Chapter 7

General discussion
Obesity has now reached epidemic proportions globally and has become a worldwide public health problem. It can lead to several chronic diseases, including cardiovascular disease and type 2 diabetes mellitus. The problem of obesity arises when food intake exceeds energy expenditure. A complex system has evolved to regulate food intake and energy homeostasis, but this is biased towards weight gain. Several peripheral signals act within the central nervous system to give information about short-term and long-term energy stores. The integration of this multitude of signals occurs in the hypothalamus, which contains a large number of neuropeptides that influence food intake.

The general hypothesis that is used throughout this thesis is, that the neuropeptides of this hypothalamic regulatory site of food intake and energy homeostasis are not only involved in regulation of food intake, but are also regulating insulin sensitivity, independently of the effects on food intake and body weight. Therefore, the aim of the studies described in this thesis was to investigate the effects of some of these neuropeptides and of some of the peripheral signals, which affect these neuropeptides, on insulin action.

All experiments described in this thesis were performed in mice. Usually wild-type (C57BL/6) mice were used, except for chapter 6, in which ob/ob mice were used as well. Sometimes a high fat diet was used to induce insulin resistance. All experiments had a similar set-up consisting of administration of a certain hypothalamic neuropeptide or compound/hormone which can affect the hypothalamic regulatory center of food intake. Subsequently, insulin sensitivity was measured by a hyperinsulinemic euglycemic clamp technique. Mice were clamped in the fasted or fed state, depending on the (an)orexigenic nature of the administered neuropeptide/hormone. To ensure low physiological levels of the used neuropeptide/hormone, mice were clamped in the fed state for orexigenic agents and the fasted state for the anorexigenic agents. This way we could artificially raise the neuropeptide/hormone of our interest in the experimental group and evaluate the results against the low levels in the control group.

Using this method, the effects of an icv infusion of the neuropeptide NPY on insulin sensitivity were studied in chapter 2. The results of this chapter show, that NPY can cause insulin resistance, specifically in the liver. Insulin-mediated glucose disposal was not affected by NPY, implying that organs like adipose tissue and muscle, were not affected.
The effect of the POMC pathway (the counter-regulating pathway of NPY) was studied in chapter 3 with the administration of MTII, an agonist of the POMC pathway. The results of that chapter show, that the POMC pathway can improve insulin-mediated glucose disposal and does not affect hepatic insulin sensitivity. Therefore, both pathways are not completely opposing each other’s effects, but seem to have a different tissue-specific effect.

In chapters 4 and 5 the results on insulin sensitivity are shown for the acute (chapter 4) or chronic (chapter 5) administration of the gut hormone PYY. Both chapters show, that PYY improves insulin sensitivity with respect to insulin mediated glucose disposal and there seems to be a tendency towards an improved hepatic insulin sensitivity as well.

Finally, in chapter 6, the role of central leptin signalling on insulin sensitivity is examined in ob/ob mice and evaluated against the contribution of the obese phenotype itself on insulin sensitivity. The results show that both the obese phenotype and the lack of leptin signals in the brain, per se, contribute to the insulin resistance of ob/ob mice.

As mentioned before, the general hypothesis, that is used as the basis for the experiments described in this thesis, is that hypothalamic neuropeptides regulate insulin sensitivity, in addition to and independently of their role in regulating food intake. However, it is important to remember that other brain regions also play an important role in the regulation of food intake. Especially the brainstem, with the nucleus of the solitary tract in particular, but also parts of the limbic system, the amygdala and the cerebral cortex play a role. The results described in this thesis are in keeping with the hypothalamic model that we used, but it is important to keep in mind that they don’t prove the involvement of the hypothalamus. NPY, by example, is one of the most abundant neurotransmitters of the brain and its receptors are widely distributed throughout the brain. MTII, which is used in chapter 3, is an agonist of the MC 3 and 4 receptors. Although the MC3 receptor is mainly expressed in the hypothalamus, the MC4 receptor is expressed in virtually all major brain regions, including the brainstem. Similarly, the leptin receptor is also expressed in the brainstem. The icv infusion/injection that we used in our experiments could therefore affect parts of the brainstem as well, since the brainstem also has easy access to the csf via the fourth ventricle. Therefore, to evaluate the role of the
hypothesised, additional experiments should be done with intra-hypothalamic
injections.

The downstream mechanism via which the neuropeptides/hormones
described in this thesis affect (insulin mediated) glucose turnover remains to be
elucidated. First of all, the effects could be mediated via an endocrine mechanistic
pathway. There are several hormones, like glucagon, growth hormone,
corticosterone and epinephrine, which can affect glucose turnover 5-8. NPY and MTII,
by example, have been shown to stimulate adrenocortical secretion via increased
release of corticotrophin-releasing hormone (CRH) and adrenocorticotropic
hormone (ACTH) 9-11. NPY is also able to increase glucagon concentration 12.
Although, we did not detect a modification of these hormones in our experimental
settings (corticosterone and glucagon were measured in chapter 2 and
corticosterone in chapter 3), the involvement of some of these hormones in the
studies described in this thesis cannot be ruled out and could be a possible
mechanism. Secondly, the effects could be mediated via a neural mechanistic
pathway. Direct multisynaptic connections have been shown between the
hypothalamus and peripheral organs that take up glucose, like liver, adipose tissue
and muscle 13-17. Many studies show that parasympathetic input to these peripheral
tissues is important for glucose uptake 15,17-20. Sympathetic neural activity in general
stimulates hepatic glucose production 21-23. However, in addition to the sympathetic
stimulation of hepatic glucose production, vagal input to the liver also modulates
hepatic glucose production 24. Therefore, the effects on glucose turnover described in
this thesis can also be mediated via a neural mechanistic pathway or perhaps a
combination of neural and endocrine mechanistic pathways control glucose turnover.

When we compare the effects of the different neuropeptides/hormones
described in this thesis, we observe that the NPY pathway causes hepatic insulin
resistance, whereas the POMC pathway (MTII) improves peripheral insulin
sensitivity. Furthermore, MTII was shown to stimulate basal glucose production.
PYY3-36 and leptin are both known to inhibit the NPY- and stimulate the POMC
pathway and would therefore be expected to improve insulin sensitivity. Indeed,
PYY3-36 improved peripheral insulin sensitivity and had a small tendency to improve
hepatic insulin sensitivity as well and leptin improved both hepatic and peripheral
insulin sensitivity. However, MTII also affected basal glucose production, an effect
that was not seen with PYY3-36 or leptin. This discrepancy can have several reasons.
First of all, we cannot rule out that some of the effects described in this thesis are not mediated via the hypothalamus but perhaps involve different brain regions, like the brain stem. The icv injections of MTII possibly affect the MC receptors in a more wide-spread brain area than the iv infusion of PYY\textsubscript{3-36} does. Secondly, it could be a simple dose effect. In the studies described in this thesis a single dose was used (based on available literature that showed effects on food intake) and we therefore cannot rule out the possibility that we might see additional effects (including an effect on basal glucose production) if a higher dose was used. Finally, other neuropeptides, like AgRP and CART, could be involved as well. The effects of these neuropeptides on insulin sensitivity are barely investigated and they might be differentially affected by PYY\textsubscript{3-36} and leptin. PYY\textsubscript{3-36} and leptin might have an additional effect on these neuropeptides as well, as they are coexpressed with NPY and POMC neurons respectively. Currently, it is unknown how these neuropeptides affect insulin sensitivity. Therefore, the possibility exists that PYY\textsubscript{3-36} and leptin additionally affect these neuropeptides, which can subsequently counteract the increase in basal glucose production that was caused by MTII.

The experiments described in this thesis show the dual involvement of neuropeptides/hormones in the regulation of both food intake and insulin sensitivity. The physiological significance of this dual regulation could consist of an extra supplemental mechanism in the body’s attempt to maintain glucose homeostasis and a fine-tuning of this mechanism through the possibility of regulating insulin sensitivity in a tissue specific way. Hypothalamic NPY levels, by example, will be increased during fasting, which will lead to hepatic insulin resistance. This could be an additional mechanism to the low insulin levels that are present during the fasted state, ensuring that the hepatic glucose production will be maintained at a high level. The opposite pathway, the POMC pathway, will be increased in the fed state and will lead to an increased insulin sensitivity of peripheral tissues like muscle and adipose tissue. This will facilitate the glucose uptake by these tissues leading to an effective decrease in blood glucose levels. Leptin and PYY are both hormones that can influence both pathways. Leptin reflects the size of fat depots and therefore acts as a long-term regulator, while PYY is increased immediately after a meal and therefore acts as a short-term regulator that will decrease food intake and simultaneously increase insulin sensitivity to decrease blood glucose levels acutely.
The central regulatory center for food intake and energy metabolism is extremely important in maintaining energy supply during times of plenty, but especially during times of famine. The experiments described in this thesis show that the neuropeptides and hormones that are involved in this regulation system, are not only involved in the regulation of food intake, but also in the regulation of insulin sensitivity. One could therefore hypothesize that disturbances in the regulation system itself, will lead to disturbances in both food intake and insulin sensitivity. Disturbances like leptin resistance and/or decreased PYY levels that were seen in obese subjects can therefore lead to a disturbed balance between the NPY and POMC pathway and can contribute to the increased food intake and insulin resistance. However, at present the role of these neuropeptides/hormones in the pathogeneses of obesity and type 2 diabetes in humans is still unknown.

**Implications for human pathophysiology**

Leptin was long thought of as the new therapeutic cure for obesity after successful experiments in several obese rodent models. However except for a few rare cases, obese humans turned out to be leptin resistant instead of leptin deficient. However, leptin resistance can eventually lead to the same metabolic consequences as leptin deficiency. We show in chapter 6 that leptin deficiency in \textit{ob/ob} mice, particularly leptin deficiency in the brain, can lead to insulin resistance. For obese humans these findings imply that leptin resistance, particularly leptin resistance of the brain, can ultimately contribute to insulin resistance as well.

For PYY\textsubscript{3-36}, human studies have shown that obese subject respond to PYY\textsubscript{3-36} by reduced food intake and are therefore not PYY\textsubscript{3-36} resistant\textsuperscript{25}. Furthermore, it was shown that obese subjects have decreased PYY\textsubscript{3-36} levels compared to lean subjects. For NPY and the POMC pathway, there are only a few studies that measured NPY or \textit{Į}-MSH levels in plasma or csf in obese and lean subjects. For NPY, some studies find higher NPY levels in obese subjects\textsuperscript{26,27} and some studies find no differences\textsuperscript{28,29}. There is one study in which the distinction between obese non-diabetic and obese diabetic subjects was made and plasma NPY levels were found to be significantly higher in the diabetic subjects\textsuperscript{30}.

For \textit{Į}-MSH, there are a few studies that find higher plasma levels in obese subjects and a negative correlation with insulin resistance\textsuperscript{31,32}. However, very little is known regarding the function of circulating \textit{Į}-MSH, as currently a great deal of
attention has focused on the central role of α-MSH and its antagonism at the MC4 receptor by AgRP. There is one study that measured α-MSH in csf in obese and lean subjects, but no difference was found. However, the csf-concentrations do not reflect hypothalamic concentrations, as NPY and α-MSH are not only confined to the hypothalamic region. There is one study that examined the hypothalamic NPY protein in normal and obese subjects and they did not find any differences. However that study was based on four obese subjects only and did not make any distinction between insulin sensitive or insulin resistant subjects. Therefore, the possibility exists that obese and insulin resistant subjects, like mice susceptible to diet induced obesity, have increased hypothalamic NPY levels and decreased POMC levels, which could be of consequence in the pathogenesis of obesity and type 2 diabetes mellitus.

Additional studies should be done that unravel the mechanism(s) by which the brain is capable of regulating insulin sensitivity. Both the endocrine or neural (sympathetic and parasympathetic) mechanistic pathways should be investigated. In addition, there are other neuropeptides and hormones that will play a role in the regulation of food intake and insulin sensitivity and these should be explored as well. It should be investigated in more detail whether disturbances in the balance of the NPY and POMC pathway or disturbances in other neuropeptides/hormones of this regulation system play a role in the pathogenesis of obesity or insulin resistance in humans. Furthermore, the possibility of (ant)agonists of these neuropeptides/hormones as a tool in the battle against obesity, type 2 diabetes and the metabolic syndrome should be investigated. There is still a lot of research that has to be done and several questions that still arise. The research described in this thesis is therefore a starting-point showing that neuropeptides/hormones that are involved in the regulation of food intake also, and independently of their effect on food intake, affect insulin sensitivity.

Reference list


7 Kahn CR, Goldfine ID, Neville DM, Jr., De Meyts P. Alterations in insulin binding induced by changes in vivo in the levels of glucocorticoids and growth hormone. Endocrinology 1978; 103: 1054-1066.


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