The role of hypothalamic pathways in the metabolic side effects of Olanzapine
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Chapter 2.3

Chronic treatment with Olanzapine increases adiposity by changing fuel substrate and causes desensitization of the acute metabolic side effects

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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 metabolic side effects are unknown at the moment. In this study, we investigated the metabolic changes induced by a chronic treatment, as well as the influence of a chronic treatment on the acute effects of Ola on glucose metabolism.

The effect of chronic Ola treatment (6.5 mg/kg/day, administered via drinking water) on body weight, locomotor activity, body temperature, fat distribution and energy expenditure was investigated in male rats. After 5 weeks, the animals received an acute Ola challenge (intragastric, IG) at 3 mg/kg/h during 160 min to investigate the acute effects of Ola on glucose metabolism.

Chronic Ola-treated animals showed a slight decrease in nocturnal body temperature, and increased perirenal fat pad weights as well as plasma leptin. In addition, chronic Ola-treated animals showed hyperinsulinemia with unchanged blood glucose concentrations. The acute challenge with IG Ola elevated blood glucose levels and endogenous glucose production (EGP) in control animals, but not in chronic Ola-pre-treated rats. Chronic Ola-treated animals also showed reduced locomotor activity and a higher respiratory exchange ratio.

Chronic treatment with Ola in rats causes desensitization to its acute effects on glucose metabolism but promotes adiposity probably due to a shift from lipids to carbohydrates as an energy source. Chronic exposure to Ola changes body fat distribution and insulin sensitivity in an unfavourable direction, but it is still unclear what the primary mechanism is.
INTRODUCTION

Chronic treatment with atypical antipsychotics is a major and effective therapeutic modality in schizophrenic patients (Lieberman et al. 2005). However, it is associated with metabolic side effects such as weight gain and hyperglycemia leading to obesity and type 2 diabetes (Nasrallah 2008). Olanzapine (Ola) is one of the atypical antipsychotics causing the most profound weight gain in patient care (Nasrallah 2008), occurring in up to 80% of patients (Umbricht et al. 1994; Masand 2000) and leading to decreased patients compliance. Considerable attention has been paid to these metabolic side effects. Rodent studies showed that not only chronic but also sub-chronic and acute administration of Ola lead to impaired insulin sensitivity (Chintoh et al. 2008a). Chronic administration of Ola has been reported to lead to fat accumulation (Cooper et al. 2005; Albaugh et al. 2010; Minet-Ringuet et al. 2006; Shobo et al. 2011; van der Zwaal et al. 2010), decreased locomotor activity (Albaugh et al. 2010; Liebig et al. 2010; van der Zwaal et al. 2010) and increased meal size (van der Zwaal et al. 2010) but decreased meal frequency (van der Zwaal et al. 2008). Due to the different modes of administration, the absence of plasma Ola measurements and single outcome measures in most studies, it is difficult to compare one study to another and to this day, the primary mechanism for the metabolic side effects of Ola is still unclear. In an attempt to pinpoint the primary event, we combined measures for energy intake, energy expenditure, glucose metabolism and plasma Ola measurements in rats chronically treated with Ola. Amongst others, we determined whether the glucoregulatory effects of an acute IG treatment with Ola were changed when rats were treated with Ola for a longer period of time. We hypothesized that chronic treatment with Ola would result in an increased adiposity as a consequence of reduced energy expenditure and that long term treatment would alter the acute metabolic side effects of the drug.

MATERIALS AND METHODS

Ethic statement

All experiments were approved by the animal care committee of the Royal Academy of Arts and Sciences and are in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Animals

Male Wistar rats (Harlan Nederland, Horst, The Netherlands) weighing 170-200 g were
individually housed (cages 40x25x25cm) and maintained on a 12h/12h light/dark cycle (light on at 7:00am) at 21±1°C and 60±5% relative humidity. Food (standard rodent chow, Harlan) and water were available ad libitum.

**Drugs**

To prevent degradation of Ola as a result of storage at body temperature in an osmotic minipump (van der Zwaal et al. 2008) and to assure a continuous delivery of the drug, we administered Ola via the drinking water. This technique has already proven to be efficient in numerous studies (van der Zwaal et al. 2010; Llorente-Berzal et al. 2012; Terry, Jr. et al. 2008; McNamara et al. 2011; Ishii et al. 2003). Water bottles were refreshed every 2-3 days to make sure the animals received the intact compound. Adding Ola to the drinking water (6.5-7mg/kg/day) reduces total water intake by 30% due to the bitter taste of the drug (van der Zwaal et al. 2010). We used water flavoured with quinine (Qui) for the control group to balance the reduced drinking as well as eating behaviour. The dose of Qui was chosen based on previous studies using Qui administration for the same purpose (Ishii et al. 2003; van der Zwaal et al. 2010). All the animals received treatment for 32 days. Water and food intake were monitored weekly to allow titration of the Qui concentration in the water. Body weight was assessed regularly.

**Experiment 1: Glucose metabolism, Ola measurements and adiposity**

1. **Animals**

36 animals were divided in 2 groups; half received Qui (control; Qui group) and the other half Ola (Ola group) chronically via the drinking water. Each of these 2 groups was further divided into 2 experimental groups on the final experimental day: one received MilliQ water (at pH=6 to mimic the pH of Ola solution, Veh) and the other Ola intragastrically (3 mg/kg/h for 3h). We therefore ended up with 4 groups in total: “Qui+Veh”, “Qui+Ola”, “Ola+Veh”, “Ola+Ola”.

2. **Surgery**

During the 4th week of treatment, 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, the left carotid artery and the stomach according to previously described methods (Girault et al. 2012).

A third of the animals (n=5 receiving Qui and n=6 receiving Ola) were implanted subcutaneously with a Thermochron iButton (DS 1922, Maxim, Dallas, Texas) data logger to
measure body temperature every 5 min during the data collection period.

3. **Experimental day**
The acute experiment was performed only after recovery of the pre-surgical body weight and with animals in healthy state, i.e. 7 – 10 days post-operative recovery (end of the 5th week of chronic treatment). The route of acute Ola administration was chosen such that a continuous 3-hours infusion was possible in unrestrained animals. During the acute experiment, animals were permanently connected to blood-sampling and infusion lines, allowing all manipulations to be performed outside the cage without handling the animal (Girault et al. 2012). Food was restricted to 20 g overnight. Rats were handled only once on the experimental day to connect them to the blood sampling and infusion lines. Remaining food was removed 2h before the experiment.

To assess EGP, [6,6-2H2]glucose was used as a tracer (Ackermans et al. 2001). Blood samples were taken at t=5min for background enrichment (t=0 was 11.00 a.m.), at t=90, t=95 and t=100min to determine enrichment at the equilibrium state and every 20min from t=120 until t=260min to determine enrichment during the experiment.

At t=0, together with the intravenous infusion of the stable glucose isotope an IG infusion of vehicle (1 ml/h) was started. At t=100 min (i.e., around 12.40 a.m.), the IG infusion of vehicle was changed to Ola or vehicle solution (36 mg/kg/h during 5min and 3 mg/kg/h till the end of the experiment).

At the end of the experiment, animals were sacrificed by an intravenous injection of pentobarbital and trunk blood was collected for Ola measurements. Perirenal and epidydimal fat pads were dissected and weighed.

4. **Laboratory methods/analysis**
Glucose concentration was 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 and corticosterone concentrations were measured using radioimmunoassay kits (Millipore, Billerica, USA for insulin and MP Biomedicals, Orangeburg, USA for corticosterone). Plasma [6,6-2H2]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).

Plasma Ola concentrations were measured by LC/MS/MS coupled to Quattro Premier Xe, using a 7 points calibration curve from 0.2 to 200 ng/ml and 3 quality standards (1 – 10 and
100 ng/ml) (Laboratoires Fournier, Abbott, Daix, France). Based on the results of the untreated animals’ samples, we considered 2 ng/ml as the limit of detection for Ola. The stored information of the iButtons was transferred using a Blue Dot receptor adapter (DS1402D) to a computer and averages of every 15-min interval were calculated. Analysis was performed using iButton Viewer software (Mission Start Delay and One Wire Viewer; Maxim, Dallas, Texas).

**Experiment 2: Locomotor activity and energy balance**

1. **Animals**

16 animals were divided in 2 groups: one received Ola in their drinking water and the other one received Qui.

2. **Food and water intake and locomotor activity**

Food intake, water intake and locomotor activity were assessed individually (TSE drinking & feeding Monitor version 3.26 and TSE InfraMot, TSE systems Bad Homburg, Germany). Locomotor activity was assessed as distance travelled in cm per 15 min. Individual analysis of O₂ consumption (VO₂) and CO₂ production (VCO₂) was done every 15 minutes per cage for 90 seconds (gas analyzers: Magnos 16 and Uras 14; ABB, Frankfurt Main, Germany). Respiratory Exchange Ratio (RER) was calculated according to the formula: VCO₂/VO₂. Energy expenditure (EE) was calculated according to the formula: (CVO₂*VO₂+CVO₂*VCO₂)/1000, where CVO₂ and CVCO₂ were pre-set values (CVO₂=3.941 and CVCO₂=1.106).

3. **Energy status: energy expenditure and respiratory exchange ratio**

The energy status of the animals was assessed during three light/dark cycles on two occasions during the course of the chronic treatment: 2 weeks after the start of the treatment (Week 2) and during the final week of the treatment (Week 5).

**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. In experiment 1, an ANOVA analysis with repeated measures was performed to compare blood glucose levels, EGP, plasma corticosterone and insulin levels and body temperature. This analysis consisted of a within-subjects factor Infusion, with two conditions: basal and experimental and two between-subjects factors: Chronic and Acute. Each between-subject
factor consisted of two conditions: Chronic (Qui or Ola), and Acute (Vehicle or Ola). Post-hoc analysis was performed using T-tests with Bonferroni correction.

In experiment 2 (calorimetric cage data), an ANOVA with repeated measures with two within-subject factors: L/D (for the difference between the light and the dark phase) and Week (for the different experimental sessions in Week 2 and 5) and one between-subjects factor Chronic (with 2 conditions Qui or Ola) was performed. Post-hoc analysis was performed using ANOVAs for separate weeks or L/D phase and T-tests with Bonferroni correction.

RESULTS

Metabolic effects of chronic treatment with Olanzapine (Experiment 1)

1. Food and water intake

Measurements of water intake showed that Ola-treated rats drank less than the controls (Chronic p=0.001, Figure 1A). However, food intake showed no significant difference between the groups (Chronic p=0.579, Figure 1B).

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<th>Experiment 1</th>
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<td>Chronic treatment</td>
<td>Quinine</td>
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<td></td>
<td>221,2±3,1</td>
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<tr>
<td>Body weight at day 0</td>
<td>307,5±7,5</td>
<td>316±5,5</td>
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<td>Body weight at the end</td>
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<td>341±4</td>
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<td>of the experiment (grams)</td>
<td>385,9±11,5</td>
<td>390,3±7,1</td>
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<tr>
<td>Body weight increase</td>
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<td>78,4±5,1</td>
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Table 1: Body weight during chronic treatment with Olanzapine.

2. Plasma olanzapine levels

The drinking behaviour of the Ola animals resulted in a drug consumption of 7.15±0.2 mg/kg/day over the 5 weeks of Ola treatment. Plasma Ola measurements performed at the end of the acute experiment in the 5th week showed higher plasma Ola levels after the acute IG infusion regardless of the chronic treatment, i.e. 1.5±1.0 ng/ml for “Qui+Veh” group and 1.0±0.3 ng/ml for “Ola+Veh” group compared to 1005.1±410.6 ng/ml for “Qui+Ola” group and 1338.9±548.9 for “Ola+Ola” group (Acute p=0.001, Figure 2A). Note that in the animals treated chronically with Ola but acutely with vehicle (i.e., “Ola+Veh”) the nocturnal consumption of Ola in the chronic treatment did not result in detectable drug levels at the end
of the acute experiment (i.e., approximately 8 hours after the last major drinking event). Unfortunately no plasma Ola measurements were possible during the dark phase.

3. Adiposity and leptin levels

Body weight measurements showed that during the 5 weeks animals grew from 221±1 g at day 1 to 355±9 g for the Qui group and 341±4 g for the Ola group at day 31 (Table 1; T-test for the body weight at day 31: p=0.003). A lower body weight for chronically Ola-treated male rats has also been described previously (van der Zwaal et al. 2010; Albaugh et al. 2011; Shobo et al. 2011).
Ola treatment induced a slight and non-significant increase (16%) of epidydimal (0.63±0.04% vs. 0.73±0.05% of body weight; Chronic p=0.172, Figure 2C) and a significant 49% increase of perirenal fat pad weight (0.35±0.02% vs. 0.53±0.05% of body weight; Chronic p=0.011, Figure 2D).

Also plasma leptin levels were elevated after chronic treatment with Ola (Chronic p=0.016, Figure 2B), which is in line with the increased adiposity. No additional effects of the acute Ola treatment on plasma leptin concentrations were detected (Chronic*Acute p=0.738 and Acute p=0.367, Figure 2B).

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Table 1: Body weight during chronic treatment with Olanzapine.

Figure 3: Body temperature over 24-h period. Ola-treated animals show a decreased body temperature especially during the dark phase (Chronic p=0.021). *p<0.05 for post-hoc analysis.
4. Body temperature

Chronic Ola treatment induced a decrease in body temperature compared to the controls (Chronic p=0.021, Figure 3) during most of the dark phase, i.e., when the animals started drinking the solution. After lights on, and most likely the arrest of drinking, the hypothermia was maintained for about one more hour. During the remainder of the light (i.e., sleep) period, body temperature did not differ between control and Ola-treated animals.

Figure 4: Basal EGP assessment. A: The overall analysis of blood glucose levels shows significant effects of Infusion (p=0.001), Chronic (p=0.029) and an almost significant effect for the Infusion*Acute interaction (p=0.055). Post-hoc analysis of the chronic Qui and the chronic Ola groups separately shows that only in the Qui group the interaction effect of Infusion*Acute is significant (p=0.044 vs. p=0.858), with acute Ola infusion increasing glycemia (p=0.026). B: The overall analysis of EGP shows a significant effect of the interaction Infusion*Acute*Chronic (p=0.005). Post-hoc analysis of the chronic Qui and the chronic Ola groups separately shows that only in the Qui group the interaction effect of Infusion*Acute is significant (p=0.005 vs. p=0.243), with acute Ola infusion increasing EGP (p=0.034). C: The overall analysis of plasma corticosterone levels shows significant effects of Infusion (p<0.001), Acute (p=0.001), the interaction Infusion*Acute (p=0.01) and an almost significant effect for Chronic (p=0.066). Post-hoc analysis of the chronic Qui and the chronic Ola groups separately shows that in both groups the effect of Infusion is significant (p=0.015 for Qui and p=0.005 for Ola). For the chronic Qui group, also the effects of Infusion*Acute (p=0.019) and Acute (p=0.011) were significant whereas, for the chronic Ola group, only the overall effect of Acute (p=0.015) was significant. D: The overall analysis of plasma insulin levels shows significant effects of Chronic (p=0.008) and the interaction effect for Acute*Chronic (p=0.03). Post-hoc analysis of the chronic Qui and the chronic Ola groups separately showed no further significant effects. For Figures A, B and C, basal samples (before the Ola treatment) are t=90, t=95 and t=100 and experimental samples (at the end of the Ola treatment) are t=220, t=240 and t=260. For plasma restriction reasons, for Figure D basal samples are t=90 and t=100 and experimental samples are t=180 and t=220. The IG Ola infusion started at t=100. *p<0.05 for ANOVAs, *p=0.05 for post-hoc analysis.
5. **Glucose metabolism**

At the end of the 5th week, an acute experiment was performed to assess the changes in whole-body glucose metabolism consecutive to the chronic treatment with Ola. Without chronic pre-treatment with Ola, the IG infusion of Ola (“Qui+Ola” group) resulted in a significant increase in glycaemia compared to the control group (“Qui+Veh” group) (within the chronic Qui groups: *Infusion*\**Acute* \(p=0.044\) and *Acute* \(p=0.073\), Figure 4A). The chronic treatment with Ola largely dissipated the hyperglycemic effect of the acute Ola treatment (within the chronic Ola groups: *Infusion*\**Acute* \(p=0.723\) and *Acute* \(p=0.858\), Figure 4A). The “Qui+Ola” group also showed a significant increase in EGP between the basal and the experimental state, whereas the “Qui+Veh” group showed a slight decrease. Within the animals treated chronically with Ola, EGP was unchanged in both the Ola- and Veh-treated groups (*Infusion*\**Acute* \(p=0.243\) and *Acute* \(p=0.58\), Figure 4B), indicating that the chronic treatment blunted the increased EGP due to the acute Ola infusion.

The acute administration of Ola had a clear stimulatory effect on plasma corticosterone levels (*Infusion*\**Acute* \(p=0.01\) and *Acute* \(p=0.001\), Figure 4C). When analysed separately, a significant increase of corticosterone was seen after acute Ola in both chronic Qui- \(p=0.022\) and chronic Ola-treated animals \(p=0.012\). In accordance, no significant effects of the chronic pre-treatment per se were noticed.

Chronic treatment with Ola caused a significant increase of plasma insulin levels (*Chronic* \(p=0.008\), Figure 4D), but the acute treatment with Ola had no significant effects. Neither the acute nor the chronic treatment with Ola significantly influenced plasma glucagon concentrations during this experiment (data not shown).

### Chronic Olanzapine-induced changes in energy metabolism (Experiment 2)

1. **Locomotor activity**

Analysis of locomotor activity showed a clear L/D rhythm with a greater distance travelled during the dark phase (L/D \(<0.001\), Figure 5A). During the dark phase of Week 2, Ola-treated animals moved significantly less (L/D*Chronic* \(<0.001\) and post-hoc analysis for Week 2 separately *Chronic* \(p=0.049\), Figure 5A).

2. **Energy expenditure**

For both groups, EE was higher during the dark phase than the light phase (L/D \(<0.001\), Figure 5B), but no significant differences between the groups were found (*Chronic* \(p=0.647\), Figure 5B). Similar results were found after correcting the data for body weight (*Chronic* \(p=0.223\), data not shown).
3. *Respiratory exchange ratio*

Finally, the mean RER was always above 0.85. RER levels were higher during the dark phase as compared to the light phase for both groups (*L/D* *p*<0.001, Figure 5C). RER decreased for both the Qui and Ola groups from Week 2 to Week 5 (*Week* *p*<0.001, Figure 5C). Ola-treated animals showed a significantly higher RER during the dark phase than the controls (*L/D*-*Chronic* *p*<0.001 and post-hoc analysis for only the dark phase *Chronic* *p*=0.008, Figure 5D), indicating that the Ola-treated animals were burning more carbohydrates.

![Graphs](image)

**Figure 5:** Calorimetric measurements during the chronic treatment with Olanzapine. A: All animals were walking a larger distance during the dark phase as compared to the light phase (*L/D* *p*<0.001). Chronic Ola rats showed a lower nocturnal activity than controls during Week 2 (*L/D*-*Chronic* *p*<0.001; *Week*-*Chronic* *p*=0.004; post-hoc analysis for Week 2 *p*=0.014). No other significant differences between the groups were found. B: During the dark phase, EE was significantly higher for both groups (*L/D* *p*<0.001). No further significant differences between the groups were detected. C: During the dark phase, RER was significantly higher for both groups (*L/D* *p*<0.001). Moreover, chronic Ola animals showed a higher RER than the controls during the dark phase (*L/D*-*Chronic* *p*=0.01; post-hoc analysis for dark phase *Chronic* *p*=0.008), this effect was more pronounced in Week 2 than Week 5 (*Week*-*Chronic* *p*=0.029; post-hoc analysis for Week 2 dark phase *p*=0.008). No other significant differences between the groups. D: The RER *L/D* ratio was significantly lower in chronic Ola animals than in controls (*Chronic* *p*<0.001). *p*<0.05 for ANOVAs, *p*<0.05 for post-hoc analysis.
DISCUSSION

Acute Ola administration resulted in hyperglycemia in the chronic-control group, which was partly explained by an increased endogenous glucose production. Interestingly, this acute effect of Ola had disappeared in the chronically Ola-treated animals. On the other hand, chronic Ola treatment resulted in hyperinsulinemia with no changes in glucose concentrations pointing towards insulin resistance as observed previously (Chintoh et al. 2008b). Chronic treatment with Ola for 5 weeks also resulted in increased relative adiposity (i.e. increased perirenal fat pad weights in our study) with increased leptin concentrations. Others also showed the same increased perirenal fat pad weights (van der Zwaal et al. 2010), or only an increase in visceral adiposity (Weston-Green et al. 2012; Raskind et al. 2007) or even an increase in epidydimal, perirenal and subcutaneous fat pad weights (Minet-Ringuet et al. 2006). However, this is the first time that this increase in adiposity is shown to be associated with changes in body substrate utilization; i.e. rats with chronic Ola treatment burned less fat but more carbohydrates compared to controls. Thus, our data suggest that chronic Olanzapine treatment affects both substrate utilization and hepatic glucose metabolism.

Chronic Ola changes energy substrate

Ola-treated animals showed an increase in adiposity without changing their total energy intake or EE. The data from the calorimetric cages indicate that this might be due to the fact that chronic Ola treatment results in a shift of whole body substrate metabolism towards a higher use of carbohydrates and less fat oxidation, i.e. higher RER for the chronic Ola animals during the dark phase. In a previous study, Albaugh et al. (2012) injected rats twice daily with Ola and showed a decreased RER right after the injection, indicating a higher fat oxidation. However, the decline in RER slowly returned to control levels over the course of the dark cycle with a time course consistent with Ola half-life (2.5 hours in rats) (Albaugh et al. 2012). The dose used by Albaugh et al. might be the reason for the different results between our studies: 10 mg/kg per bolus injection (intraperitoneal) for Albaugh et al. and only 7.15 mg/kg orally over 24h in our study. Moreover, in view of the extensive first-pass metabolism of Ola (Mattiuz et al. 1997), the systemic availability of Ola is likely to have been much lower in our study as compared to Albaugh et al.

Chronic Ola causes hypothermia

We observed a decreased body temperature during the dark phase which is in line with previous studies in rodents (Evers et al. 2010; Stefanidis et al. 2009). Stefanidis et al. (2009)
showed that Ola decreases uncoupling protein 1 (UCP1) in brown adipose tissue (BAT), a protein used in mitochondria of BAT to generate heat by non-shivering thermogenesis. This Ola-induced hypothermia was also observed in patients (Blass and Chuen 2004; Hagg et al. 2001; Phan et al. 1998). However, our data indicate that this hypothermia did not result in a significant decrease in EE.

*Chronic Ola blunts the hyperglycemia- and increased EGP-induced by an acute Ola administration*

The hyperglycemic effect of acute Ola is well known (Girault et al. 2012; Chintoh et al. 2008a). However, the hyperglycemia and increased EGP induced by the acute Ola treatment was blunted by the chronic pre-treatment with Ola. A comparable desensitizing effect of chronic Ola was recently reported by Boyda et al. (2012). But despite this apparent favourable effect of the chronic exposure to Ola, another pronounced effect of the chronic treatment with Ola was an increase in basal plasma insulin levels. The increased plasma insulin levels without a similar increase in plasma glucose levels and the tendency to increase basal EGP indicates that the chronic treatment induces insulin resistance, as already extensively documented in the literature (for example (Chintoh et al., 2008b). Thus the absence of an increased EGP after the acute Ola challenge in the chronic Ola group might be explained by the fact that these animals are already insulin resistant and that their insulin sensitivity cannot be reduced any further.

Finally, basal plasma corticosterone levels and acute Ola-induced hypercorticosteronemia is not affected by the chronic treatment, and thus does not seem to be involved in changes in glucose and EGP induced by the chronic treatment.

*Chronic Ola treatment via drinking water: a good model?*

Ola-treated animals showed a decreased body weight compared to the controls. This is not the first time such an observation has been made using male rats (van der Zwaal et al. 2010; Albaugh et al. 2011; Shobo et al. 2011). Females seem to be more prone to body weight gain after the chronic Ola treatment (Davey et al. 2012). However, since the male animals do show changes in adiposity and in order to avoid the influence of oestrus cycle, we decided to use male animals for our study. Moreover, the absence of body weight gain enabled us to show that effects of chronic Ola on insulin sensitivity are not mediated by obesity per se.

In this study, it was of great importance to be able to compare groups of animals with similar patterns of food and water intake in view of the metabolic phenotype expected. Ola treatment via the drinking water decreases water intake due to the bitter taste of the solution. Our
choice to use Qui-enriched water as a control allowed us to compare groups of animals showing a similar reduction in food and water intake. In addition, as humans take Ola orally, another advantage of treatment via the drinking water in our rat model is the comparability with the human situation where Ola is being taken up via the gastrointestinal tract. Moreover, the model also simulates Ola intake during the wake period similar to the human situation. Indeed, plasma Ola levels were below the detection level during the light period in animals treated chronically with Ola, i.e., the nocturnally consumed Ola was completely cleared from the circulation halfway the sleeping phase. Despite this rapid clearance, the long term effects of chronic Ola prove that the dose was sufficient for the purpose of our study.

Ola-induced increased leptin levels with no changes in energy expenditure possibly due to leptin resistance

Our study confirmed that chronic treatment with Ola results in an increased adiposity and leptin concentrations as previously reported for both rodents and humans (Albaugh et al. 2010; van der Zwaal et al. 2010; Haupt et al. 2005; Wathen et al. 2012). Increased levels of leptin are supposed to reduce food intake and increase EE. In our chronic Ola-treated animals, the increase in leptin levels did not seem to have these physiological consequences suggesting that Ola may have induced leptin resistance. At present, it is not known via what mechanism Ola could induce leptin resistance, but several mechanisms have been proposed. Ola may block receptors that interact with the downstream effects of leptin in the hypothalamus. Kim et al. (2007) showed that Ola-induced weight gain involves stimulation of adenosine monophosphate-activated protein kinase (AMPK) in the hypothalamus, which in turn causes an increase in food intake (Kim et al. 2007). Indeed, our data also showed an increased food intake during the dark phase but also a reduced food intake during the light phase resulting in overall similar 24h food intake (data not shown, Experiment 2). Another study using female rats found that Ola treatment decreased food intake during the dark phase (especially the second half) but increased it during the light phase, again resulting in a similar 24h food intake as the controls (Evers et al. 2010). A second possible mechanism was indicated by the experiments of Leinninger et al. as they showed that leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system to suppress feeding (Leinninger et al. 2009). Ola has a high affinity for dopamine receptors (Bymaster et al. 1996); therefore it seems worthwhile investigating the possibility that Ola causes leptin resistance by blocking dopamine receptors.
CONCLUSION

Chronic Ola treatment results in increased adiposity that is not explained by increased energy consumption or decreased EE. The most likely explanation at present is that the increased adiposity is secondary to the shift in substrate utilization as a consequence of insulin and, possibly, leptin resistance. We also showed that chronic treatment with Ola leads to hypothermia and to a desensitization to the acute glucoregulatory effect of the drug. Unfortunately, at present the mechanism by which Ola might induce insulin and/or leptin resistance remains to be clarified.

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Reference List


