The role of hypothalamic pathways in the metabolic side effects of Olanzapine
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Citation for published version (APA):
Girault, E. M. (2013). The role of hypothalamic pathways in the metabolic side effects of Olanzapine 's-Hertogenbosch: Boxpress

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Chapter 3.1
Orexin, MCH and energy metabolism

Adapted from:
INTRODUCTION

In the previous chapter, we focused on the effect of peripheral administration of Olanzapine (Ola) on glucose metabolism and insulin sensitivity in rats. Ola, however, also influences feeding behavior, locomotor activity and body weight in rats (Evers et al. 2010; van der Zwaal et al. 2010). Also, in patients, major weight gain has been described as one of the metabolic side effects (Sacher et al. 2008) and there are also reports on Ola inducing increased caloric intake (Gothelf et al. 2002). These Ola-induced behavioral effects imply a role for the brain in Ola-induced changes in energy metabolism. The lateral hypothalamus (LH) is an interesting candidate for these effects of Ola as it has been implicated in arousal, feeding and other motivated behaviors (Sakurai et al. 1998). Moreover it is also an important area for the regulation of glucose metabolism. Two neuropeptides orexin (Ox) and melanin-concentrating hormone (MCH) are expressed abundantly in the LH and known to be involved in the control of feeding behavior. Thus, Ox and MCH may be involved in Ola-induced metabolic side effects.

THE OREXIN SYSTEM

In the late 1990’s, screening of high-resolution high-performance liquid chromatography (HPLC) fractions in a functional assay using multiple cells expressing individual orphan G-protein-coupled receptors resulted in the isolation of two peptides, named Ox-A and Ox-B (Sakurai et al. 1998). Although currently best known for their effects on sleep and arousal, the Ox peptides were first identified as regulators of feeding behavior in 1998, which explains their name (orexis is the Greek word for appetite) (Sakurai et al. 1998). In the same year, an analysis of mRNAs that were restricted to or enriched in the rat hypothalamus, resulted in the isolation of a nucleotide sequence encoding a 130 residue protein called prepro-hypocretin (HCRT). Sequence analysis indicated that prepro-HCRT yields two peptides, HCRT-1 and HCRT-2 (deLecea et al. 1998). Chemical analyses subsequently revealed that HCRT-1 and HCRT-2 were identical to Ox-A and Ox-B.

The neuropeptides Ox-A and Ox-B are expressed by a specific population of neurons in the LH area (Elias et al. 1998). Ox-A and Ox-B are derived from a precursor peptide, the product of the prepro-Ox gene (Sakurai et al. 1998). Two G-protein-coupled receptors, the Ox-receptor type 1 (OxR1) and type 2 (OxR2), respond to Ox (Sakurai et al. 1998). OxR1 and OxR2 are distributed widely but differentially throughout the brain (Vanitallie 2006).
Narcolepsy is a chronic sleep disorder characterized by disrupted nocturnal sleep, excessive daytime sleepiness and symptoms of dissociated rapid eye movement sleep. Studies showing that Ox KO mice presented a narcoleptic phenotype (Chemelli et al. 1999) led to the investigation of Ox levels in narcoleptic patients. Interestingly, patients affected by narcolepsy appeared to show low or undetectable levels of Ox in their cerebrospinal fluid (Nishino et al. 2000).

Antagonism of OxR1 is characterized by reduced food intake and weight reduction in rodents (Smart et al. 2002). Despite the reduced food intake, the narcoleptic patients with Ox deficiency and the animal model with genetic ablation of the Ox neurons tend to be obese (Hara et al. 2001; Kok et al. 2003). These findings indicate that the link between Ox and energy homeostasis involves an effect of Ox on appetite (Cai et al. 2001; Sakurai et al. 1998), as well as additional mechanisms implicated in the control of energy metabolism, such as energy expenditure. Indeed, when Ox is administered centrally in rodents, they are reported to increase not only arousal and food intake but also blood glucose levels, sympathetic tone, plasma corticosterone levels, metabolic rate, and locomotor activity (Vanitallie 2006; Yamanaka et al. 2003).

Ox-A regulates plasma glucose concentrations via central and peripheral mechanisms (Nowak et al. 2000). Data from our own group clearly showed that increased availability of Ox in the central nervous system, either by ICV infusion or by local activation via removal of GABA inhibition, increases plasma glucose concentrations through an increase in hepatic glucose production (EGP). The stimulatory effect of Ox on EGP is blocked by a hepatic sympathetic, but not parasympathetic, denervation (Yi et al. 2009).

**THE MELANIN-CONCENTRATING HORMONE SYSTEM**

In 1983, the MCH peptide was discovered as a 17 amino-acid cyclic peptide in the pituitary of the chum salmon that regulates the aggregation of melanosomes in the skin, thus lightning skin color, in response to stress and other environmental stimuli (Kawauchi et al. 1983). Processing of prepro-MCH results in the production of 3 neuropeptides: neuropeptide glycine-glutamic acid (N-GE), neuropeptide glutamic acid-isoleucine (N-EI) and MCH (Saito and Maruyama 2006). In rodents, MCH binds only to MCH-receptor 1 (MCHR1), whereas humans also express a second MCH receptor, MCHR2 (Sailer et al. 2001). MCHR1 is a G-protein coupled receptor expressed throughout the brain (Chambers et al. 1999; Lembo et al. 1999; Saito et al. 1999; Saito et al. 2001). Peripheral prepro-MCH expression has been observed in stomach, intestines and testis (Hervieu and Nahon 1995), whereas peripheral
MCHR1 expression is present in many tissues including stomach, eye, adipocytes, pituitary, heart, kidney, ovaries, skeletal muscle and tongue (Kolakowski, Jr. et al. 1996; Saito et al. 1999; Saito et al. 2000; Bradley et al. 2002). Mature and immature RNAs were observed in rat brain, thymus, pancreas and adrenal gland (Hervieu and Nahon 1995). Magnocellular neurons expressing prepro-MCH are predominantly present in the LH and the zona incerta, and project to many brain regions including the olfactory bulb, the prefrontal cortex, the striatum, and hindbrain nuclei including the nucleus of the tractus solitarius and the parabrachial nucleus (Bittencourt et al. 1992; Sita et al. 2007).

Prepro-MCH mRNA is up-regulated after fasting or leptin deficiency (Qu et al. 1996; Kokkotou et al. 2001), while intracerebroventricular (ICV) injections of MCH increase food intake and body weight (Rossi et al. 1997; Della-Zuana et al. 2002; Gomori et al. 2003; Ito et al. 2003; Guesdon et al. 2009). Deletion of prepro-MCH in rats resulted in decreased food intake, fat mass, leptin levels and energy expenditure (Mul et al. 2010). Likewise, prepro-MCH KO mice are lean due to decreased food intake and increased metabolic rate (Shimada et al. 1998; Kokkotou et al. 2005), while overexpression of MCH in mice causes obesity (Ludwig et al. 2001). MCHR1 antagonism decreases food intake and weight gain in adult rodents (Shearman et al. 2003; Mashiko et al. 2005; Palani et al. 2005; Handlon and Zhou 2006; Luthin 2007).

Kong et al. genetically altered the glucose excitability of MCH neurons (decreasing it by expressing ATP-resistant K_{ATP} channels and increasing it by deleting uncoupling protein 2 (UCP2), which is expected to increase glucose-stimulated ATP production). They showed that glucose sensing by MCH neurons (either decreased or increased) impacts on peripheral glucose homeostasis. They concluded that MCH neurons regulate glucose homeostasis and that a combination of glucose-excited neurons (MCH neurons, POMC neurons, and possibly other glucose-excited neurons) are likely to play a key role in the maintenance of a euglycemic state (Kong et al. 2010).

OREXIN AND MCH: COMPLEMENTARY SYSTEMS OF THE HYPOTHALAMUS

Ox neurons are intermingled with MCH neurons in the same region of the LH but represent a distinct neuronal population as no co-localization has been found (Sakurai 2005). Ox and MCH neurons also display opposite functions regarding their firing pattern. Specifically, Ox neurons discharge during waking (Wake-On/Sleep-Off) (Lee et al. 2005), whereas MCH
neurons do the opposite: Wake-Off/Sleep-On (Hassani et al. 2009). It is also known that there is a reciprocal innervation between Ox and MCH neurons and that MCH attenuates the efficacy of glutamatergic synapses on the Ox neurons (Rao et al. 2008). Blocking GABAergic activity in the LH will activate Ox neurons, but not the MCH neurons (Alam et al. 2005; Yi et al. 2009). The two groups of neurons thus appear to fulfill complementary roles in various hypothalamic functions (Tsuneki et al. 2010). In the mouse LH, Ox neurons are of the glucose inhibited type, whereas MCH neurons are of the glucose-excitied type.

![Diagram of orexin system](image)

**Figure 1: Arousing the arousal system.** Two mechanisms for activating the orexin system are shown: (left) via an arrest of the inhibitory GABAergic input from the SGN during the active phase and (right) via an activation of the NPY-containing projections from the Arc NPY system by for instance food or sleep deprivation. LC: locus coeruleus, TMN: tuberomamillary nucleus, NTS: nucleus of the tractus solitarius, DMV: dorsal motor nucleus of the vagus, RVLM: rostroventrolateral medulla, LHA: lateral hypothalamic area, PeF: perifornical area, NPY: neuropeptide Y, AgRP: agouti-related peptide, POMC: pro-opiomelanocortin, CART: cocaine and amphetamine-regulated transcript, BAT: brown adipose tissue.)
Both Ox- and MCH-neurons receive synaptic input from neurons in the arcuate nucleus (Arc). Within the Arc, two populations of neurons, POMC/CART (pro-opiomelanocortin/cocaine- and amphetamine-related transcript) and NPY/AgRP (neuropeptide Y/agouti-related peptide), convey their information to second-order neurons in the paraventricular nucleus of the hypothalamus (PVN) and LH, such as the corticotropin-releasing hormone (CRH), thyrotropin releasing hormone (TRH), oxytocin, Ox and MCH neurons (Figure 1) (Elias et al. 1998). ICV injection of AgRP, the endogenous antagonist for the melanocortin 3 and 4 receptors, results in the activation of Ox neurons, but not of MCH and NPY neurons (Zheng et al. 2002). NPY profoundly increases feeding behavior after central administration, the most sensitive area being the perifornical area (PeF) (Stanley and Thomas 1993) that contains a dense population of Ox and MCH neurons. Indeed, NPY neurons in the Arc have been shown to project to the LH (Backberg et al. 2002). mRNA levels of prepro-Ox, the Ox precursor, were higher during 24–96 h of sleep deprivation, while MCH was unchanged. NPY neurons thus seem to activate Ox neurons during sleep deprivation. On the other hand, the Arc also receives projections from the LH, where Ox and MCH neurons reside. Thus an increased orexinergic activity during sleep deprivation might also activate arcuate NPY neurons, resulting in hyperphagia independent from endocrine and metabolic cues from the periphery (Yamanaka et al. 2000).

Leptin is a hormone secreted by adipose tissue (Zhang et al. 1994); its role is to inform the brain about the energy status of the adipose tissue so that appropriate changes in appetite, metabolism and nutrient partitioning can be initiated by the hypothalamus (Zhang et al. 1994). Increased levels of leptin reduce food intake and increase energy expenditure. A functional link between leptin and Ox is indicated by observations in ob/ob mice, which are leptin deficient and show a lower expression of Ox than wild-type mice. If ob/ob mice are treated with leptin, the expression of Ox is increased (Yamanaka et al. 2003). Leptin inhibits activation of NPY/AgRP neurons and thus, conversely, absence of leptin will increase not only NPY levels but also Ox neuronal firing, and thereby Ox release.

Since it has been demonstrated that peripheral injections of Olanzapine result in a strong activation of neurons in the LH, in the next two chapters we investigated the possible involvement of these two complementary neuropeptide systems on the metabolic side effects of Olanzapine.


hypothalamic MCH neurons involves K(ATP) channels, is modulated by UCP2, and regulates peripheral glucose homeostasis. *Cell Metab* 12: 545-552.


Metabolism 55: S30-S35.


