Progress in Neuro-Psychopharmacology & Biological Psychiatry 34 (2010) 945–954

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

# A parametric study of the acute effects of antipsychotic drugs on glucose sensitivity in an animal model

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ARTICLE INFO

*Article history:*
Received 19 January 2010
Received in revised form 19 April 2010
Accepted 26 April 2010
Available online 7 May 2010

*Keywords:*
Clozapine
Haloperidol
Metabolic
Olanzapine
Rat
Risperidone

ABSTRACT

The therapeutic use of atypical antipsychotics is associated with a high incidence of metabolic side-effects. In the present study we examined the acute effects of both high and low-dose atypical antipsychotic drugs and one typical drug on alterations in glucose and insulin parameters using a rodent model. The effects of administration of clozapine (2 mg/kg; 20 mg/kg), olanzapine (1.5 mg/kg; 15 mg/kg), risperidone (0.5 mg/kg; 2.5 mg/kg) and haloperidol (0.1 mg/kg; 1.0 mg/kg) on glucose sensitivity and insulin resistance were determined through HOMA-IR values in fasted rats and glucose clearance during a glucose tolerance test. Acute effects were determined 60, 180 or 360 min following drug administration. The atypical antipsychotics produced significant dose and time dependent effects on fasting plasma glucose and insulin concentrations, HOMA-IR values, insulin resistance and glucose intolerance. The greatest effect on glucose dysregulation was noted primarily with clozapine and olanzapine; however, all four treatments caused significant increases in fasting glucose and/or insulin levels with the high dose, 60 min post-drug administration. Together, these findings indicate that acute administration of antipsychotic drugs has potent effects on metabolic regulation of glucose and insulin sensitivities, which may contribute to metabolic side-effects seen in humans.


## 1. Introduction

The second generation, or "atypical", antipsychotic drugs represent the first line of pharmacotherapeutic treatment for most forms of chronic psychosis (Honer et al., 2007), and are increasingly being prescribed for additional indications (Procyshyn et al., 2010). While the use of these compounds is associated with a significantly lower incidence of both early- and late-onset extrapyramidal side-effects than the first generation drugs, recent evidence indicates that the atypical drugs cause a constellation of metabolic side-effects (Henderson, 2007; Newcomer, 2007; Procyshyn et al., 2007). Numerous clinical studies have reported that chronic treatment with certain atypical antipsychotics causes weight gain, hyperlipidemia and insulin resistance, resulting in metabolic syndrome and Type II diabetes mellitus (DM) (Newcomer, 2005; Reynolds, 2007).

Symptoms of metabolic syndrome are risk factors for Type II DM and cardiovascular disease. However, impaired glucose tolerance, hyperglycemia and insulin resistance are the defining characteristics of Type II DM, and directly contribute to physical sequelae, such as microvascular damage and neuropathies (AHA, 2005). High fasting glucose levels, impaired glucose tolerance and insulin resistance are frequently noted in patients treated with most of the atypical antipsychotic drugs (Ramaswamy et al., 2006).

Alterations in glucose levels and insulin resistance in patients treated with atypical antipsychotic drugs may feasibly occur as a result of a number of different mechanisms, none of which are necessarily exclusively involved. Firstly, changes may be related to the psychiatric disorder itself, independent of treatment and prior to exposure to antipsychotic drugs (Ryan et al., 2003). Secondly, the atypical drugs may cause behavioral changes, such as increased appetite and food consumption, or decreased activity (Elman et al., 2006). Thirdly, chronic treatment with the drugs could cause one biological parameter to change directly, such as hyperlipidemia (Procyshyn et al., 2007), which would result in secondary changes, such as weight gain, causing chronic downstream changes in glucose metabolism and insulin resistance. Finally, there may be acute and direct effects of the drugs themselves on glucose regulation and insulin resistance, which occur rapidly, and only in the presence of the drug. Clearly, it is important to study each of these potential factors independently before more complex models of interacting factors are developed.

*Abbreviations:* DM, Diabetes mellitus; IGTT, Intraperitoneal glucose tolerance test; HOMA-IR, Homeostatic model assessment of insulin resistance; ISI, Insulin sensitivity index.

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doi:10.1016/j.pnpbp.2010.04.024

Rodent studies of the metabolic side-effects of atypical antipsychotic drugs have demonstrated that some aspects of the metabolic syndrome can be modeled more successfully than others. For instance, weight gain in rodents after chronic antipsychotic drug treatment remains inconsistent across research groups e.g. (Cooper et al., 2008; Cooper et al., 2007; Fell et al., 2007; Patil et al., 2006), whereas a growing number of studies have reported reliable alterations in glucose regulation and insulin resistance (Chintoh et al., 2009; Chintoh et al., 2008a; Houseknecht et al., 2007; Smith et al., 2008). Given the above noted concerns about dissociating between the acute and chronic effects of atypical antipsychotic drugs on metabolic indices, it is important to conduct research that evaluates in detail how potential metabolic effects relate to the acute administration of atypical antipsychotics.

The purpose of the present series of experiments was therefore to conduct a comprehensive study of the acute effects of antipsychotic drugs on glucose tolerance and insulin resistance in a rat model, using multiple supporting indices of both measures. We have directly compared the effects of two atypical drugs with a higher risk for metabolic side-effects (clozapine and olanzapine), an atypical drug with lower risk of metabolic side-effects (risperidone) and a common typical antipsychotic drug with low risk of metabolic side-effects (haloperidol). In order to verify the acute nature of metabolic effects, all drugs were administered to naïve animals with high or low doses, and effects on glucose tolerance and insulin resistance were assessed at three different time points after drug administration.

## 2. Materials and methods

### 2.1. Animals

Adult, nulliparous female Sprague–Dawley rats (225–250 g) were purchased from an animal supplier (Charles River, Montreal, Canada) and allowed to habituate to the UBC colony for one week before experiments commenced. Rats were group-housed in groups of 3–4 and given *ad libitum* access to food and water; all animals were maintained on a 12-hour light–dark cycle (lights on at 0700 h) in a temperature controlled colony at 22 ± 1 °C. Experimental procedures were conducted during the light cycle, and 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. Pharmaceutical agents and solutions

Doses of antipsychotic drugs were chosen to represent physiologically relevant levels *in vivo*, and therefore based on previously reported behavioral studies in rats, such as reversal of prepulse inhibition (PPI) deficits of the acoustic startle reflex. Two doses were chosen for each drug, with the higher dose representing the upper limit commonly seen in dosing for behavioral studies and the lower dose an order of five to tenfold lower (see Appendices 1 and 2 of reference (Geyer et al., 2001) for a summary of drug doses used in PPI studies). Doses for the present study included clozapine (2 mg/kg; 20 mg/kg), olanzapine (1.5 mg/kg; 15 mg/kg), risperidone (0.5 mg/kg; 2.5 mg/kg) [purchased from Toronto Research Chemicals Inc, Toronto, ON, Canada] and haloperidol (0.1 mg/kg; 1.0 mg/kg) [purchased from Sigma Aldrich, St. Louis, MO]; sedation effects were only evident in rats treated with the higher dose of haloperidol. Dosing solutions were prepared fresh daily: clozapine, olanzapine and risperidone were formulated in a vehicle composed of 50% polyethylene glycol 400, 40% distilled water and 10% ethanol. Haloperidol was formulated in a vehicle of 0.3% tartaric acid. All other chemicals were commercially available and of reagent grade. Each rat received a 1 ml/kg intraperitoneal (i.p.) injection of the vehicle control or antipsychotic formulation. The results of the studies indicated no differences between the two types of vehicle on glucose tolerance or insulin levels.

### 2.3. Intraperitoneal glucose tolerance test: (see Fig. 1 for representation of sequence of events)

Animals were randomly assigned to one of the three treatment groups (vehicle, low dose or high-dose antipsychotic [$n = 8$ per group]): each animal was only tested once. 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-guage 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).

The antipsychotic drug or vehicle was then administered acutely as a single i.p. injection, and separate cohorts of rats were tested either 60, 180 or 360 min after injection. At the appropriate time after injection, a second measurement of glucose levels was taken, to assess the effects of drug treatment on fasting glucose levels. Immediately afterwards, 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 future 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. Each animal handler was blinded to respected drug treatment. Analysis of results for haloperidol-treated animals indicated that there was no effect of the drug by 180 min on the IGTT, and so the 360 min group was not run.

In order to measure changes in insulin levels and insulin resistance throughout the entire duration of the IGTT, a separate cohort of two groups of rats ($n = 8$ per group) was treated with either vehicle or the high dose of olanzapine (15 mg/kg, i.p.); olanzapine was chosen as a representative drug due to its large effects in the first series of experiments and its widespread use clinically. One hour after drug treatment, an initial saphenous blood draw was carried out, and then every 30 min from the challenge glucose injection throughout the 120 min IGTT. Plasma samples were collected and stored in −80 °C for future analysis of insulin levels.

### 2.4. Insulin measurement

Plasma levels of insulin were measured using Enzyme-Linked ImmunoSorbent Assay (ELISA). Plasma samples were thawed and assayed for insulin using the Rat Insulin 96-well ELISA kit (Mercodia, Uppsala, Sweden). 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 0.95 (range 0.92–0.99).

### 2.5. Insulin resistance

To determine whether acute treatment with antipsychotic drugs created a state of insulin resistance, the homeostatic model assessment of insulin resistance (HOMA-IR) was utilized for fasting measurements. This technique has been previously validated as a measure of insulin