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Obesity exacerbates the acute metabolic side effects of olanzapine

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ABSTRACT

Olanzapine is a second-generation antipsychotic used in the management of schizophrenia and various off-label conditions. The acute metabolic responses of olanzapine recapitulate many of the side effects associated with obesity. Obesity rates are high in the schizophrenic population, but it is unknown whether pre-existing obesity-associated metabolic dysfunction augments the acute side effects of olanzapine. To address this question, we compared the responses to olanzapine in lean and high-fat diet-induced (HFD) obese mice. Four weeks of HFD (60% kcal from fat) led to obese, hyperglycemic, and insulin resistant mice. Olanzapine-induced hyperglycemia and systemic insulin resistance were exacerbated in HFD-induced obese mice. Olanzapine also profoundly inhibited insulin signalling in skeletal muscle and liver, which appears to be exacerbated by obesity. The greater olanzapine-induced hyperglycemia may also result from increased hepatic glucose output in obese mice as pyruvate challenge led to significantly higher blood glucose concentrations, with associated increases in hepatic content of gluconeogenic enzymes. Olanzapine also suppressed RER while acutely increasing oxygen consumption in obese mice. A single olanzapine treatment reduced physical activity for up to 24 h, regardless of obesity. Considering obesity is very common in the schizophrenic population, these data suggest that previous research may be under-estimating the severity of olanzapine's acute side effects.

1. Introduction

Olanzapine (OLZ) is a second-generation antipsychotic drug (SGA) widely used for the treatment of schizophrenia. Recently, OLZ and other SGAs are being used in the management of off label conditions, such as anxiety (Pringsheim and Gardner, 2014) and attention-deficit disorder (Devlin and Panagiotopoulos, 2015). Unfortunately, OLZ has severe metabolic side effects, including hyperphagia (Coccurello et al., 2008a,b), weight gain (Allison and Casey, 2001), hepatosteatosis (Coccurello et al., 2009), dyslipidemia, and impairments in glucose homeostasis (Newcomer, 2005). These factors ultimately contribute to an increased risk of type 2 diabetes (Rojo et al., 2015; Sernyak et al., 2002) and cardiovascular disease (Rojo et al., 2015), which are leading causes of death for those with schizophrenia (Lawrence et al., 2013).

While the development of type 2 diabetes with SGAs was initially thought to be due to weight gain, direct diabetogenic effects of these compounds have emerged (Coccurello and Moles, 2010). In rodents (Chintoh et al., 2008) and humans (Albaugh et al., 2011b), SGAs cause hyperglycemia within minutes to hours, an effect that does not dissipate even after months of regular SGA use (Boyda et al., 2012b, 2010). Rapid hyperglycemia is associated with increased hepatic glucose production (Chintoh et al., 2009; Ikegami et al., 2013), systemic insulin resistance (Chintoh et al., 2009, 2008), impaired pancreatic insulin secretion (Boyda et al., 2010; Chintoh et al., 2009), and greater reliance on fat oxidation (Coccurello et al., 2006; Klingerman et al., 2014). Such repeated excursions in blood glucose may be even more harmful than chronically elevated blood glucose (Ceriello et al., 2008; Qiao et al., 2003) as transient oscillations in glucose produce greater endothelial dysfunction and oxidative stress, two factors implicated in the cardiovascular complications of diabetes, compared to consistently high blood glucose (Ceriello et al., 2008).

Regardless of SGA usage, obesity is more prevalent in individuals with schizophrenia than those without (Allison et al., 1999; Subramaniam et al., 2014), raising the important question of whether pre-existing metabolic dysfunction alters the acute effects of SGAs on glucose metabolism. Indeed, OLZ's side effects recapitulate many of the metabolic disturbances that characterize obesity, including hyperglycemia, insulin resistance, glucose intolerance, and increased fat oxidation (Alberti et al., 2005; Coccurello and Moles, 2010). Thus, it would be extremely undesirable if OLZ and obesity synergistically disturbed metabolism, conceivably accelerating the development of cardiovascular complications or diabetes.

Despite relatively thorough characterization of the acute metabolic responses to OLZ, all of the mechanistic work using animal models has been performed in healthy lean animals (Chintoh et al., 2009, 2008; Coccurello et al., 2009; Hahn et al., 2014; Houseknecht et al., 2007;

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Ikegami et al., 2013; Remington et al., 2015; Smith et al., 2008) which could lead to inaccurate interpretations or underestimations of OLZ's acute metabolic side effects. With this in mind, the purpose of the present investigation was to assess the acute metabolic responses to OLZ in lean healthy animals compared to their high-fat diet-induced obese counterparts. We hypothesized that OLZ would induce greater glucose excursions in obese mice with concomitantly increased markers of hepatic gluconeogenesis and insulin resistance compared to lean mice.

2. Methods

2.1. Ethics

All experimental procedures were approved by the University of Guelph Animal Care Committee and followed Canadian Council on Animal Care guidelines.

2.2. Materials

Olanzapine was purchased from Cayman Chemicals (CAT# 11937) (Ann Arbor, MI). Dimethyl Sulfoxide (DMSO) was from Wako Pure Chemical Industries (CAT# 67-68-5; Richmond, VA). Kolliphor EL was from Millipore Sigma (CAT# C5135; Etobicoke, ON). Antibodies against total AKT (CAT# 9272), phospho AKT^{T308} (CAT# 9275), phospho AKT^{S473} (Cat# 9271) were from Cell Signaling (Danvers, MA). An antibody against PEPCK was from Cayman Chemicals (CAT# 10004943). G6Pase antibody (CAT# 25840) was from Santa Cruz Biotechnology (Mississauga, ON). An antibody against GAPDH (CAT# Ab8245) was from Abcam (Toronto, ON). An antibody against Vinculin (CAT# 05386) was purchased from Millipore Sigma. Ponceau S staining loading control was used for epididymal white adipose tissue (CAT# P7170, Millipore Sigma).

2.3. Study design

Upon arrival, animals (~7 week old male C57BL/6 mice, n = 60, Charles River) were given ~1 week to acclimate to our facility during which time they had free access to standard rodent diet and tap water. Mice were housed individually on a 12:12 h light:dark cycle. After acclimation, half of the mice were switched to a high-fat diet (HFD; 60% kcal from fat, Research Diets D12492) for 4 weeks while the others remained on the standard rodent diet. After 4 weeks we performed glucose and insulin tolerance tests and measured fed blood glucose to assess metabolic health. We then performed a series of tests in response to OLZ; all OLZ injections were given at ~0900.

2.4. Insulin tolerance test (ITT)

To assess HFD-induced insulin resistance, non-fasted mice were injected intraperitoneally (IP) with insulin (0.75 IU kg^{-1}) at ~0900 and glucose measured from a tail vein at baseline and 15, 30, 45, 60, 90, and 120 min after injection (Frendo-Cumbo et al., 2016).

2.5. Glucose tolerance test (GTT)

IP glucose tolerance tests were performed on fasted (6 h) mice at ~1400. Glucose measures were obtained from a tail vein at baseline and 15, 30, 45, 60, 90, and 120 min after injection of glucose (2 g kg^{-1}) (MacPherson et al., 2015; Peppler et al., 2016).

2.6. Olanzapine tolerance test (OTT)

OLZ was dissolved in DMSO (1 mg/100 µl) before being combined with a saline and Kolliphor EL solution (900 ml/500 µl). Mice were IP injected with OLZ (5 mg kg^{-1}) or vehicle solution (DMSO, Kolliphor EL, saline) at the beginning of the light cycle; we (Castellani et al., 2017) and others (Ikegami et al., 2013) have previously used this dose as it mimics human dosing requirements based on dopamine-binding occupancy in rats given OLZ by subcutaneous injections (Kapur et al., 2003). Blood glucose was measured prior to, 15, 30, 45, 60, 90, and 120 min post-OLZ (Castellani et al., 2017).

2.7. Pyruvate tolerance test (PTT)

Fed mice were injected with OLZ (5 mg kg^{-1}) or vehicle and 60 min later blood glucose was measured and mice were injected with pyruvate (2 g kg^{-1}) (Castellani et al., 2017). Blood glucose was measured at 15, 30, 45, 60, 90 and 120 min post pyruvate-injection.

2.8. Terminal ITT

Fed mice were injected with OLZ or saline, and 60 min later blood glucose was measured and mice were injected IP with insulin (0.75 IU kg^{-1}) or saline. Blood glucose concentrations were measured 5 and 15 min post-insulin (Peppler et al., 2016). After 15 min, mice were anesthetized with sodium pentobarbital (~5 mg 100 g^{-1}) and liver, triceps, and epididymal white adipose tissue were rapidly removed, weighed, and snap frozen in liquid nitrogen. Cardiac blood was taken, centrifuged at 5000G for 10 min at 4 °C. All samples were stored at −80 °C.

2.9. Comprehensive lab animal monitoring system (CLAMS)

A separate cohort of mice was placed in a CLAMS at the beginning of their light phase (~0900 h) for a 24-h acclimatization period. At the beginning of the following light cycle (0900), mice were injected with OLZ (5 mg kg^{-1}), immediately placed back into the CLAMS, and measurements were taken over the following light and dark cycle (24 h). Mice spent a total of 48 h in the CLAMS system but only the final 24 h were analyzed. VCO_2, VO_2, RER (VCO_2/VO_2), and physical activity were measured.

2.10. Western blotting

Tissues were homogenized, protein extracted, and quantified by BCA (Peppler et al., 2016). Membranes were incubated overnight with antibodies diluted (1:1000) in TBST/%5 BSA at 4 °C. Membranes were briefly washed in TBST and incubated for 1 h at room temperature with horseradish peroxidase conjugated secondary antibodies (1:2000) and signals were detected using chemiluminescence. Phosphorylated and total protein content was expressed relative to a within-gel loading control (GAPDH or Ponceau). Phosphorylated proteins were then expressed relative to total protein content. Hepatic PEPCK and G6Pase were expressed relative to a vinculin loading control, all of which were from the same gel.

2.11. Statistical analysis

Normality was assessed by a Shapiro-Wilks test and if failed ($p < 0.05$) data were $\log_{10}$ transformed. Data were then compared by unpaired 2-tailed *t*-test (tissue weights, GTT AUC, fed blood glucose), two-way ANOVA (GTT, PTT, ITT, liver PEPCK and G6Pase, CLAMS), or three-way ANOVA (tissue insulin signalling). Post hoc tests with Bonferroni corrections were performed when significant interactions were observed. All data are presented as mean ± SE. Significance level was set at $p < 0.05$. Statistical tests were completed using Graph Pad version 6.0 (La Jolla, CA) except 3-way ANOVAs which were run using SPSS (Armonk, NY).