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Intermittent treatment with olanzapine causes sensitization of the metabolic side-effects in rats

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ABSTRACT

The second generation antipsychotic drugs are effective treatments for psychotic disorders. Many of these compounds, including the drug olanzapine, have been associated with metabolic side-effects, including weight gain, impaired glucose tolerance and insulin resistance, which increase the risk of developing cardiometabolic disorders. Rodent models of olanzapine-induced metabolic side-effects have been used to study the physiology of these effects, but only at a single time point after drug treatment. The purpose of the present study was to examine longitudinal changes with chronic antipsychotic drug treatment. Adult female rats were treated with either olanzapine (15 mg/kg) or vehicle for five consecutive days each week, followed by a 48 h washout period. Animals were then challenged with either olanzapine (15 mg/kg) or vehicle, and fasting glucose and insulin values were recorded, as well as glucose clearance in the glucose tolerance test. Treatment with olanzapine was continued for 10 weeks, with weekly tests of metabolic indices. Rats treated acutely with olanzapine showed both glucose dysregulation and insulin resistance; for the group treated during the week with olanzapine, these effects did not change by the end of ten weeks of treatment. However, in the group of animals challenged only once per week with olanzapine, the metabolic side-effects markedly intensified with the passage of time, whereby glucose intolerance and insulin resistance increased significantly compared to both baseline values and all other treatment groups. This previously unreported sensitization phenomenon represents a novel finding that may have clinical implications for patients receiving intermittent antipsychotic drug dosing or with variable adherence to treatment.

This article is part of a Special Issue entitled 'Schizophrenia'.

1. Introduction

The atypical antipsychotic drugs (also known as "second generation" antipsychotics) are effective pharmacological treatments for chronic psychotic disorders, which include schizophrenia and bipolar disorder (Honer et al., 2007). Furthermore, these antipsychotic drugs are increasingly being prescribed for additional psychiatric conditions, such as mood and anxiety disorders (Procyshyn et al., 2010b). Importantly, these drugs exhibit a lower incidence of neurological side-effects, i.e. extrapyramidal symptoms and tardive dyskinesia, compared to the earlier, "first generation" antipsychotic drugs (Rummel-Kluge et al., 2010a). However, in the past decade, there has been significant evidence indicating that atypical antipsychotic drugs are linked to serious adverse effects. These primarily include metabolic side-effects, which substantially increase the risk of developing cardiometabolic disorders such as Type II diabetes mellitus (DM) and cardiovascular disease (Boyda et al., 2010a; Honer et al., 2009; Kessing et al., 2010; Newcomer, 2007; Procyshyn et al., 2007). In the clinical setting, the identifying characteristics of antipsychotic drug-induced metabolic disorder are weight gain, hypertension, hyperlipidemia, hyperglycemia, glucose intolerance and insulin resistance (Hasnain et al., 2010).

Substantial differences exist between the atypical antipsychotic drugs in their capacity to induce metabolic abnormalities, with some drugs having more severe metabolic effects than others (Meyer et al., 2008; Rummel-Kluge et al., 2010b). The atypical drug olanzapine represents one of the most commonly prescribed

Abbreviations: DM, diabetes mellitus; HOMA-IR, homeostatic assessment of insulin resistance; IGTT, intraperitoneal glucose tolerance test.

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atypical antipsychotic drugs worldwide (Weinbrenner et al., 2009), and results from phase I in the large Clinical Antipsychotic Trials in Intervention Effectiveness (CATIE) trial showed that in head-to-head comparison, it was superior to the other tested antipsychotic drugs in preventing re-hospitalization of patients (Lieberman et al., 2005). Nevertheless, olanzapine in this and other clinical trials was shown to induce potent metabolic side-effects (Foussias and Remington, 2010; Meyer et al., 2008), representing a dilemma for clinicians.

At present, the biological basis of atypical antipsychotic drug-induced side-effects is poorly understood. Translational research utilizing animal models is therefore imperative to understand better this phenomenon, which becomes considerably more complicated in the clinical setting. We have recently summarized in detail the effectiveness of preclinical models of antipsychotic drug-induced metabolic syndrome (Boyda et al., 2010a). The most consistent and homologous preclinical findings that parallel the clinical data are drug effects on glucose dysregulation and insulin resistance (Assie et al., 2008; Chintoh et al., 2009; Houseknecht et al., 2007). For olanzapine, this has been confirmed using a number of different techniques. While fasting glucose levels provide a useful measure of drug-induced hyperglycemia, more accurate and quantitative measures of glucose intolerance and insulin resistance are obtained with more sophisticated techniques. These include the glucose tolerance test, which measures glucose clearance after a glucose challenge, and the hyperinsulinemic-euglycemic clamp, which measures whole-body insulin resistance (Boyda et al., 2010a; Muniyappa et al., 2008). Using both of these techniques, it has been shown that either acute or chronic treatment with olanzapine can result in metabolic dysregulation (Chintoh et al., 2009, 2008a; Houseknecht et al., 2007).

We recently demonstrated that the metabolic effects of olanzapine are both dose and time dependent, indicating that the drug can acutely affect both glucose regulation and insulin sensitivity (Boyda et al., 2010b). However, what remains unknown is whether the acute metabolic effects of olanzapine show any adaptation over time. This is an important issue, as clinical studies typically assess metabolic indices only after chronic treatment (Haupt et al., 2009), and it would be important to know if changes can occur over time: it has previously been shown that some properties of antipsychotic drugs can exhibit tolerance in rodents with repeated treatment (e.g. Samaha et al., 2007). Studies using the euglycemic clamp in rodents have only ever tested for insulin resistance at a single time point after drug treatment (Chintoh et al., 2008b; Park et al., 2007), due to technical issues, and so it has not been possible to measure putative changes in the metabolic effects of antipsychotic drugs over time. The goal of the present study was therefore to address this issue in the first longitudinal study of glucose intolerance and insulin resistance with an atypical antipsychotic drug, using the glucose tolerance test in animals treated chronically with olanzapine for 10 weeks.

2. Materials and methods

2.1. Animals

Female, Sprague-Dawley rats (Charles River, Montreal, Canada) weighing 250–275 g were group housed and maintained on a 12-h light–dark cycle (lights on at 07:00 h) in a temperature controlled colony at 22 ± 1 °C. Females are used because they exhibit much more consistent metabolic dysregulation than male rats (e.g. Baptista et al., 2002; Choi et al., 2007), and so are routinely the preferred sex in rodent models of antipsychotic drug-induced metabolic dysregulation; previous studies have indicated that there are no significant effects of olanzapine on uterine weights and estrous cycle synchronicity with chronic treatment (Fell et al., 2004, 2005). Rats were allowed to habituate to the UBC colony for one week prior to experimental testing. Food and water were available *ad libitum*. Animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Approval by the University of British Columbia's Animal Care and Use Committee was established for all methods.

2.2. Drug treatment and follow-up

The dose of olanzapine (15 mg/kg, i.p.) [Toronto Research Chemicals Inc, Toronto, ON, Canada] was carefully chosen, based on previously reported studies (Boyda et al., 2010b; Geyer et al., 2001), to represent the higher end of physiologically relevant levels *in vivo*, so that potential tolerance effects could be observed. Doses as high as 20–40 mg/kg are commonly used in the literature to examine the metabolic side-effects of olanzapine (Albaugh et al., 2006; Assie et al., 2008; Kalinichev et al., 2005). The vehicle solution consisted of 50% polyethylene glycol 400, 40% distilled water and 10% ethanol. All solutions were compounded fresh daily and administered via the intraperitoneal route in a volume of 1 ml/kg. Individual rat weights were monitored and recorded on a daily basis throughout the study. Standard rat chow consumption was recorded once a week at 16 ± 2 h prior to the glucose tolerance test and was measured on a cage-by-cage basis ($n = 3$ per cage).

2.3. Weekly intraperitoneal glucose tolerance test (IGTT) (see Fig. 1 for representation of sequence of events)

In the current protocol, the effects of olanzapine were monitored once per week for a 10-week period. Two separate groups of rats [$n = 18$ per group] were randomly assigned to receive chronic treatment during this period with either olanzapine or vehicle. As we wished to compare changes in the acute response to an antipsychotic drug challenge over time, rats were weekly administered olanzapine (or vehicle) for five days continuously, followed by a two-day "washout" period. This was so that when rats were re-challenged with olanzapine at the end of the washout period, over 10 drug half-lives had passed, and there was no residual drug from the previous five days of treatment. The drug challenge consisted of a single treatment with either the olanzapine dose (15 mg/kg, i.p.) or vehicle.

This resulted in a matrix of four different treatment groups [$n = 9$ per group]: one group treated for five days per week with olanzapine and challenged following the washout period two days later with olanzapine [O–O], one group treated for five days per week with olanzapine and challenged with vehicle [O–V], one group treated for five days per week with vehicle and challenged with olanzapine [V–O], and one group treated and challenged with only vehicle [V–V]. The experiment began with an acute challenge with olanzapine or vehicle for the appropriate groups, to allow subsequent comparison over time to the initial effects of the antipsychotic drug on glucose tolerance and insulin sensitivity: this represented the "baseline" response to olanzapine against which subsequent challenges over the next 10 weeks were compared.

Rats were fasted overnight for 16 ± 2 h. On the morning of testing, rats were transferred to the laboratory, weighed and allowed to rest for approximately 15–20 min. A baseline blood glucose level measurement was then taken from the hind leg using a 25-gauge needle to procure a drop of venous blood, which was measured by a glucometer (One Touch Ultra 2). For all blood draws, animals were wrapped in a towel and the hind leg was exposed; this technique minimizes stress to the animals and so no anesthesia is required (Hem et al., 1998). Olanzapine or vehicle was then administered acutely as a single i.p. injection. Sixty minutes after olanzapine treatment, a second measurement of glucose levels was taken, to assess the effects of drug treatment on fasting glucose levels. Immediately afterward, a saphenous blood draw (200 μl) using heparinized collecting tubes, was performed to obtain plasma for measurement of insulin levels; blood samples were centrifuged (10,000 RPM, 10 min, 4 °C) and plasma samples were stored at −80 °C for analysis. The intraperitoneal glucose tolerance test (IGTT) commenced approximately 5 min after saphenous blood draws, as all rats were given a challenge i.p. injection of 1 g/ml/kg of glucose. Glucose levels were then measured every 15 min for 120 min duration: combined "area-under-the-curve" glucose values for this period are used for analysis (Boyda et al., 2010b). Each animal handler was blinded to respected drug treatment. This procedure was repeated identically for all subsequent weeks.

2.4. Insulin measurement

Individual plasma samples extracted during each IGTT were analyzed for insulin content using Enzyme-Linked ImmunoSorbent Assay (ELISA, Mercodia, Uppsala, Sweden), using techniques similar to previously (Barr et al., 2008, 2004). Briefly, 50 μl plasma samples were added and analyzed, in duplicate, on each plate according to the specific time points studied. Samples were incubated at room temperature for 2 h followed by repeated washes. Substrate was added for 15 min and absorbance was measured at 450 nm. Calibrators provided with the kit were prepared and used to generate a calibration curve to interpolate sample data values. In addition, a reference (non-fasted) animal's plasma was added to all plates to serve as a reference standard; this confirmed a high inter-plate reliability, with the mean run-to-run correlation of 0.98 (range 0.97–0.99).

2.5. Insulin resistance

Determination of insulin resistance in treated rats was calculated through the use of the homeostatic model assessment of insulin resistance (HOMA-IR). This equation takes into account the product of both fasting levels of glucose (expressed as mmol/L) and insulin (μU/ml) 60 min post-challenge treatment and divides