The role of hypothalamic pathways in the metabolic side effects of Olanzapine
Girault, E.M.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 4

General discussion
Many atypical antipsychotic drugs exhibit metabolic side effects in humans. Obesity, diabetes mellitus, hypertension, osteoarthritis and lipid abnormalities are the most common threats associated with long-term treatment with atypical antipsychotics (Fertig et al. 1998; Taylor and McAskill 2000; Wetterling 2001; Newcomer 2005). Unraveling the mechanisms underlying these side effects might be advantageous in two ways. First, a better understanding of the mechanisms responsible for this drug-induced increased risk of type 2 diabetes or obesity may lead to new targets or therapies for the treatment of these disorders. Secondly, such research may reveal ways to design new antipsychotic drugs with fewer (metabolic) side effects. One of the atypical antipsychotics well-known for its metabolic side effects is Olanzapine (Ola). But despite its major clinical impact, the mechanisms underlying the etiology of its side effects are still poorly understood. In this thesis, we reported several experiments aimed to determine the mechanism(s) responsible for the metabolic side effects of Ola.

We hypothesized that just like its antipsychotic action also the metabolic side effects of Ola are mediated via the central nervous system. More specifically, we hypothesized that Ola acts, either directly or indirectly, on the pre-autonomic neurons in the hypothalamus that are involved in the control of glucose and/or energy metabolism.

1. Summary of main findings

In Chapter 1, the general introduction, we first describe how energy balance is maintained and investigated the main mechanisms leading to obesity, insulin resistance and diabetes. Then, we give an overview of the pharmacological treatment of schizophrenia, with typical and atypical antipsychotics, focusing specifically on Ola and its metabolic side effects. In Chapter 2, the first experimental chapter, we studied the acute and chronic effects of Ola on glucose and energy metabolism in rats. In the 1st part of this chapter (2.1 and 2.2), we compared the acute effects of peripheral and central Ola administration. Acute intragastric (IG) Ola administration induced hyperglycemia and hepatic as well as extra-hepatic insulin resistance, adverse metabolic side effects also seen in patients. Surprisingly, acute central administration of Ola did not induce any of these metabolic side effects. In the second part of the chapter (2.3), we assessed energy intake, energy expenditure, temperature, glucose metabolism and plasma Ola levels in rats treated chronically (5 weeks) with Ola via drinking water. In these animals, basal insulin levels were increased whereas glycemia was unchanged, which points towards insulin resistance. In addition, chronic treatment induced hypothermia and desensitization to the acute glucoregulatory effects of the drug upon IG
treatment, i.e. hyperglycemia and increased endogenous glucose production (EGP). The chronic treatment also resulted in increased adiposity with unaltered body weight, energy intake and energy expenditure. Our calorimetric study indicated that the increased adiposity was associated with a shift in substrate utilization, mainly from lipids to carbohydrates, as a consequence of insulin and possibly leptin resistance. The main effects of Ola administration are summarized in Figure 1.

In Chapter 2.1 and 2.3, we focus on the effects of peripheral administration of Ola, since, as described in 2.1, we found no gluco regulatory effects of intracerebroventricular (ICV) administered Ola. However, Martins et al. showed that Ola administered ICV led to hepatic insulin resistance (Martins et al. 2010). As plasma Ola measurements in our study (Chapter 2.2) showed traces of Ola in the plasma after ICV administration, we concluded that the changes seen by Martins et al., using a 10-fold higher ICV dose, may well be due to a leakage of Ola from the ventricle compartment to the periphery. However, the apparent primary site of action in the periphery does not exclude an involvement of the central nervous system in the metabolic side effects of Ola. For instance, the peripheral action of Ola can

---

Figure 1: Main effects of Ola administration. Straight line = IG or drinking water administration; dashed line = ICV administration; dotted lines = Ola absorbed from the gastro-intestinal tract and travelling through the general circulation to the brain and other tissues.
induce an afferent signal that is transmitted to the central nervous system. This was also indicated by the results of Stefanidis et al. who showed that peripheral administration of Ola activates neurons in the hypothalamus (Stefanidis et al. 2009). Ola could also penetrate the brain from the blood stream. Indeed, as described in chapter 2.2, high levels of Ola were found in the brain after IG Ola infusion. Orexin and melanin-concentrating hormone (MCH) are two hypothalamic orexigenic peptides. In Chapter 3, we investigate the possible implication of orexin and MCH in the metabolic effects of IG administered Ola. ICV orexin-receptor-1 (OxR1) antagonist administration was able to block the increased EGP induced by IG Ola administration, but not its hyperglycemic effect (Chapter 3.2; Figure 2).

The stimulatory effect of Ola on EGP did not seem to be mediated solely by an orexin-induced increased release of glucagon, as no differences between OxR1 antagonist-treated and vehicle-treated animals were observed in their plasma glucagon response. Therefore, it is most likely that the ICV OxR1 treatment blocks an Ola-induced stimulatory effect of orexin on the sympathetic input to the liver. Prepro-MCH knock-out (PMCH KO) rats were used to study the possible involvement of the MCH system in the Ola-induced changes in glucose metabolism. The crosses represent the changes occurring when administering OxR1 antagonist ICV.

Figure 2: Hypothalamic orexin, but not MCH, system is involved in Ola-induced effects on glucose metabolism. The crosses represent the changes occurring when administering OxR1 antagonist ICV.
metabolism. IGF Ola administration induced increased glucose levels, EGP and corticosterone levels in both wild-type and PMCH KO rats. The absence of hypothalamic MCH expression did not have a significant effect on the Ola-induced changes in glucose metabolism. However, since the PMCH KO rats (vs. wild-type (WT) rats) did not show an increase in insulin levels after Ola treatment, we hypothesized that the Ola-induced hyperinsulinemia in WT animals is mediated via a MCH-dependent mechanism, probably within the pancreas.

2. Pharmacological aspects of Olanzapine-induced metabolic side effects

A definitive cause of schizophrenia has not been established yet, but a prominent hypothesis regarding its pathogenesis involves excess neurotransmission at the dopamine (DA) receptor. This hypothesis emerged from the discovery of antipsychotic drugs in 1952 (Delay et al. 1952) and the work of Carlsson and Lindqvist who identified that these drugs increased degradation of DA when administered to animals (Carlsson and Lindqvist 1963). In line with this hypothesis, reserpine, another drug effective for treating psychosis, blocked the reuptake of DA and other monoamines leading to their dissipation (Carlsson et al. 1957). However, the launch of atypical antipsychotics in 1990s questioned the DA theory as the primary cause of schizophrenia, since those drugs, which had a much lower affinity for DA receptors, were found to be just as effective as classic typical antipsychotic drugs in controlling psychosis, and even more effective in controlling the negative symptoms. So even if DA plays an important role in the development of schizophrenia, other neurotransmitters might also play a pivotal role in its pathogenesis.

Yoon et al. studied the involvement of the DA system in the metabolic side effects of three atypical antipsychotics, Ola, ziprasidone and risperidone. They showed that all three drugs induced decreased body weight, food intake, body fat mass and locomotor activity when administered to WT male mice which are unexpected results in view of Ola-induced metabolic side effects in humans (Yoon et al. 2010). However, when administered to DA D₂ receptor (D₂R) KO mice, only animals treated with ziprasidone and risperidone exhibited those changes, suggesting that the dominant effect of Ola on metabolic regulation may be associated with DA D₂R signaling (Yoon et al. 2010). Indeed, Ola acts as a D₂R antagonist (Bymaster et al. 1996; Moore et al. 1992). D₂R KO mice exhibit an impaired insulin response to glucose overload, high fasting blood glucose levels, glucose intolerance and possess a reduced β-cell mass at 7 months of age (Garcia-Tornadu et al. 2010). In addition, Li et al. showed that administration of Ola to the medial prefrontal cortex (mPFC) induced an increased mPFC DA efflux that could be blocked by a systemic injection of a serotonin 5-
HT₂₅-receptor antagonist, WAY-100635, but not by telenzepine, a preferential M₁-receptor antagonist (Li et al. 2009). This shows that 5-HT₁₅-receptor antagonism, WAY-100635, but not M₁-receptors in the mPFC are involved in cortical DA efflux induced by Ola (Li et al. 2009). Furthermore, the study of Kirk et al., using a female rat model, suggested that 5-HT₂C-receptor antagonism, perhaps with inverse agonism, and concurrent D₂R antagonism may be the underlying cause of Ola-induced weight gain (Kirk et al. 2009). Other studies demonstrated that chronic administration of atypical antipsychotics causes desensitization and down regulation of central 5-HT₁₅R signaling (Singh et al. 2010). Moreover, Muma et al. showed that 7 days of treatment with Ola decreases serotonin-stimulated phospholipase C (PLC) activity in rat frontal cortex (Muma et al. 2007). Ola is also a potent H₁-receptor antagonist (Richelson and Souder 2000). As mentioned already in Chapter 1, acute (1-week) and chronic (12-weeks) Ola treatment significantly down-regulated H₁-receptor mRNA expression in the hypothalamic arcuate (Arc) and ventromedial nucleus (VMH). Haloperidol or aripiprazole, antipsychotics with a lower risk of weight gain side-effect did not have these effects (Han et al. 2008). In addition, Ola decreased H₁-receptor binding density in the VMH. This altered H₁ signaling was accompanied by an increase in food intake and weight gain in Ola-treated rats compared to those treated with aripiprazole or haloperidol (Han et al. 2008). Coinciding with these findings, a study by Kim et al. (2007) found that Ola activates hypothalamic 5'-adenosine monophosphate-activated protein kinase (AMPK), which increases food intake and weight gain (Minokoshi et al., 2004), via H₁-receptor antagonism. These findings suggest that a possible mechanism for Ola-induced weight gain is through a drug-induced decrease in the hypothalamic expression of the H₁-receptor, blockade of which is linked to downstream AMPK activation, resulting in increased food intake that, when coupled with an insufficient increase in locomotor activity, contributes to weight gain (Kim et al. 2007; Han et al. 2008). In conclusion, the metabolic side effects of Ola can be linked to its action on different types of receptors.

### 3. Olanzapine and impaired mobilization of stored fuel

As demonstrated in Chapter 2.3 and also by Albaugh et al. (2010), impaired mobilization of stored fuels may be a factor contributing to the increased adiposity observed during chronic Ola administration. In a physiological state, stored adipose triglycerides are mobilized through lipolysis during the post-absorptive state to conserve glucose as a fuel for the brain. In 14h food-restricted rats, Ola administration blunted the increase in plasma FFA and
glycerol, suggesting lipolytic impairment (Albaugh et al. 2012). This decreased lipolysis could be due to a decreased sympathetic tone. The lowered plasma FFA levels resulted both from impaired lipolysis and accelerated fat oxidation (Albaugh et al. 2011). Especially decreased lipolysis would contribute to the increased fat accumulation we observe after chronic Ola treatment (Chapter 2.3). Vestri et al. also reported stimulatory effects of Ola on lipogenesis and inhibitory effects on lipolysis (Vestri et al. 2007). These effects are consistent with the results from Albaugh et al. (2012) and help to understand the Ola-induced increased adiposity as reported previously.

4. Olanzapine metabolic side effects and the insulin receptor cascade

Mondelli et al. showed that the acute administration of Ola has a direct effect on the hepatic insulin signaling pathway, with a reduction of insulin receptor substrate-2 (IRS-2) levels, reduced phosphorylation of glycogen synthase kinase 3-Ser21 (GSK-3α-Ser21) and increased phosphorylation of GSK-3β-Ser9 (Mondelli et al. 2013). These effects appeared in the absence of an effect on body weight or visceral adipose tissue deposition. The decrease in IRS-2 suggests that Ola-induced changes in glucose metabolism are caused by an inhibition of the insulin signaling cascade. These data on hepatic insulin resistance are in line with ours showing a reduced insulin-induced inhibition of EGP, a decreased insulin-stimulated glucose uptake and a reduced liver glycogen storage in rats treated with Ola (Chapter 2.1.). Moreover in Chapter 2.3, we showed that chronic administration of Ola leads to increased insulin levels while basal blood glucose levels are unchanged which points towards insulin resistance. Chintoh et al. showed that, indeed, chronic Ola treatment leads to both hepatic and extra-hepatic insulin resistance by diminishing the ability of insulin to facilitate uptake of glucose into liver and muscle cells (Chintoh et al. 2008).

5. The role of the hypothalamus

The rate of hepatic glucose production depends on the activities of unidirectional enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase). PEPCK catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, which is an important step of gluconeogenesis. G-6-Pase catalyzes the production of free glucose from glucose-6-phosphate, which is the final step in both gluconeogenesis and glycogenolysis. Ikegami et al. showed that ICV Ola administration leads to increased mRNA levels of G-6-Pase in the liver (Ikegami et al. 2013). Previous studies have indicated that the
overexpression of G-6-Pase increases glucose production, and causes hyperglycemia and glucose intolerance (Trinh et al. 1998). Similarly, Martins et al. also showed increased G-6-Pase, but also PEPCK gene expression levels after intravenous and ICV Ola treatment (Martins et al. 2010). Together these findings strongly suggest that Ola accelerates glucose production by increasing G-6-Pase and PEPCK activity. Furthermore, Ikegami et al. also demonstrated that ICV Ola activates AMPK in the hypothalamus of non-fasted mice but not of fasted mice (Ikegami et al. 2013). They propose that this activated AMPK in the hypothalamus can, in turn, stimulate the sympathetic nervous system, and subsequently increase G-6-Pase levels via hepatic β-adrenergic receptors thus inducing hyperglycemia. Of note, unlike in the study of Martins et al., the dose of Ola injected in the study of Ikegami et al. is about 10-fold lower than that is in our study and thus less likely to result in a leakage to the periphery. The main difference between ICV experiments in this study compared to the study of Martins et al. is the fasting state of the animals. Ikegami et al. showed that ICV Ola induces changes only in non-fasted animals and not in fasted animals. On the other hand, in our study, all animals were food restricted which might explain the absence of effect.

When injected centrally MCH, orexin, as well as neuropeptide Y (NPY), increase food intake. Sahu et al. showed that orexin-A and NPY administration have a synergistic action in the rat hypothalamus in stimulating feeding, but that orexin-A and MCH have no interaction in the regulation of food intake and that NPY and MCH have very little synergistic effect on feeding (Sahu 2002). Although MCH and orexin neurons are located in the same brain region, i.e., the lateral hypothalamus (LH), and both project to several hypothalamic areas implicated in feeding and body weight regulation, the action of those two neuropeptides on feeding seems to be independent of each other. This suggests that their action on feeding behaviour is via two distinct mechanisms. Ola administration results in the activation of neurons in the LH that are orexin- but not MCH-positive (Wallingford et al. 2008). Since part of Ola metabolic side effects are blunted by a blockade of the orexin system but not by the absence of MCH expression, it will be of great interest to investigate further the activity of the orexin pathway under Ola treatment. Interestingly, adult MCH-KO animals show an increased hypothalamic orexin mRNA expression (Mul et al. 2010).

It has been shown that subchronic treatment with Ola decreases the expression of proopiomelanocortin (POMC)/cocaine- and amphetamine-related transcripts (CART) mRNA and increases the expression of NPY/ agouti-related peptide (AgRP) mRNA expression in the arcuate nucleus (Arc) (Ferno et al. 2011). The increased NPY/AgRP activity in turn may
increase orexin expression in the LH, but could also act independently from orexin to increase EGP (Bruinstroop et al. 2012). Decreased POMC/CART and increased NPY/AgRP mRNA expression levels seen after Ola treatment are also observed when ghrelin is administered centrally (Gao et al. 2013). In fact, some animal studies report increased levels of plasma ghrelin after Ola treatment (Weston-Green et al. 2012; van der Zwaal et al. 2012). On the contrary, clinical studies report no changes (Smith et al. 2012) or even decreased levels of ghrelin (Stip et al. 2012; Tanaka et al. 2008). More investigations will be necessary to rule out or establish a possible role of ghrelin in the Ola-induced metabolic side effects, perhaps by the use of ghrelin or ghrelin receptor KO animals. Finally, an increase in orexin levels in the LH was also shown after acute peripheral Ola administration (Fadel et al. 2002). Stefanidis et al. showed that neurons containing orexin were activated after an acute Ola treatment (Stefanidis et al. 2009). This is in line with our findings, wherein administration of an OxR1 antagonist blunts the increased EGP induced by Ola (Chapter 3.2). Fadel et al. showed that a non-orexin set of neurons were also activated in the LH area. More importantly, acute treatment with Ola leads to activation of several other hypothalamic nuclei, amongst those are the Arc, the dorsomedial nucleus of the hypothalamus (DMH) and the paraventricular nucleus of the hypothalamus (PVN) (Stefanidis et al. 2009). Identification of the neurotransmission system in those neurons would help to determine further the hypothalamic mechanism of Ola metabolic side effects. In the light of those evidences, it seems clear that the hypothalamus is involved in the metabolic side effects of Ola; however, the exact pathways need further investigation.

In Figure 3, we summarize the information gathered thus far. Ola-increased orexin levels may be caused by a direct action of Ola on the LH, via an activation of NPY/AgRP neurons in the Arc or via a signal from the brainstem. Once activated, orexin neurons can signal to the metabolic organs via the sympathetic nervous system. It is known that orexin plays a key role in maintaining hypothalamic and peripheral insulin sensitivity. Shen et al. showed that low dose ICV administration of orexin-A decreased the activity of autonomic nerves innervating white adipose tissue, thus orexin might affect lipolysis and leptin release via the autonomic nervous system (Shen et al. 2008). In Chapter 3.2., we demonstrate that ICV OxR1 antagonist administration prevented the stimulatory effect of Ola on EGP. Data from our group showed that ICV orexin-A administration increases blood glucose concentration through an increased hepatic EGP, which can be blocked by a sympathetic – but not a parasympathetic- hepatic denervation (Yi et al. 2009).
Moreover, via its action in the medial hypothalamus, orexin also stimulated glucose uptake in skeletal muscle, mediated via a sympathetic nervous system (Shiuchi et al. 2009). Thus Ola-induced changes in energy metabolism could involve the hypothalamic orexin- and subsequently the sympathetic nervous system. Ola is also known to affect substrate
utilization and body fat gain. It is thus plausible that the hypothalamic system might be involved in those changes (Joly-Amado et al. 2012). Ola administration affects ghrelin and leptin levels, these hormones have physiologically antagonizing effect on POMC/CART and NPY/AgRP neurons in the Arc, and lead to increased and decreased food intake respectively and have reverse effects on energy expenditure.

Some of the neurotransmitters involved in the metabolic side effects of Ola, cited in section 2, are also modulating the activity of orexin neurons. Although orexin neurons do not have DA receptors, DA does inhibit orexin neurons by acting on \( \alpha_2 \)-adrenoreceptors (Yamanaka et al. 2003; Yamanaka et al. 2006). Serotonin is also known to send inhibitory projections to orexin neurons (Tsujino and Sakurai 2009). Thus it is important to keep in consideration that Ola can modulate the activity of orexin neurons via its action on those neurotransmitter systems as well.

6. Technical difficulties and justification of the animal model

Generally, animal models are used to perform invasive experiments that, for ethical reasons, cannot be performed in healthy volunteers or patients. However, those models have certain limits and one should always keep a critical eye on the results obtained using animal models.

One of the main challenges in these experiments was to deal with the major difference in Ola metabolism between humans and rodents, i.e. the plasma half-life time of Ola in humans is 21-54 hours whereas in rodents it is only 2.5 hours (Aravagiri et al. 1999). As described in chapter 2.2., there are a few possibilities to circumvent this problem in animal studies, i.e. usage of implantable osmotic minipumps or administration via drinking water. However, each approach will have secondary effects that must be taken into account. In chapter 2.2, we also discussed that central administration of Ola is problematic, due to poor penetration in the brain tissue and leakage to the periphery. Furthermore, Ola clearance has been shown to be 25% lower in women than in men and also age has a significant impact (Callaghan et al. 1999). A major sex difference is also seen regarding the effects of Ola treatment in rodents. Contrary to humans, Ola administration in rodents causes a significant body weight gain, albeit only in females (Davey et al. 2012). However, both genders display an increased adiposity upon chronic treatment. The less pronounced effects of Ola on body weight in male animals allowed us to study the Ola effects on glucose metabolism independent of changes in body mass.
Whilst studying the metabolic side effects of Ola, it is also important to consider the background of the patients experiencing side effects possibly explaining in part why patients suffering from schizophrenia are prone to obesity. For instance, symptoms such as apathy and social withdrawal will contribute to the lack of adherence to a proper diet and stimulate an overall sedentary lifestyle (Davidson 2002). However, Ola has been shown to result in increased body weight gain in both schizophrenic patients and healthy volunteers (Sacher et al. 2008), as well as in patients suffering from acute manic episodes. Thus, body weight gain after Ola treatment cannot be attributed solely to the patients’ background and symptoms.

In this thesis, we showed that both acute and chronic administration of Ola lead to metabolic side effects with regards to glucose metabolism. We found that the long-term exposure to the drug modifies its effect during an acute challenge. Ola has affinity for several receptors and an extensive literature concerning the involvement of those receptors is available. We decided to focus on probable hypothalamic mechanisms because this brain structure plays a key role both in body weight regulation and glucose metabolism. The results in this thesis indicate that the metabolic side effects of Ola are partly mediated by the hypothalamic orexin system. The mechanisms by which Ola affects this system remain unclear but several mechanisms have been proposed in this discussion. Ola might influence the orexin neurons in the LH directly, via the POMC/CART and NPY/AgRP neurons in the Arc or even via the brainstem (Figure 3). In conclusion, lowering OxR1 activity seems to be efficient in reducing the glucoregulatory effects of Ola in rat models and, by inference seems to be an attractive therapeutic target. However, the orexin system is involved in various functions including sleep regulation, and an OxR1 antagonist concomitantly administered with Ola might lead to unwanted effects in patients including disturbed vigilance, therefore use of OxR1 antagonist merits further research.


