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Clozapine directly increases insulin and glucagon secretion from islets: Implications for impairment of glucose tolerance

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ABSTRACT

Second generation antipsychotics cause derangements in glucose metabolism that are often interpreted as insulin resistance. In previous studies we have shown that this is not classical insulin resistance but the drugs were actually inducing a hyperglycaemic state associated with elevated hepatic glucose output (HGO) and increased levels of glucagon and insulin. However, it remains unclear whether these effects are directly elicited by drug actions in the liver and pancreas, or whether they are indirectly mediated. Here we investigated if clozapine is capable of inducing insulin resistance in the liver or enhancing insulin and glucagon secretion from the pancreas. It was observed that insulin signalling was elevated in livers from animals treated with clozapine indicating there was no insulin resistance in the early steps of insulin signalling. To explore whether the defects arise at later stages of insulin action we used an isolated perfused liver system. In this model, clozapine had no direct effect on insulin's counter regulatory effect on epinephrine-induced HGO. In isolated mouse islets clozapine significantly increased glucose-stimulated insulin secretion while simultaneously blocking glucose-induced reductions in glucagon secretion. We also show that the non-peptidic glucagon receptor like peptide-1 (GLP-1) receptor agonist Boc5 was able to overcome the inhibitory effects of clozapine on glucose metabolism. Taken together these results suggest that clozapine does not have any direct effect on glucose metabolism in the liver but it simultaneously stimulates insulin and glucagon secretion, a situation that would allow for the concurrent presence of high glucose and high insulin levels in treated animals.

1. Introduction

Schizophrenia is a major mental disorder that is generally diagnosed in early adolescence causing major disruptions in the lives of individuals affected (Jablensky, 2000; Tandon et al., 2008). The atypical antipsychotics (*i.e.*, olanzapine and clozapine) are the most widely prescribed class of drugs used to treat schizophrenia. However, they have recently been associated with serious metabolic side-effects including weight gain, dyslipidemia and type 2 diabetes (T2D) (Girgis et al., 2008). Given the large number of people who receive these drugs such adverse effects therefore represent a major health issue in their own right. The pathogenesis of T2D is often thought to be secondary to the development of insulin resistance brought on by weight gains, following long-term dosing of either olanzapine or clozapine (Girgis et al., 2008). However, impairments of glucose metabolism are seen in both drug naïve schizophrenic patients and normal controls after short-term dosing with olazapine, *i.e.*, before any signs of obesity (Vidarsdottir et al., 2010; Ou et al., 2013), suggesting a direct effect of drug on the mechanisms regulating glucose homeostasis.

Our group and others have previously used animal models to better understand how clozapine and olanzapine can acutely cause defects in glucose metabolism (Houseknecht et al., 2007; Smith et al., 2008; Chintoh et al., 2009; Smith et al., 2009; Boyda et al., 2010; Smith et al., 2011; Boyda et al., 2012). These studies have established that a single acute dose of clozapine or olanzapine induces derangements in glucose metabolism within minutes of dosing (Houseknecht et al., 2007;

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Smith et al., 2008; Chintoh et al., 2009; Smith et al., 2009; Boyda et al., 2010; Smith et al., 2011). Our studies have also shown that the derangements in glucose metabolism were not due to reduced glucose uptake in fat or muscle (classical insulin resistance phenotype), but instead due to increased hepatic glucose production (Smith et al., 2008, 2009, 2011). Work from other groups using clamp techniques also indicates that these drugs are inducing an increase in hepatic glucose output (HGO) (Houseknecht et al., 2007; Chintoh et al., 2009).

Three possible mechanisms could be envisaged for the increase in HGO: one would be a centrally mediated effect on the liver acting by efferent nerves; a second would be a direct effect in the islets on insulin and glucagon releases (or possibly by efferent nerves) which would then act on the liver; the third would be a direct action of the drug on the liver to induce insulin resistance such that insulin would not be able to counter the effects of hormones that stimulate HGO such as glucagon and adrenaline. Some studies have attempted to address these three possibilities. There are conflicting results on the centrally mediated effects of olanzapine on hepatic glucose metabolism as there is one report that ICV administration of olanzapine induces increases in HGO (Martins et al., 2010) but another group of researchers found no effect (Girault et al., 2012). Several groups have studied the acute effects of clozapine and olanzapine on insulin secretion in isolated islets and β-cell models but results are again conflicting with some finding inhibition of secretion and others observing a stimulation (Melkersson, 2004; Johnson et al., 2005; Sasaki et al., 2006; Menga et al., 2013). There are currently no reports of the direct effects of these drugs on glucagon secretion from islets. Further, the direct effects of atypical antipsychotic drugs on insulin action and glucose metabolism in the liver have not been previously documented. Thus, we assessed the effect of clozapine on insulin signalling pathways in rat liver and used an isolated liver model to test the direct effect of clozapine on hepatic glucose metabolism. We also examined the effect of clozapine on insulin and glucagon secretion from isolated islets and finally tested the novel non-peptidic GLP-1 receptor agonist Boc5 (Su et al., 2008) to further understand whether restoring defective GLP-1 signalling would improve clozapine induced defects in glucose metabolism.

2. Materials and methods

2.1. Chemicals

Boc5 was prepared as previously described (Su et al., 2008). Clozapine was kindly provided by Douglas Pharmaceuticals (Auckland, New Zealand). The insulin used was ActRapid (Novo Nodisk) and glucagon was GlucGen Hypo. Other chemicals were from Sigma unless otherwise stated.

2.2. Animal experiments

All animal experimentation was conducted in accordance with the regulations adopted either by the University of Auckland Animal Ethics Committee, by the A* Animal Ethics Committee, Singapore or by the Animal Care and Use Committee, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (approval number: 2012-05-WMW-03). Only male rats were used in this study due to the oestrous cycle having an effect on metabolism.

2.3. Isolated liver experiments

Male Sprague–Dawley rats (190–210 g) were maintained up until the experiment on 12 h day-night cycle; water *ad lib*; and food *ad lib* (Harlan Teklad 2018 diet, Madison, WI, USA). Non-fasted rats underwent laparotomy under general anaesthesia (75 mg/kg body weight ketamine, 10 mg/kg body weight xylazine, intraperitoneal administration, i.p.). The liver was perfused *in vivo* and then removed (non re-circulating mode) in a similar manner to that previously reported (Gores et al., 1986; Cheung et al., 1996). Briefly, the portal vein was cannulated *in situ* and the atria vented to allow the liver to be perfused initially with 20 ml of heparinised perfusion media (final; NaCl 128 mM, MOPS 23.9 mM, KCl 6 mM, $MgSO_4 \cdot 7H_2O$ 1.18 mM, Ca_2 1.29 mM, BSA(FFA) 0.2%, pH 7.4). The *in situ* liver was then perfused at 2 ml/min (connected to a custom-made, temperature-controlled organ perfusion system and perfusion medium oxygenated using carbogen, O_2: 95%; CO_2: 5%; 37 ºC) while the superior vena cava was cannulated and the liver excised. The liver was weighed and re-connected to the organ perfusion system whereby perfusion was continued at a rate of 2 ml g^{-1} min^{-1} with oxygenated and warmed perfusion medium. The liver was stabilised for 40 min (t = 40). Organ effluent (1 ml) was then collected every 5 min for glucose and lactate measurement, using a GEM3500 glucose/gas analyser. At t = 40 min, clozapine or vehicle (DMSO) was delivered *via* a side arm infusion (13.3 μl g^{-1} min^{-1}) to achieve a final organ perfusion concentration of 1 μM. Finally, glucagon (1.15 nM), insulin (1 nM) or epinephrine (50 nM) was infused at the stated time point (see Figs. 1 and 3 for more information on the experimental design and analysis) *via* a side arm.

2.4. Insulin signalling in the liver

Drug naive male Sprague–Dawley rats (350 g; VJU, New Zealand) were weight-matched into groups (n = 9/group): vehicle (Veh; 5% acetic acid pH 6), vehicle plus octreotide (Veh + Oct; 250 μg/kg), clozapine (Cloz; 10 mg/kg; Douglas Pharmaceuticals, New Zealand), and clozapine plus octreotide (Cloz + Oct). After 1 h clozapine exposure the liver and blood was collected for analysis. The activation status of downstream components of the insulin signalling pathway in the liver homogenates was assessed using Luminex based phospho-antibody sandwich assays, as we have previously described (Smith et al., 2012). Blood glucose was analysed using a hand held glucose meter (Perfoma, Roch).

2.5. Isolated pancreatic islets experiments

Islets were isolated by liberase digestion and cultured for 24 h at 11 mM glucose in hcell medium (Gustavsson et al., 2008). Subsequent experimental handling was performed in Krebs–Ringer–Hepes (KRH) (containing mM: 130 NaCl, 4.7 KCL, 1.2 KH_2PO_4, 1.2 $MgSO_4$, and 2.56 $CaCl_2$, 1 mg/ml BSA, 20 mM Hepes, pH 7.4) supplemented with 10 mM glucose. Similar size islets (5 islets per tube) were first washed with 10 mM glucose containing KRH medium. Islets were incubated in 3 mM glucose containing KRH medium for 30 min prior to clozapine or vehicle (DMSO) and glucose experiments as detailed in Fig. 4. Glucagon concentration was measured using a glucagon RIA (Millipore) and insulin by a Mouse insulin ELISA (Mercodia). Each experiment was repeated 5 times.

2.6. Oral glucose tolerance testing following Boc5 exposure

Male Sprague–Dawley rats (190–210 g) were assigned to one of 4 experimental groups: 1) vehicle (no Boc5), 2) Boc5 alone (100 mg/kg), 3) vehicle (no Boc5) + clozapine (10 mg/kg) and Boc5 (100 mg/kg) + clozapine (10 mg/kg), *n* = 9/group. Each group received a daily i.p. injection of vehicle (no Boc5) (1% DMSO, 20% PEG400 in saline, pH 7.0–7.5) or Boc5 (purity > 90% dissolved in 1% DMSO, 20% PEG400 in saline to a final concentration of 6 mg/ml, pH 7.0–7.5 with a final dose of 100 mg/kg) for 14 days. On the 14th day all animals were fasted overnight and given their final (15th) vehicle (Boc5) or Boc5 injection at 8 in the morning. After a 2 h period animals were injected with another vehicle (no clozapine) (5% acetic acid) or clozapine (10 mg/kg in 5% acetic acid). After 1 h of clozapine or vehicle (no clozapine) each animal was given a glucose bolus (2 mg/kg of D-glucose in 40% [wt./vol.]) *via* a feeding tube ((1.2 mm OD × 0.7 mm) ID × 75 mm).

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