Neural control of hepatic lipid metabolism: A (patho)physiological perspective
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GENERAL DISCUSSION
AUTONOMIC INNERVATION IN PHYSIOLOGY: FASTING AND FEEDING

Our body is well designed to store energy in times of nutrient excess and release energy in times of need. This adaptation to the external environment is achieved by both humoral factors and the autonomic nervous system. Already in the 19th century, Claude Bernard pointed out the importance of the autonomic nervous system in the control of glucose metabolism. In the next century, the discovery of insulin and the development of techniques to measure hormone concentrations shifted the focus of the control of metabolism to the secretion of hormones, thus functionally “decapitating” the body. Just before the end of the 20th century, starting with the discovery of leptin in 1994, the control of energy metabolism went back to our heads. Today, the autonomic nervous system is acknowledged as one of the important determinants of liver metabolism and as a possible treatment target.

This thesis investigates the role of the autonomic nervous system in the control of hepatic lipid metabolism during different physiological conditions. We found that the sympathetic and parasympathetic nervous system represent complimentary forces, fine-tuning hepatic lipid metabolism during different nutritional states.

Fastig – In the fasted state, lipids are the main source of energy for peripheral tissues, mobilized as free fatty acids or VLDL-triglycerides (VLDL-TG). In chapter 3 we show that both the hypothalamic arcuate nucleus and the sympathetic nervous system are necessary to stimulate VLDL-TG secretion by the liver during fasting. It is known that fasting, amongst others, stimulates the release of neuropeptide Y (NPY) at the level of the hypothalamic paraventricular nucleus (PVN) and that these neurons are connected to the sympathetic nervous system (1,2). We showed that the stimulatory effect of intracerebroventricular (ICV) infusion of NPY on VLDL-TG secretion is abolished by a sympathetic hepatic denervation. We now believe that the increased activity of arcuate nucleus NPY neurons during fasting, amongst others, stimulates the sympathetic nervous system to increase VLDL-TG secretion by the liver, thereby representing a hypothalamic route of hepatic lipid control during fasting. Recently, it was shown that the ICV administration of an Y1 receptor agonist shows the strongest effect on VLDL-TG secretion, whereas an Y2 receptor agonist had the strongest effect on feeding behaviour (3). This shows that the differentiation of the effects of NPY on feeding behaviour and lipid metabolism is partially accomplished by the involvement of different receptors. The parvocellular portion of the PVN shows dense Y1 receptor processes, c-fos activation in response to ICV NPY and retrograde labeling in response to tracing from sympathetic motor neurons (1,4). Therefore, the dense NPY-immunoreactive projections from the arcuate nucleus towards the pre-autonomic neurons in the paraventricular nucleus could certainly be involved in the control of lipid metabolism. A recent study also elucidated a crucial role for arcuate nucleus NPY neurons modulating tyrosine hydroxylase mRNA expression in the PVN and brown adipose tissue (BAT) activity via Y1 receptor mediated mechanisms (2). However, other hypothalamic nuclei containing Y1 receptors (e.g. ventromedial hypothalamus, dorsomedial hypothalamus) cannot be ruled out until a study comparing the effects of NPY agonists in those different brain nuclei will be performed. In the fasted state, a parasympathetic denervation had no effect on VLDL-TG secretion.
We hypothesized that the parasympathetic nervous system is important for storing lipids postprandially. In chapter 4, rats with a selective liver denervation were fed a meal after an overnight fast. In an additional experiment, we kept rats in the postprandial state around the clock with the help of a six-meals-a-day feeding schedule. In both experiments, we found that rats with a parasympathetic denervation showed increased plasma triglyceride levels postprandially. Therefore, we believe that the parasympathetic nervous system is activated postprandially to inhibit VLDL-TG secretion by the liver, thereby promoting the storage of triglycerides. Lam et al. (5) showed that an ICV infusion of glucose inhibits VLDL-TG secretion. This effect was dependent on an intact vagal nerve, in line with the results from our experiments, i.e., feeding and vagal nervous activity inhibit VLDL-TG secretion. In addition and also in line with our previous results, these authors found no effects of a selective parasympathetic denervation in fasted rats, emphasizing that a meal or ICV glucose should be given in order to observe an effect of a parasympathetic denervation. Additionally, we found that cutting the sympathetic nerve also increased plasma triglycerides after a meal. This led us to believe that the sympathetic outflow to the liver is activated during fasting to increase VLDL-TG secretion and inhibited during feeding to inhibit VLDL-TG secretion.

Based on these experiments, we propose the following model for autonomic activity towards the liver (Figure 1). We assume that in the basal state there is a basal sympathetic tone,
whereas parasympathetic activity is low. During fasting, sympathetic activity is increased to stimulate VLDL-TG secretion, thereby mobilizing triglycerides. Parasympathetic activity does not change and remains low. During feeding both the increased parasympathetic activity, and the decreased sympathetic activity function to inhibit VLDL-TG secretion, favouring the storage of triglycerides (Figure 1).

Previous studies investigating the role of the autonomic nervous system in glucose metabolism have shown similar results. Sympathetic hepatic activation increases hepatic glucose output by stimulating glycogen breakdown, while parasympathetic hepatic activation decreases blood glucose levels by promoting glycogen synthesis (for review 6).

Based on our experiments, the concept that the sympathetic nervous system is important during fasting to mobilize nutrients and the parasympathetic nervous system is important during feeding to promote storage of nutrients, also holds true for hepatic lipid metabolism.

**ACTIVITY OF THE AUTONOMIC NERVOUS SYSTEM DURING FASTING AND FEEDING**

Our hypothesis that sympathetic activity is increased during fasting is supported by the concept that the absence of food in evolution is a life-threatening stressor and catecholamines are important substrate mobilizing hormones. However, the denervation technique applied in our experiments is a “loss-of-function” model and the actual changes in nerve activity have not been measured. There have been conflicting data on the activity status of the autonomic nervous system towards the liver during feeding and fasting. Niijima et al. showed that, after an intravenous administration of D-glucose, the firing rate in efferent vagal nerves innervating the liver was increased, whereas the activity of the sympathetic splanchnic nerve was decreased. Conversely, with decreasing glucose concentrations they found an increased activity of the sympathetic splanchnic nerve (for review 7). These electrophysiological findings of Niijima et al. are in accord with our proposed model.

However, several studies measuring noradrenaline turnover with tritiated noradrenaline or pharmacological blockade of noradrenaline secretion, observed a decreased noradrenaline turnover in livers of rats fasted for 18 or 48 hours compared to those of ad libitum fed animals (7,8). These authors hypothesized that the decreased sympathetic activity is part of an adaptive response to the fasted state in order to conserve energy (9). It should be noted that this fasting-induced decrease of noradrenaline turnover was stronger in heart and pancreas (8). The authors acknowledge that the measurement of noradrenaline turnover is challenging in liver, as the liver metabolizes circulating catecholamines and receives recirculating tritiated noradrenaline not only via arterial blood flow but also via portal blood flow. They concluded that the difference between the apparent decreased sympathetic activity in heart and pancreas compared to the less robust effect in liver may reflect insensitivity of the technique or a solid difference in sympathetic outflow towards different organs as had also been suggested by others (for review 11). This notion was supported by a study by Migliorini et al. (12), who measured sympathetic activity towards white adipose tissue (WAT) and BAT. They concluded that, whereas fasting indeed decreased sympathetic activity towards BAT, the activity of the sympathetic nervous system
towards WAT was increased. This supports both the view of energy conservation by reduced BAT activity and increased mobilization of nutrients by WAT for lipolysis, during fasting. Whereas we hypothesize that activation of NPY neurons increase sympathetic outflow towards the liver, others show that these neurons decrease sympathetic outflow towards brown adipose tissue (2). Anatomical data show that the sympathetic innervation towards different compartments originates from distinct motor neurons in the hypothalamus (13). In summary, the evidence on the activity of the sympathetic nervous system towards the liver during fasting and feeding is inconclusive due to the following reasons:

- The direct measurement of sympathetic activity in liver in intact, undisturbed animals during fasting and feeding is technically challenging.
- There may be different phases of fasting and sympathetic activity that are dependent on the duration of the fasting period. The appropriate response to fasting is, on the one hand to stress the body to search for food and mobilize energy for this search. On the other hand, during prolonged fasting, it is necessary to conserve energy for longer survival.
- There is a possible divergence of autonomic activity towards different organs, so that increased sympathetic activity towards one organ and decreased sympathetic activity towards another organ may occur simultaneously.

**MECHANISM IN THE LIVER**

We conclude that the sympathetic nervous system is involved in the stimulation of VLDL-TG secretion and that the parasympathetic nervous system is predominantly involved in the inhibition of VLDL-TG secretion. However, in this thesis we were unable to elucidate the mechanism by which autonomic liver innervation regulates VLDL-TG secretion.

**Noradrenaline** – We hypothesize that increased sympathetic activity stimulates VLDL-TG secretion. This might be mediated via secretion of noradrenaline at the sympathetic nerve endings in the liver. However, noradrenaline infusion into the portal vein decreases VLDL-TG secretion (14). This may be explained by a difference between the effects of noradrenaline released locally from nerve endings and noradrenaline released into the circulation by the adrenals. However, in our experiments we have not shown a direct relation between activation of the sympathetic nervous system and VLDL-TG secretion. Stimulating the splanchnic nerve and measuring VLDL-TG secretion could clarify this relation, as was done for glucose metabolism (15).

**Gene expression** – We investigated if the autonomic nervous system could directly modulate gene expression of key genes involved in lipid metabolism. In our experiments in chapter 3, combining fasting with a hepatic denervation, we found lower VLDL-TG secretion but no effects on mRNA levels of important genes involved in the lipid pathway. Other studies found decreased carnitine palmitoyltransferase 1 (CPT1) activity after denervation in fasted rats, but this decreased activity does not explain the decreased VLDL-TG secretion also observed in those studies (16,17). After ICV infusion of NPY we observed decreased mRNA expression of CPT1α and increased mRNA expression of ADP-ribosylation factor (ARF1), indicative of decreased β-oxidation and increased
VLDL-TG assembly. Others have indicated that stearoyl-coenzyme A desaturase 1 (SCD1) mRNA is increased after ICV NPY (3,18), a finding later replicated in our rats receiving ICV NPY. SCD1 catalyzes the limiting step of monounsaturated fatty acid synthesis and is an important player in triglyceride generation. SCD -/- mice have low concentrations of VLDL-TG (19). Interestingly, when glucose is infused centrally, a decrease of SCD1 mRNA expression is observed (5). Therefore, SCD1 is a possible candidate in the central regulation of hepatic lipid metabolism.

Other mechanisms – In the field of glucose metabolism, studies suggest that parasympathetic innervation alters insulin sensitivity of the liver (20). A parasympathetic denervation caused insulin resistance in the postprandial state. As insulin inhibits VLDL-TG secretion and insulin resistant males show higher post-prandial triglycerides, it is indeed possible that also for lipid metabolism the autonomic nervous system acts by regulating the sensitivity to insulin (21). Finally, sympathetic stimulation has been shown to alter both whole liver perfusion and distribution (22).

In summary, although several possible mechanisms of autonomic control of hepatic lipid metabolism have been described, the exact mechanisms are yet to be elucidated.

**THE ROLE OF THE HYPOTHALAMUS AND AUTONOMIC INNERRATION IN PATHOPHYSIOLOGICAL CONDITIONS**

In essence, the autonomic nervous system protects us from life-threatening challenges to our homeostasis. However, the continuous abundance of food in our Western society may unanticipated from an evolutionary perspective. Therefore, we have directed our focus not only to the involvement of the autonomic nervous system in maintaining homeostasis, but also to the role of the autonomic nervous system in the development of life-threatening conditions including hypertension, obesity and diabetes. Increasing evidence shows that increased sympathetic activity correlates with components of the metabolic syndrome. Licht et al. (23) investigated the relation between autonomic activity and triglycerides by measuring respiratory sinus arrhythmia, as a measure of parasympathetic activity, and pre-ejection period, as a measure of sympathetic activity in a large cohort of patients. They concluded that increased sympathetic and decreased parasympathetic activity correlates with hypertriglyceridemia, which is in accord with our study. We hypothesized that this increased activity of the sympathetic nervous system would lead to a stimulation of VLDL-TG secretion, resulting in dyslipidemia. We investigated this hypothesis in an animal model of dyslipidemia, the obese Zucker (fa/fa) rat. Obese Zucker rats display low sympathetic nerve activity to BAT, but high levels of renal sympathetic nerve activity and increased baseline splanchnic nerve activity, indicating increased sympathetic nervous activity to the liver (24-26). In chapter 5 we show that a sympathetic liver denervation in obese Zucker rats lowers VLDL-TG secretion and plasma triglycerides. In addition, a parasympathetic liver denervation elevates plasma cholesterol levels. We believe that this study introduces new possibilities of modulating dyslipidemia, possibly at the level of the hypothalamus (NPY/POMC) or at the level of the sympathetic nerve and its endings.
THE HYPOTHALAMUS AND THE AUTONOMIC NERVOUS SYSTEM: A POSSIBLE TREATMENT TARGET?

Hypothalamic NPY – In chapter 3 we show that central infusion of NPY stimulates VLDL-TG secretion, which is prevented by a sympathetic denervation of the liver. Genetic obesity models, e.g. obese Zucker ras, ob/ob and db/db mice, are characterized by increased NPY mRNA levels and protein levels (27-30). In ob/ob and db/db mice, NPY neurons are not only activated during fasting, as in wildtype mice, but also in the postprandial condition (30). We hypothesize that the increased release of NPY at the level of the PVN results in a constant activation of the pre-autonomic neurons that are in charge of the sympathetic input to the liver, therefore overstimulating VLDL-TG secretion. It is striking that indeed many effects of chronic intracerebroventricular (ICV) infusion of NPY are similar to the abnormal characteristics of obese Zucker rats (31,32). Future studies are necessary to investigate if the increased release of NPY is indeed involved in the hypertriglyceridemia of these obese animal models by treating for instance obese Zucker rats with an ICV NPY antagonist. The treatment of obesity related diseases with an NPY antagonist has been subject of many animal studies showing beneficial effects on food intake and body weight (for review 33). However, specifically targeting the right NPY receptor subtype (Y1-6) and delivery of the drug to the right target in patients is challenging due to poor transport across the blood-brain barrier. The highly selective Y5 antagonist, MK-0557, has been tested in a 52 week, multicenter, randomized, double-blind, placebo-controlled trial involving 1661 overweight and obese patients (34). Although the authors reported a small but significant weight loss in the treatment group, no effects on serum triglycerides were observed. However, a recent study suggests that the effects of NPY on VLDL-TG secretion are mediated via the Y1 receptor (3).

Hypothalamic POMC – In addition to increased concentrations of NPY, obese Zucker rats also exhibit decreased pro-opiomelanocortin (POMC) mRNA in the ARC and decreased α-MSH levels in the PVN (35,36). However, contrary to the effect of chronic ICV NPY administration, chronic blockade of the central melanocortin system does not change plasma levels of triglycerides or fatty acids independently of increased food intake, although it does result in an obese phenotype (37-39). Furthermore, contrary to acute NPY infusions, acute infusions with a melanocortin antagonist do not increase VLDL-TG secretion (18). However, both rats infused ICV with a melanocortin antagonist and mice with a melanocortin 4 receptor deficiency do display higher levels of total cholesterol (40). Furthermore, POMC gene delivery to the arcuate nucleus in obese Zucker rats decreases plasma cholesterol levels (41). Therefore it can be hypothesized that the low POMC tone in obese Zucker rats is responsible for increased cholesterol levels and that this effect might be mediated by a lowered activity of the parasympathetic nervous system.

Sympathetic nervous system – In this thesis we show that cutting the sympathetic nerve to the liver in obese Zucker rats lowers VLDL-TG secretion and plasma triglycerides compared to sham-operated obese Zucker rats. Although surgical denervation is an invasive method as compared to drug treatment, renal denervation is under investigation in treatment-resistant hypertension (42). Pharmacological blockade of the beta-receptor is
less invasive and can be applied in a more patient-tailored way. In chapter 7 we measured serum triglycerides in healthy post-menopausal women receiving the β-blocker propranolol. If a hepatic sympathetic denervation lowers triglycerides in obese Zucker rats, it can be hypothesized that β-adrenergic blockade will have beneficial effects on serum triglycerides levels in humans. However, from our study we conclude that exactly the opposite is true. Unexpectedly, β-adrenergic blockade with propranolol appeared to increase serum triglycerides in our study and in many other studies published previously. In addition, serum HDL-cholesterol was often decreased by the treatment. Although the exact mechanism of the increased triglycerides and decreased HDL-cholesterol concentrations remain to be elucidated, it is proposed that propranolol affects LPL activity in the adipocyte by concurrent α-adrenergic stimulation. The difficulty of pharmacological sympathetic blockade is that we cannot target the reduction of sympathetic activity towards one single organ, and the off-target effects may overrule the benefits of the target effects.

FUTURE PERSPECTIVES

To further investigate the role of the autonomic nervous system in hepatic lipid metabolism in animal and human studies, techniques directly stimulating or repressing sympathetic or parasympathetic nerve activity and specific measures of autonomic activity should be further developed. In animal studies, in vivo electrical stimulation of the hepatic nerves has been used for the study of glucose metabolism. Most human studies on the correlation between metabolic factors and autonomic activity use cardiac or muscle measures of autonomic activity or whole body noradrenaline turnover (23,43-45). However, we believe that sympathetic outflow towards different organs is not uniform and we are specifically interested in measures of autonomic activity of the abdominal compartment representative of liver autonomic activity. A reflection of this could be postprandial gallbladder contractility. Furthermore, further studies are necessary to understand how the sympathetic and parasympathetic nervous system impact the hepatocyte to further elucidate the nervous regulation of VLDL-TG secretion.

Although the brain and autonomic nervous system seem inaccessible for treatment of metabolic disease in humans, fascinating research is being performed to open this door. Several animal studies found associations between decreased body weight gain and vagal nerve stimulation, although small retrospective studies in humans with a vagal nerve stimulator for the treatment of epilepsy are inconclusive on this matter (46-49). Larger prospective studies including different components of the metabolic syndrome are necessary to show that this could indeed be a novel treatment as previously hypothesized (50). Finally, new combinatorial compounds are being developed that are able to target dedicated hypothalamic sites more specifically, making the study of the hypothalamic mechanisms even more important and fascinating also in relation to human disease (51).
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