The role of hypothalamic pathways in the metabolic side effects of Olanzapine

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Central administration of an orexin receptor 1 antagonist prevents the stimulatory effect of Olanzapine on endogenous glucose production

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ABSTRACT

Atypical antipsychotic drugs such as Olanzapine (Ola) induce weight gain and metabolic changes associated with the development of type 2 diabetes. The mechanisms underlying these undesired side effects are currently unknown. It has been shown that peripheral injections of Ola activate neurons in the lateral hypothalamus/perifornical area and that a large part of these neurons are orexin (Ox) A-positive. We investigated further the possible involvement of the central Ox system in the metabolic side effects of Ola by comparing the hyperglycemic effects of an intragastric (IG) Ola infusion between animals treated intracerebroventricularly (ICV) with an Ox-1 receptor antagonist (SB-408124) or vehicle. As observed in previous studies IG Ola caused an increase in blood glucose, endogenous glucose production and plasma glucagon levels. ICV pre-treatment with the Ox-1 receptor antagonist did not affect the Ola-induced hyperglycemia or increased plasma glucagon concentrations, but the increased endogenous glucose production was blunted by the ICV SB-408124 treatment.

From these results, we conclude that the metabolic side effects of Ola are partly mediated by the hypothalamic Ox system.
INTRODUCTION

Treatment with atypical antipsychotic drugs (AAPDs) is associated with significant weight gain and metabolic disturbances including hyperglycemia and insulin resistance. Over the past decade, particular attention has been paid to one of the AAPDs that causes dramatic weight gain, Olanzapine (Ola). However, the exact mechanisms underlying the metabolic changes induced by Ola are still far from clear.

In a first attempt to elucidate the mechanism of the metabolic side effects of Ola, we compared the effects of peripheral and central administration of Ola on glucose metabolism and showed that intragastric (IG), but not intracerebroventricular (ICV), administration of Ola induces hyperglycemia and insulin resistance (Girault et al. 2012). Previously it has also been shown that a peripheral injection of Ola (10mg/kg) induces activation of neurons in the parvocellular paraventricular nucleus of the hypothalamus (PVN) and the lateral hypothalamus/perifornical area (LH/PFA) (Stefanidis et al. 2009) and that a large part of these latter neurons are orexin-positive (Stefanidis et al. 2009; Wallingford et al. 2008). Fadel et al. reported that among four AAPDs associated most prominently with weight gain, Ola activated more orexin (Ox) neurons in the medial LH/PFA than the other three AAPDs (Fadel et al. 2002). The Ox neurons in the LH/PFA are potent regulators of body weight, mood, arousal and reward (Sakurai 2005). For example, it has been shown that subcutaneous injections of both Ox-A and –B stimulate insulin secretion (Nowak et al. 2000). Moreover, activation of Ox neurons in the LH/PFA increases blood glucose concentrations via a sympathetic stimulation of endogenous glucose production (Yi et al. 2009). Shiuchi et al. demonstrated that Ox stimulates glucose uptake in skeletal muscle via its action in the ventromedial hypothalamus, which is also mediated via the sympathetic nervous system (Shiuchi et al. 2009). Moreover, a peripheral injection of Ola (10mg/kg) blocks the sympathetic and hyperthermic response to an ICV injection of Ox-A (Monda et al. 2008). Finally, the intravenous injection of an Ox-1 receptor (OxR1) antagonist reverses the number of A9 and A10 dopamine cells activated by a subcutaneous injection of Ola (Rasmussen et al. 2007b), as well as some of the behavioural effects of antipsychotic drugs (Rasmussen et al. 2007a).

Together, these observations point towards an involvement of the central Ox pathway in the metabolic side effects of Ola. We therefore investigated whether an ICV infusion of the OxR1 antagonist SB-408124 would inhibit the effects of a peripheral injection of Ola on glucose metabolism.
MATERIALS AND METHODS

Ethic statement
All experiments were approved by the animal care committee of the Royal Netherlands Academy of Arts and Sciences (Permit number: NIN09.35) and followed the EC Directive 86/609/EEC for animal experiments.

Animals
32 male RccHan:WIST rats (Charles River Breeding Laboratories, Sulzfeld, Germany) weighing 300–350 g were individually housed (cages 40x25x25 cm) and maintained on a 12h/12h light/dark cycle (lights on at 7:00am) at 21±1°C and 60±5% relative humidity. Food (standard rodent chow, Teklad) and water were available ad libitum.

Drugs
The OxR1 antagonist 1-(6,8-difluoro-2-methyl-quinolin-4-yl)-3-(4-dimethylamino-phenyl)-urea (SB-408124; 50 mmol/L; Sigma-Aldrich Corp., St. Louis, MO, USA) was infused via an ICV probe at 0.267 mg/kg/h (100 µM) for 260 minutes. The dose of SB-408124 was similar to Yi et al. (2009). The dose of Ola chosen in the present study was identical to the effective dose used in our previous experiments (Girault et al. 2012). The route of administration was chosen such that a continuous infusion of freshly made solution was possible in freely moving, undisturbed animals. Using a surgically implanted IG catheter, animals were treated with a primed 36 mg/kg/h infusion during 5 minutes followed by a continuous 3 mg/kg/h infusion for 160 minutes (i.e., in total 3.66 mg/rat) of Ola (ChemPacific Corporation, Maryland) dissolved in acidified MilliQ water (pH=6). Ola solution was prepared in MilliQ water acidified with HCl (1M) and then brought back to pH 6 using NaOH (1M).

Surgical procedures
After 7 days of habituation, animals were anesthetized by an intramuscular injection of 0.9 ml/kg Hypnorm (Janssen, High Wycombe, Buckinghamshire, UK) and a subcutaneous injection of 0.3 ml/kg Dormicum (Roche, Almere, The Netherlands). Silicon catheters were placed into the right jugular vein and the left carotid artery for intravenous infusions and blood sampling. The vascular lines were closed using a mix of polyvinylpyruvidon (PVP; Sigma-Aldrich Corp., St. Louis, MO), heparin (LEO Pharma, Ballerup, DK) and amoxicillin (Centrafarm, Etten-Leur, NL). A silicon cannula was placed in the stomach during the same
surgery. IG cannulas were placed through a 1 cm incision on the left side of the abdomen. ICV probes were placed into the lateral cerebral ventricle using a standard Kopf stereotaxic apparatus (Anteroposterior: -0.8mm, Lateral: 2.0mm, Ventral: -3.2mm, Angle: 0). Catheters, ICV probe and IG cannula were fixed on the top of the head of the animal using dental cement. These techniques allowed us to perform all our experiments in awake and freely moving animals. Experiments were performed only after recovery of the pre-surgical body weight and with animals in healthy state, i.e. 7 – 10 days post-operative recovery.

Experimental procedures
During the experiment, animals were permanently connected to blood-sampling and infusion lines, which were attached to a metal collar and kept out of reach from the rats by means of a counterbalanced arm. This allowed all manipulations to be performed outside the cages without handling the animals. The metal collars were attached the day before the experiment. Before the day of the experiment, food was restricted to 20 g overnight which is only slightly less than the average consumption per night (i.e., 24±2 g) for a rat of that body weight. Two hours before the experiment, rats were handled to connect them to the blood sampling and infusion lines and all remaining food was removed.

Endogenous glucose production
To assess endogenous glucose production (EGP), [6,6-²H₂]glucose was used as a tracer. Blood samples were taken at t=-5min for background enrichment (t=0 was at 11.00 a.m.), at t=90, t=95 and t=100 min to determine enrichment during the equilibrium state and every 20 min from t=120 until t=260 min to determine enrichment during the experimental state.

**Figure 1: Experimental protocol**, Experimental protocol showing the time line of the different infusions (IV, ICV and IG) and the timing of blood sampling.
Vehicle (MilliQ water at pH=6 to mimic the pH of the Ola solution) for the IG infusion (1 ml/h) and SB-408124 (or Vehicle) (5 µl/h) for the ICV infusion started together with a continuous [6,6-\(^2\)H\(_2\)]glucose infusion via the jugular vein at t=0. After the t=100 min blood sample (at about 12.40 a.m.), vehicle IG infusion was changed to Ola or vehicle solution (36 mg/kg/h during 5 minutes and 3 mg/kg/h until the end of the experiment) (Figure 1). At the end of the experiment, animals were sacrificed by a lethal intravenous injection of pentobarbital.

Thus the experiment consisted of 4 experimental groups: “ICV-Veh + IG-Veh” (n=6), “ICV-Veh + IG-Ola” (n=8), “ICV-SB-408124 + IG-Veh” (n=5) and “ICV-SB-408124 + IG-Ola” (n=8). Five of the 32 animals had to be excluded from the experiment because of technical problems, i.e. blocked cannulas.

Laboratory methods/analysis

Glucose concentrations were determined using a glucometer (Abbott). Blood samples were collected in tubes containing heparin on ice and centrifuged at +4°C. Plasma was stored at -20°C until further analysis.

Plasma insulin, glucagon and corticosterone concentrations were measured using radioimmunoassay kits (Millipore, Billerica, USA for insulin and glucagon and MP Biomedicals, Orangeburg, USA for corticosterone). Plasma [6,6-\(^2\)H\(_2\)]glucose enrichment was measured by gas chromatography-mass spectrometry (GCMS) (Ackermans et al. 2001), and EGP was calculated by the methods of Steele (Steele 1959).

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS version 17.0. A p<0.05 was considered statistically significant. An ANOVA with repeated measures was performed to compare glucose levels, EGP, corticosterone, insulin and glucagon levels. If appropriate, post-hoc analysis was performed using One-Way ANOVA.

RESULTS

Experiments were performed according to the experimental protocol outlined in Figure 1. The IG Ola infusion resulted in a marked increase in blood glucose levels (Time*IG \(F_{10,230}=5.632; \ p<0.001\) and \(IG \ F_{1,23}=12.893; \ p=0.002\); Figure 2). Post-hoc analysis showed that IG infusion of Ola increased blood glucose in both Veh- and SB-408124-treated animals (\(IG \ F_{1,12}=7.95; \ p<0.05\).
p=0.015 and \( IG F_{1,11}=5.167; p=0.044 \) respectively). No effect of ICV or interaction effect of Time*ICV was detected. Treatment with SB-408124 therefore did not influence the hyperglycemia induced by Ola. This Ola-induced hyperglycemia could be established via different mechanisms. The first mechanism we investigated was a change in endogenous glucose production (EGP).

Figure 2: Glucose levels during treatment with intragastric Ola and central SB-408124. Variation of blood glucose levels during the ICV SB-408124, the IG Ola administration and the combination of those 2 drugs. IG Ola significantly increases blood glucose (Time*IG \( F_{1,23}=5.632; p<0.001 \) and IG \( F_{1,12}=12.893; p=0.002 \). Post-hoc analysis showed that this effect is significant in both SB-408124 pre-treated and non-pre-treated animals (respectively IG \( F_{1,23}=7.95; p=0.015 \) and IG \( F_{1,12}=5.167; p=0.044 \)). Data are expressed as the absolute increase (delta) compared to the mean value of the 3 basal samples (i=90, 95 and 100 min). Absolute values of the means of basal samples are presented in Supplementary Table 1. *p<0.05 between "ICV-Veh + IG-Veh" and "ICV-Veh + IG-Ola" groups; #p<0.05 between "ICV-SB-408124 + IG-Veh" and "ICV-SB-408124 + IG-Ola" groups.

Figure 3: EGP during treatment with intragastric Ola and central SB-408124. A: Variations in EGP during the ICV SB-408124, the IG Ola and the combination of those 2 drugs. IG Ola significantly increases EGP (IG \( F_{1,23}=6.281; p=0.02 \). Post-hoc analysis showed that this effect is significant only in ICV-Veh-treated animals (IG \( F_{1,23}=6.402; p=0.026 \) and not in ICV-SB-408124-treated animals (IG \( F_{1,12}=1.664; p=0.224 \). B: AUC of the four different EGP responses. IG Ola increases AUC of EGP significantly (IG \( F_{1,23}=6.875; p=0.015 \), but post-hoc analysis shows that this effect is only significant in the "ICV-Veh" group (IG \( F_{1,23}=0.236; p=0.632 \). Data are expressed as the absolute increase (delta) compared to the mean value of the 3 basal samples (i=90, 95 and 100 min). Absolute values of the means of basal samples are presented in Supplementary Table 1. *p<0.05 between "ICV-Veh + IG-Veh" and "ICV-Veh + IG-Ola" groups.
IG infusion of Ola caused a significant increase of EGP (Time*IG F_{10,230}=2.865; p=0.002 and IG F_{1,23}=6.281; p=0.02, Figure 3a). However, post-hoc analysis of the “ICV-Veh” and “ICV-SB-408124” groups separately showed that Ola only significantly increased EGP in the “ICV-Veh” group (IG F_{1,23}=6.402; p=0.026, Figure 3a), and not in the “ICV-SB-408124” group (IG F_{1,12}=1.664; p=0.224).

These results indicate that the ICV infusion of SB-408124 changed (i.e., decreased) the Ola-induced increase in EGP. The decreased efficiency of Ola in the “ICV-SB-408124” group is most clearly reflected in the AUC of the different EGP responses (IG F_{1,23}=6.875; p=0.015, Figure 3b), with a reduction of the Ola effect in the “ICV-SB-408124” group of >50%.

Also changes in plasma corticosterone levels can influence EGP and thus glucose levels. In our study, however, plasma corticosterone levels were not changed by the treatments (IG F_{1,23}=2.478; p=0.129 and ICV F_{1,23}=0.175; p=0.68, Figure 4).

![Corticosterone levels during treatment with intragastric Ola and central SB-408124.](image)

We also assessed possible changes in plasma insulin and glucagon levels, since these 2 hormones have profound effects on hepatic glucose regulation. ANOVA analysis of the plasma insulin levels showed no significant effect of the treatments (IG F_{1,23}=0.288; p=0.597 and ICV F_{1,23}=0.47; p=0.5, Figure 5).

Plasma glucagon levels were significantly increased by the Ola treatment as indicated by the significant interaction of Time*IG (F_{4,92}=4.208; p=0.004, Figure 6), however, the lack of a significant effect of ICV indicates that the ICV-SB-408124 infusion did not affect this Ola-induced increase of plasma glucagon (ICV F_{1,23}=0.215; p=0.647).
DISCUSSION

We showed that ICV treatment with an OxR1 antagonist blunts the Ola-induced increase in EGP, but not its hyperglycemic effect. Previously it had been shown that Ola activates neurons in the LH/PFA and that part of these activated neurons contained Ox (Fadel et al.)
In addition, it was known already that intravenous injections of an OxR1 antagonist block Ola-induced catalepsy, another side-effect of the drug (Rasmussen et al. 2007a). Our study is the first to show that also some of the metabolic side effects of Ola are mediated via a central mechanism involving the Ox system.

Our group and others have shown previously that Ola administration leads to insulin resistance both in experimental animals and humans (Girault et al. 2012; Chintoh et al. 2009; Chintoh et al. 2008; Houseknecht et al. 2007; Sacher et al. 2008). Hyperinsulinemic-euglycemic clamp experiments showed that administration of Ola leads to both hepatic and extra-hepatic insulin resistance (Chintoh et al. 2009; Girault et al. 2012). In the current experiment, we observed an undisturbed increase in glucose levels in the “ICV-SB-408124 + IG-Ola” group while the Ola-induced increase in EGP was blunted by the OxR1 antagonist. Since our animals were deprived of food during the experiment (i.e. from 7.00 a.m. onwards), the only way to explain the persistent hyperglycaemia is a decrease in glucose uptake that was not blocked or even might have been reduced further by the SB-408124 treatment. Indeed, Ox has been shown to enhance both glucose production (Yi et al. 2009) and glucose uptake (Shiuchi et al. 2009). Therefore, it is to be expected that treatment with an OxR1 antagonist will reduce both EGP and glucose uptake. However, as our experiments were not designed to assess glucose uptake in a quantitative way this remains to be demonstrated using a hyperinsulinemic-euglycemic clamp experiment.

ICV administration of Ox stimulates EGP, but only when the sympathetic innervation of the liver is intact, indicating a stimulatory effect of central Ox on the sympathetic neuronal outflow to the liver (Yi et al. 2009). However, also in this previous experiment, a sympathetic denervation of the liver was not able to prevent the stimulatory effect of an ICV Ox-A infusion on plasma glucose levels (Yi et al. 2009). The separation of plasma glucose and EGP responses indicates that increased central Ox signalling may affect plasma glucose concentrations not only through changes in hepatic glucose production but also by affecting peripheral glucose uptake. Indeed, Shiuchi et al. demonstrated that, via its action in the ventromedial hypothalamus, Ox stimulates glucose uptake in skeletal muscle (Shiuchi et al. 2009). This effect also turned out to be mediated via the sympathetic nervous system (SNS). Conflicting results are reported concerning changes in plasma insulin levels after Ola administration. On one hand, patient studies report an increase in insulin levels after chronic or sub-chronic treatment (Ou et al. 2012; Melkersson et al. 2011; Melkersson et al. 2000) and no changes after acute administration (Kopf et al. 2012). On the other hand, animal studies
showed that Ola-induced glucose dysregulation can be associated with increased (Boyda et al. 2012a; Smith et al. 2011), decreased (Weston-Green et al. 2012) and even unaltered insulin levels (Ferno et al. 2011) with no relation with the duration of the treatment. However, Boyda et al. showed that chronic treatment with Ola leads to a desensitization of the insulin response to an acute challenge with the same drug (Boyda et al. 2012b). Moreover, Ox administration leads to an increased insulin secretion in vitro (Nowak et al. 2005; Colombo et al. 2003) and in vivo (Adeghate and Hameed 2011). But although Ox is known to be involved in the hypothalamic projections to the pancreas (Buijs et al. 2001), Yi et al. found no changes in insulin levels after the administration of SB-408124 (Yi et al. 2009). Our results are confirming this since none of the treatments we used affected insulin levels.

Clearly the rise in glucagon levels in both Ola-treated groups will also contribute to the hyperglycemia and increased EGP. Remarkably, only EGP, and not the increase in glucagon, was lowered in the ICV-SB-408124 + IG-Ola group. Thus the increased release of glucagon is not mediated by the Ox system. On the one hand, this observation fits with our previous results that the ICV infusion of Ox did not affect glucagon release (Yi et al. 2009). On the other hand, this also explains why EGP is not completely suppressed in the ICV-SB-408124 + IG-Ola group, i.e., the non-Ox mediated increased release of glucagon still stimulates EGP.

In the current study, we observed no effects of the OxR1 antagonist on corticosterone levels. Also in our previous study we found no evidence for an involvement of the hypothalamo-pituitary-adrenal (HPA) axis in the Ola-induced glucoregulatory effects (Girault et al. 2012). However, Ox is known to have clear neuroendocrine effects, amongst others on plasma concentrations of luteinizing hormone (LH), prolactin and thyroid stimulating hormone (TSH) (Pu et al. 1998; Hagan et al. 1999; Jones et al. 2001). Various studies reported increased plasma concentrations of adrenocorticotropic hormone (ACTH) and corticosterone after ICV administration of Ox, suggesting a central effect of the peptide on the activity of the HPA axis (Hagan et al. 1999; Ida et al. 2000; Jaszberenyi et al. 2000; Kuru et al. 2000; Russell et al. 2001; Samson et al. 2007). ICV Ox also leads to a rise in plasma vasopressin (AVP) and enhances the release of corticotropin-releasing hormone (CRH) in the PVN (Date et al. 1999; Marcus et al. 2001). Thus Ox neurons in the hypothalamus are involved in the central circuits that control the activity of the HPA axis (Koob 2008; Winsky-Sommerer et al. 2004). Administration of Ola reduces ACTH and cortisol secretion in patients (Cohrs et al. 2006), but increases plasma corticosterone levels in rodents (Assie et al. 2008; Girault et al. 2012). Other groups have also studied the role of OxR1 and Ox-A in relation with the metabolic side
effects of atypical antipsychotics including Ola, be it to a limited extent (Basoglu et al. 2010; Stefanidis et al. 2009; Davoodi et al. 2009; Wallingford et al. 2008). Although the localization of the OxR1 and Ox-2R has been established, the role of each receptor in glucose metabolism has not been clearly defined yet. Funato et al. showed that Ox improves the sensitivity to leptin and insulin by an Ox-2R-dependent mechanism (Funato et al. 2009). Both leptin and insulin sensitivity seem to be reduced under treatment with atypical antipsychotic (Girault et al. 2012; Albaugh et al. 2006), and the current results indicate the implication of the OxR1 in the Ola-induced insulin resistance. Clearly more research is needed to understand the full implication of Ox, OxR1 and Ox-2R in the atypical antipsychotic-induced metabolic side effects.

In conclusion, we showed that the central Ox system is involved in the increased EGP induced by the peripheral administration of Ola. The stimulatory effect of Ola on EGP does not seem to be mediated via an increased release of glucagon, but an Ox-induced increased sympathetic input to the liver. In addition, it may involve Ola-induced changes in glucose uptake.

ACKNOWLEDGEMENTS

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Supplementary table:

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<th>Groups</th>
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**Supplementary table 1:** Absolute values of the means of basal samples of glucose, EGP, corticosterone, insulin and glucagon.
1. Ackermans MT, Pereira Arias AM, Bisschop PH, Endert E, Sauerwein HP, Romijn JA (2001). The quantification of gluconeogenesis in healthy men by \((2)\text{H}_2\text{O}\) and \([2-(13)\text{C}]\text{glycerol}\) yields different results: rates of gluconeogenesis in healthy men measured with \((2)\text{H}_2\text{O}\) are higher than those measured with \([2-(13)\text{C}]\text{glycerol}\). J Clin Endocrinol Metab 86: 2220-2226.


