to the impairment of glucose regulation. In the present study, we examined that the effects of various atypical antipsychotics on insulin resistance were examined without the potential confounding effects of genetic predisposition or drug-induced changes in body composition.

The pronounced hepatic insulin resistance induced by CLOZ is rapid in onset, occurs within 60 min following a single dose in normal animals, and can be elicited after repeated dosing for 5 days. These findings suggest that this acute response does not tolerate over time, a result that is in agreement with extensive published evidence that chronic treatment with OLAN and CLOZ results in weight gain and whole-body insulin resistance. Our data fully support the

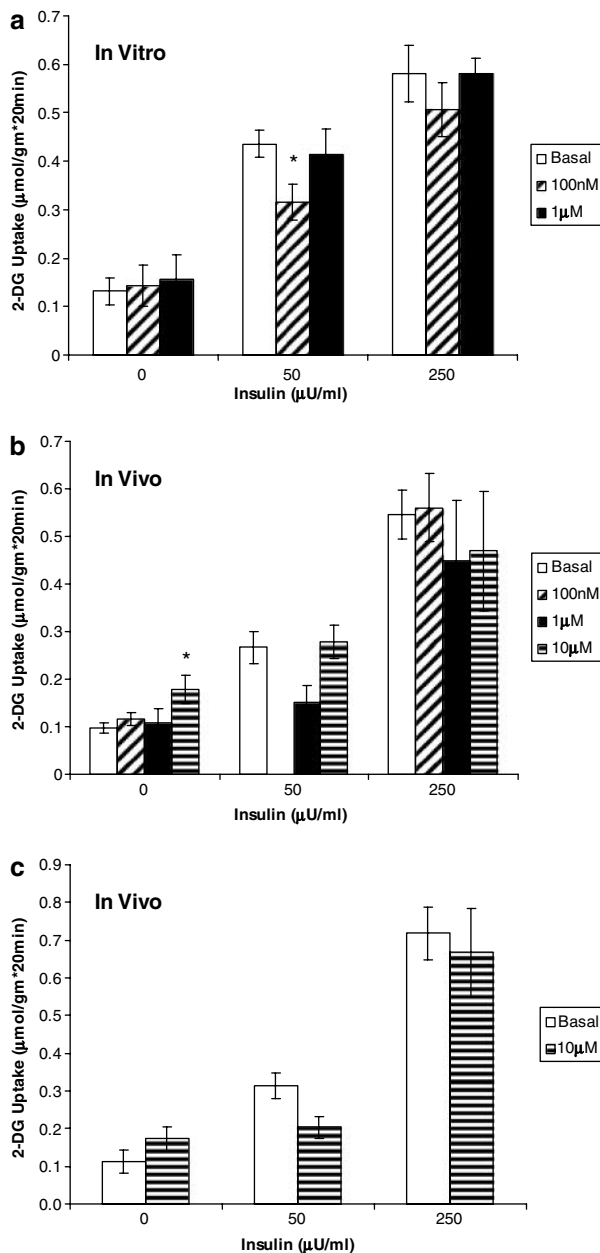


Figure 4 Effect of *in vitro* and *in vivo* OLAN and CLOZ treatment on 2-DG in rat epitrochlearis (EPI) muscle *in vitro*. (a) 2-DG uptake into isolated rat EPI muscles treated with OLAN *in vitro*; (b) 2-DG uptake into isolated EPI muscles from rats treated with OLAN (10 mg/kg) *in vivo*; (c) 2-DG uptake into isolated EPI muscles from rats treated with CLOZ (10 mg/kg) *in vivo*. Additional OLAN was added to the *in vitro* incubation bath as described in Methods. * $P < 0.05$; $n = 4-6$ per treatment.

findings of Ader *et al* (2005), who used the glucose clamp model in dogs and observed severe whole-body and hepatic insulin resistance in dogs chronically treated with OLAN for up to 6 weeks. The magnitude of the hepatic insulin resistance these authors reported, about 70% reduction in hepatic insulin sensitivity, was similar to what we observed upon acute dosing in either in naive rats or rats that were dosed for 5 days. It seems likely that the hepatic insulin resistance develops with acute dosing, whereas drug-induced weight gain and hyperlipidemia occur following repeated dosing.

Two human clamp studies have been performed to examine effects of antipsychotics on β -cell function (Sowell *et al*, 2002) and insulin resistance (Sowell *et al*, 2003) in healthy volunteers. In the latter study, Sowell *et al* (2003) used the hyperinsulinemic, euglycemic clamp methodology. Despite the fact that both drugs caused significant weight gain, which would be expected to induce insulin resistance in and of itself, they reported that OLAN and RISP had no effect on insulin sensitivity after 3-week treatment. As carefully discussed by Bergman and Ader (2005), Sowell *et al* (2002, 2003) did not report initial GIRs and other important details related to experimental design, making it difficult to reconcile the differences in their published results and those of this study. Importantly, Sowell *et al* (2003) did not indicate the timing of the last drug dose before the euglycemic clamp study. As we demonstrate in the present study that the effect is reversible 24 h after dosing (in acute and sub-chronic dosing paradigms), the timing of the clamp study relative to the last dose may be a critical aspect of the experimental design in order to detect a change in insulin sensitivity.

Together with recent clinical and pre-clinical data, our results provide strong evidence that the atypical antipsychotics OLAN and CLOZ have unique pharmacological activities that contribute to their increased propensity to produce disruptions in glucose homeostasis. Although the data show that insulin resistance could be one of the first steps in a series of events leading to hyperglycemia and diabetes, the underlying molecular mechanisms are unknown and have not been studied in much detail. One possible mechanism is inhibition of glucose transport into peripheral tissues, as proposed by Dwyer and co-workers (Ardizzone *et al*, 2001; Dwyer and Donohoe, 2003) based on their observation that antipsychotics induce acute hyperglycemia in mice and inhibit glucose transport *in vitro*. Although the drug-induced acute hyperglycemia in mice (Dwyer and Donohoe, 2003) could represent a very useful *in vivo* model for antipsychotic-induced impairment of glucose regulation, several factors argue against a mechanism involving inhibition of glucose transport. First, PC12 cells used for those experiments do not express the major insulin-responsive glucose transporter, GLUT4 (Bouche *et al*, 2004; Bjornholm and Zierath, 2005). Second, as the drug concentrations required to inhibit glucose transport are cytotoxic (Dwyer *et al*, 2003), the glucose transport inhibition is likely the result of cell death rather than of a specific effect on glucose transport. Finally, our results and recently published data (Robinson *et al*, 2006) demonstrate that OLAN does not inhibit glucose transport. In the *in vivo* clamp model, acute OLAN, ZIPR or 5-day CLOZ treatment had no significant effect on 2-DG uptake into skeletal muscles, and OLAN and CLOZ did not inhibit basal (GLUT1- and GLUT4-mediated) or insulin-stimulated (GLUT4-mediated) 2-DG uptake into isolated EPI muscles. Also, high OLAN concentrations had no effect on insulin-stimulated glucose uptake into GLUT4-expressing 3T3-L1 adipocytes, in agreement with data from Robinson *et al* (2006).

Suppression of cholinergic-stimulated insulin secretion by a direct action on the pancreas, which involves antagonism of muscarinic M_3 receptors on β cells, has been postulated as another potential mechanism underlying

metabolic effects of some atypical antipsychotics (Johnson *et al*, 2005b). However, this mechanism is not a factor in the current acute study, as prolonged hyperglycemia would be required to activate cholinergic compensatory pathways (Balkan and Dunning, 1995) and under the hyperinsulinemic experimental conditions used in this study, insulin release is suppressed via the constant infusion of somatostatin.

Given the important role of the autonomic nervous system in controlling glucose metabolism in the liver (Gardemann *et al*, 1992), it is conceivable that disruption of sympathetic and parasympathetic regulatory pathways underlies the drug-induced impairment of glucose regulation. For instance, pre-clinical studies have demonstrated that cholinergic input to the liver via parasympathetic nerve endings helps to control hepatic glucose metabolism (Vatamaniuk *et al*, 2003). As mentioned above, OLAN and CLOZ are potent non-selective muscarinic antagonists and could thus act through this pathway. The adrenergic pathways of the sympathetic nervous system are also linked to glucose homeostasis (Gardemann *et al*, 1992). Activation of the sympathetic system releases epinephrine from the adrenal gland, which has been shown to produce profound effects on glucose regulation. Using conditions similar to those used in our clamp study, epinephrine was shown to produce a marked reduction in insulin sensitivity and increased hepatic glucose output (James *et al*, 1986). Similarly, epinephrine-induced insulin resistance has been observed in humans (Deibert and DeFronzo, 1980).

In addition to the direct actions at peripheral tissues such as liver and pancreas, or the secondary actions of humoral substances such as epinephrine, it is likely that the central nervous system plays an important role in the development of hyperglycemia and insulin resistance in patients treated with certain atypical antipsychotic drugs. The hypothalamus regulates glucose homeostasis via sympathetic and parasympathetic pathways (Nonogaki and Iguchi, 1997) and activation of the hypothalamic cholinergic pathway has been shown to increase epinephrine and glucagon release that was associated with hyperglycemia (Iguchi *et al*, 1988). Thus, in addition to the direct peripheral effects at organs, such as the muscarinic receptors on the pancreas and liver, activation of the central nervous system that leads to stimulation of the neural pathways that innervate peripheral tissues could contribute to antipsychotic-induced hyperglycemia and insulin resistance.

In conclusion, we provide the first evidence that OLAN and CLOZ cause profound whole-body insulin resistance following a single dose, without any changes in weight or body composition. This effect is primarily due to hepatic insulin resistance and is sustained with sub-chronic dosing. Our data and the work of others (Ader *et al*, 2005) using pre-clinical models suggest that hepatic insulin resistance is an early response to OLAN or CLOZ treatment, and that hepatic effects are maintained with the development of obesity and hyperlipidemia. As insulin resistance and hyperglycemia persist, OLAN and CLOZ could further aggravate the condition by suppressing compensatory insulin secretion via blockade of muscarinic M_3 receptors on β cells (Johnson *et al*, 2005b). Clearly, additional studies are needed to further elucidate the molecular mechanisms underlying these acute pharmacological effects. A better understanding of the underlying molecular mechanisms will

guide the design and development of new agents without adverse metabolic effects. Properly designed clamp studies in human subjects could confirm whether the acute effects we observed pre-clinically translate to schizophrenic patients.

ACKNOWLEDGEMENTS

All authors of this work are employees of Pfizer Inc. Pfizer discovers, develops, and markets therapies to treat schizophrenia and diabetes.

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