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Short Communication

# Effects of acute olanzapine exposure on central insulin-mediated regulation of whole body fuel selection and feeding

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ABSTRACT

The use of antipsychotics is associated with severe disruptions in whole body glucose and lipid metabolism which may in part occur through the central nervous system and impaired insulin action at the brain. Here we investigated whether olanzapine treatment might also affect the ability of central insulin treatment to regulate food intake and fuel preference in the light and dark cycle. Male Sprague-Dawley rats were treated with olanzapine (or vehicle solution; 3 mg/kg, subcutaneous) and a simultaneous acute intracerebral ventricular (ICV) infusion of insulin (or vehicle; 3 μL at 10mU; ICV) at the beginning of the 12-h light and dark cycles. Olanzapine treatment reduced RER in the dark and light phases (most consistently in the 4-hours post-treatment), while ICV insulin reduced average RER predominantly in the dark phase, but also at the end of the light cycle. The RER lowering effect of ICV-insulin during the light cycle was absent in the group co-administered olanzapine. The reduction in RER during the dark phase was mirrored by decreased food intake with ICV insulin, but not olanzapine treated rats. The reduction in food intake by ICV-insulin was abolished in rats co-administered olanzapine suggesting rapid induction of central insulin resistance. A combination of ICV-insulin and olanzapine similarly reduced RER in the dark phase, independent of changes in food intake. Olanzapine treatment, alone or in combination with ICV-insulin, significantly reduced $VCO_2$ at regular intervals in the dark phase (specifically 3 h post-treatment), while $VO_2$ was not significantly altered by either treatment. Finally, heat production was increased by olanzapine treatment in the light phase, though this effect was not consistent.

The findings confirm that acute olanzapine treatment directly reduces RER and suggest that treatment with this drug may also override central insulin-mediated reductions in food intake at the hypothalamus (while still independently favoring fatty acid oxidation). Acute central insulin similarly reduces RER, but in contrast to olanzapine, this may represent a physiologically appropriate response to reduction in food intake.

## 1. Introduction

The use of antipsychotics (APs) is increasing at an alarming rate, owing to expanding on- and off-label uses in adults and children (Pringsheim and Gardner, 2014). This is concerning due to the severe metabolic side effects associated with chronic and acute AP-use. In addition to the increased propensity for weight gain and related co-morbidities (Bergman and Ader, 2005), acute AP-exposure is associated with rapid, unmatched fuel repartitioning (Klingerman et al., 2014). Specifically, treatment with the AP olanzapine has been shown to increase hepatic glucose output (Houseknecht et al., 2007) and decrease adipose tissue lipolysis, while simultaneously promoting reliance on fat oxidation (as demonstrated by reduced respiratory exchange ratio [RER]) for energy requirements (Klingerman et al., 2014).

The central nervous system (CNS) has recently been implicated in metabolic consequences associated with AP-use(Kowalchuk et al., 2018; Hahn et al., 2013). Therapeutically, APs are known to act in insulin sensitive brain regions and to disturb neurotransmitter systems

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involved in energy homeostasis (Hahn et al., 2011).

Central insulin signaling is integral in maintaining glucose and lipid metabolism and for the post-prandial suppression of appetite (Dodd and Tiganis, 2017). To our knowledge, the direct effects of central insulin treatment on changes in RER have not been considered.

While APs have been previously associated with peripheral insulin resistance (Chintoh et al., 2009), the effects of APs on central insulin action are less known. As reviewed elsewhere, studies examining intracerbroventricular (ICV) administration of olanzapine have variably shown impairments in whole body insulin sensitivity, with inconsistencies possibly attributable to differences in ICV olanzapine dosing (Kowalchuk et al., 2018). Recently, we have shown that systemic olanzapine administration (dosing based on clinically comparable D2 receptor occupancy) impairs the ability of a direct central insulin infusion to suppress hepatic glucose output (Kowalchuk et al., 2017). To our knowledge, the effect of antipsychotics on CNS-insulin mediated suppression of food intake and whole body substrate selection have not yet been examined.

As such, it was the purpose of the present study to assess changes in RER in response to acute central insulin and peripheral olanzapine exposure. It was hypothesized that i) central insulin exposure alone would attenuate food intake and increase RER, while olanzapine would decrease RER independent of changes in food intake, and ii) olanzapine would override the effects of central insulin, preventing insulin-mediated reductions in food-intake and increases in RER.

## 2. Methods

### 2.1. Animals

10-week old male Sprague Dawley rats (~300 g) were purchased from Charles River Laboratories (Wilmington, MA, USA) and were singly housed and maintained on a 12-h light/dark cycle. Rats had access to Standard Rodent Laboratory Diet 5001 (Lab Diets, St Louis, MO) and water ad libitum and were given one week to acclimatize to their home cages upon arrival. At the time of the indirect calorimetry experiments, average body weights were similar between treatment groups (Veh-Veh 337.32 ± 21.85; Ins-Veh 372.0 ± 16.35; Veh-Olz 413.8 ± 20.70; Ins-Olz 388.83 ± 31.58), and body composition was assumed to be the same.

### 2.2. Intracerebral ventricular surgery

After acclimatization, rats were anesthetized using inhaled isofluorane (5% followed by 3.5%). Stainless steel cannulae (HRS Scientific, Montreal, Canada) were stereotaxically implanted into the third ventricle at the coordinates A/P = −2.5 mm; M/L = 0 mm; D/V = −8 mm, based on the coordinates of the Bregma. The cannula was secured using four stainless steel screws (Lomat, Montreal, Canada) fixed to the skull and held down using dental cement (Jet Repair, Niagara Falls, Ontario). The cannula was kept patent with a stainless steel obturator (HRS Scientific, Montreal, Canada). Rats were given one week to recover before undergoing experimentation.

After a week of recovery, to assess if the cannula was correctly placed in the brain, rats were injected with 3 µl of angiotensin into the third ventricle. Increased drinking behavior was induced with central infusion of angiotensin, indicating correct cannula placement.

### 2.3. Olanzapine preparation

Olanzapine (Toronto Research Chemicals, Toronto, Canada) was dissolved in 1% acetic acid buffered with 1 M NaOH to a pH of > 5.5. Rats received a subcutaneous injection of 3 mg/kg body weight, approximating 70% $D_2$ receptor occupancy (a threshold for therapeutic efficacy), in keeping also with our previous findings that this dose acutely impairs whole body insulin sensitivity (Chintoh et al., 2009).

### 2.4. Insulin preparation

Insulin was prepared at a concentration of 10 mU to a total volume of 3 µl in 0.9% sterile saline, as this concentration has been previously shown to suppress feeding (Brief and Davis, 1984).

### 2.5. Indirect calorimetry

Rats were acclimatized to a Comprehensive Laboratory Animal Monitoring System (CLAMS; Oxymax, Columbus Instruments) for 24 h prior to the experiment. On the day of experiment rats and food were weighed immediately prior to treatment. At 7:00 (T = 0) rats received a 3 µl injection of insulin (10 mU) or vehicle into the 3rd ventricle using a 10 µl Hamilton syringe and connecting tube cut 0.5 mm below the cannula (HRS Scientific, Montreal Canada). Rats then received a subcutaneous injection of olanzapine (3 mg/kg) or vehicle using a 1 mL syringe. At 19:00 (T = 12) rats and food were removed from cages and weighed. Rats were then injected again with both insulin/saline and olanzapine/vehicle according to their assigned treatment. The following morning at 7:00 (T = 24) rats and food were removed and weighed, and the system was turned off. RER, $VO_2$, $VCO_2$, and heat production were calculated for each sealed chamber for one minute, at ten minute intervals based on $O_2$ consumption and $CO_2$ production as previously described (Albaugh et al., 2011). Specifically, $VO_2$ is calculated as the difference between volumetric oxygen flow in and flow out of the chamber ($VO_2 = V_iO_{2i} - VoO_{2o}$) Similarly, $VCO_2$ is calculated as the difference in flow of carbon dioxide in and out of the chamber ($VCO_2 = V_iCO_{2i} - VoCO_{2o}$). Calculation of heat production is based upon the rate of oxygen consumption and the calculated calorific value at a given time point (i.e. CV = 3.815 + 1.232 * RER). Heat production is calculated by the system as the product of the CV and the rate of oxygen consumption (Heat = $CV * VO_{2,subject}$). The OxyMax system relies on measurement of 3 types of gases; produced (carbon dioxide), consumed (oxygen), and inert (nitrogen).All calculations were completed automatically by the Oxymax system as described by the manufacturer (Columbus Instruments, Columbus, OH).

### 2.6. Statistical analysis

All data are presented as means ± standard error and significant relationships are defined as $p < 0.05$. The effects of subcutaneous olanzapine and central insulin treatments were compared using 2-way ANOVA for analysis of food intake followed by Tukey's post-hoc analysis. Analysis of longitudinal RER, $VO_2$, $VCO_2$ and heat production was completed using a repeated measures ANOVA with LSD post-hoc analysis in IBM SPSS statistics 25. Power and sample size calculations were not performed.

## 3. Results

To assess the effects of central insulin and peripheral olanzapine treatments on mediating whole body fuel selection, we examined changes in RER, $VO_2$, $VCO_2$ and heat production post-treatment, in both light and dark phases.

In agreement with previous work (Klingerman et al., 2014), we confirm that olanzapine treatment significantly reduced average RER, compared to vehicle treated animals in the light and dark phases. In the light phase, this effect was transient, with RER recovering ~3–4 h post olanzapine administration. Notably, the effects of olanzapine on RER remained intact in the presence of central insulin, which on its own did not have an acute effect on RER in the light cycle. Intriguingly, RER began to decline towards the end of the light cycle with ICV-insulin treatment, an effect which was not observed in the group co-administered olanzapine, suggesting that olanzapine may have over-ridden the effects of insulin on RER.

In the dark cycle, all treatments significantly decreased RER as