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Neural control of lipid metabolism and inflammation : implications for atherosclerosis

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GENERAL INTRODUCTION AND OUTLINE

Adapted from:

- Sympathetic nervous system control of triglyceride metabolism: novel concepts derived from recent studies. *J Lipid Res (2014) 55:180-9*
- Brown fat as a novel target to attenuate hyperlipidemia and atherosclerosis. *Circ Res (2015) in press*
- Regulation of brown fat by AMP-activated protein kinase. *Trends Mol Med (2015) in press*
- Hypothalamic regulation of brown fat activity. *Trends Endocrinol Metab (2015) in press*

Cardiovascular disease (CVD) is currently the leading cause of death worldwide (1). While mortality rates are declining in high-income countries, CVD morbidity and mortality rapidly increase in low- and middle-income countries. Although CVD usually affects the elderly, the antecedents of CVD including atherosclerosis, begin in early life. CVD may be prevented by behavioral adjustments including exercise, healthy eating and avoidance of smoking, thereby reducing the development of dyslipidemia and inflammation, the two main risk factors for atherosclerotic lesion development. Current pharmaceutical approaches in the treatment of atherosclerosis mainly aim at correcting dyslipidemia through reduction of plasma cholesterol levels. However, although statins effectively reduce low-density lipoprotein-cholesterol (LDL-C), only about 25-30% of all cardiovascular events are prevented by this treatment (2). Accumulating evidence indicates a prominent role of the autonomic nervous system (ANS) in the regulation of lipid metabolism as well as inflammation, but the consequences for atherosclerosis development and the potential for novel treatment strategies are still unclear (**Figure 1**).

Autonomic nervous system control of homeostatic activity

From reports describing the effects of lesioning discrete brain nuclei, through to studies of genetically engineered animal models and human genetic disorders, the primacy of the central nervous system (CNS) in the control and coordination of homeostasis is clear. The hypothalamus in particular is critical in sensing and

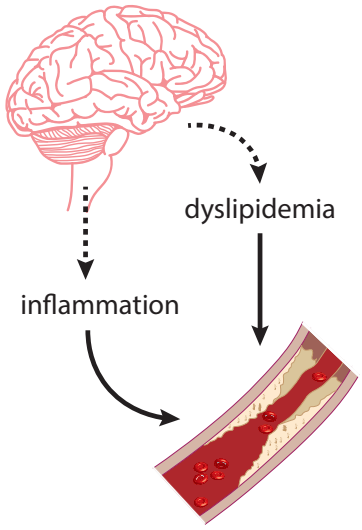

Figure 1 – Dysregulation of the inflammatory response and lipid metabolism by the autonomic nervous system may result in atherosclerotic lesion development.

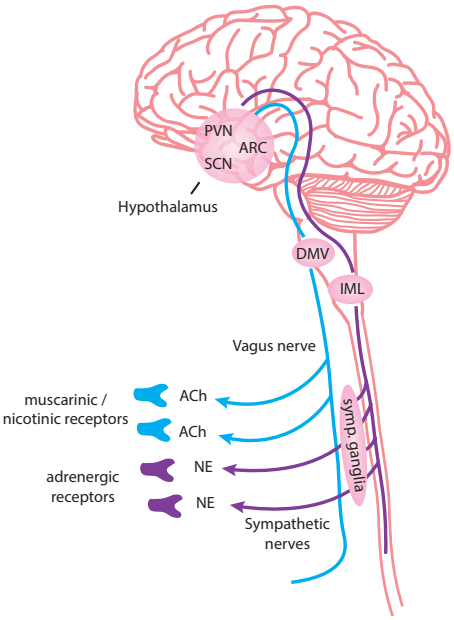

Figure 2 – Hypothalamic projection onto the autonomic nervous system. See text for details. In purple sympathetic pathways, in blue parasympathetic pathways. Ach, acetyl choline; ARC, arcuate nucleus; DMV, dorsal motor nucleus of the vagus; IML intermediolateral column of the thoracic spinal cord; NE, norepinephrine; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus.

integrating signals from the periphery and effecting appropriate physiological changes to maintain homeostasis. Classically, this has been viewed in terms of the neuroendocrine control system resulting from processing of hypothalamic signals projected to the pituitary. More recently, the hypothalamic control over the ANS has been increasingly recognized as a potent modulator of homeostatic activity in peripheral tissues.

Separate populations of pre-autonomic nerve fibers residing from hypothalamic nuclei relay to either parasympathetic or sympathetic nuclei in the brain stem and spinal cord, respectively (**Figure 2**). The efferent parasympathetic signal is conveyed via preganglionic cells in the dorsal motor nucleus of the vagus (DMV). The DMV is directly connected by the vagal nerve to ganglion cells, without involvement of the spinal cord. Postganglionic parasympathetic nerves project on target organs and use acetylcholine (ACh) as their main neurotransmitter, which acts on muscarinic and nicotinic cholinergic receptors. On the other hand, sympathetic nerve fibers arise from the intermediolateral (IML) column of the thoracic spinal cord and project onto the chain of sympathetic ganglia located just outside of the spinal cord. In turn, sympathetic ganglia give rise to postsynaptic sympathetic nerve fibers that subsequently innervate the target organ. In general, sympathetic

neurons transmit their signal by releasing norepinephrine (NE) from their nerve endings. NE subsequently binds to adrenergic receptors located at the postsynaptic membrane on the target organ. At least nine subtypes of adrenergic receptors, divided into three major classes, have been identified: $\alpha 1_{(A/B/D)}$-adrenergic receptors, $\alpha 2_{(A/B/C)}$-adrenergic receptors, and $\beta_{(1/2/3)}$-adrenergic receptors.

In addition to the hypothalamic control of both the sympathetic and parasympathetic nervous system, the ANS regulation of homeostatic activity involves certain reflexes, including the cholinergic anti-inflammatory reflex. Changes in homeostasis, *e.g.* the release of pro-inflammatory cytokines, are sensed by afferent peripheral nerve fibers that project directly onto efferent nerve fibers within the spinal cord or DMV, resulting in direct (de-)activation of efferent nerve signaling, without involvement of the hypothalamus or other brain regions.

Hypothalamic control of energy homeostasis

Before the ANS regulation of lipid metabolism can be discussed in more detail, I first have to elaborate on the role of the hypothalamus in energy homeostasis, which is a balance between energy intake and energy expenditure. It should be noted that several hypothalamic nuclei control both energy intake as well as peripheral energy expenditure, indicating the close interplay between these processes.

The arcuate nucleus (ARC) of the hypothalamus, in particular, plays an important role in energy metabolism. Within the ARC, two neuronal populations, cocaine- and amphetamine-regulated transcript (CART)/pro-opiomelanocortin (POMC)-expressing neurons and neuropeptide Y (NPY)/Agouti-related protein (AgRP)-expressing neurons, oppositely regulate energy balance (3). Activation of CART/POMC neurons leads to the production of α-melanocyte-stimulating hormone (α-MSH), which in turn stimulates the melanocortin (MC) receptors within the paraventricular nucleus (PVN) to promote satiety and induce a catabolic state of the body. In contrast, activation of NPY/AgRP neurons promotes food intake and an anabolic state, partly because AgRP acts as an endogenous antagonist for the melanocortin receptors and hereby directly inhibits the actions of CART/POMC-expressing neurons. Mutations and polymorphism within genes involved in this central melanocortin system are associated with the development of obesity and related metabolic disorders (4). Interestingly, variants in and near the melanocortin 4 receptor gene are not only associated with obesity, but also with reduced energy expenditure, suggesting that the melanocortin system directly regulates energy metabolism in peripheral organs. However, the exact mechanism remains unknown.

A second example and probably underappreciated regulator of energy balance is the central biological clock, located in the suprachiasmatic nucleus (SCN), which orchestrates diurnal and seasonal rhythms. Neuronal firing within the SCN is synchronized by light exposure, resulting in a high electrical activity during

the day and low activity during the night. The SCN subsequently signals to other hypothalamic nuclei and peripheral organs via regulation of hormonal output, *e.g.* rhythmic secretion of pituitary hormones and melatonin output, and via the ANS (5), together resulting in diurnal rhythms in metabolic processes. Disturbances in circadian rhythmicity, *e.g.* by shift-work or light pollution, are associated with obesity and related disorders including cancer and CVD (6). Causality in many of these associations still remain to be confirmed in animal studies or randomized-controlled clinical trials.

Hypothalamic control of lipid metabolism via the autonomic nervous system

Energy balance comprises energy intake and expenditure of mainly glucose and lipids (triglycerides; TG). The role of the ANS in the regulation of glucose metabolism has been firmly established (reviewed in (7)), however, considerably fewer studies have focused on its role in TG metabolism.

Important players in TG metabolism include the liver and intestines (production), white adipose tissue (WAT) (storage), heart and skeletal muscle (combustion to generate ATP), and brown adipose tissue (BAT) (combustion towards heat), the collective action of which determine plasma TG levels. While all of these organs are innervated by sympathetic nerve fibers (8), the role for parasympathetic nerves in WAT is less clear (9) and even appear to be absent in BAT (10).

Hepatic sympathetic innervation stimulates very low-density lipoprotein (VLDL)-triglyceride (TG) production (reviewed in (11)), while sympathetic denervation reduces VLDL-TG secretion (12). This reduction in VLDL-TG secretion upon sympathetic denervation was only observed in fasted rats, a situation in which lipids become the key substrate for energy metabolism. Conversely, neuropeptides that are increased upon positive energy balance reduce hepatic VLDL-TG production and hepatic parasympathetic denervation in obese Zucker rats results in elevated plasma cholesterol levels (reviewed in (13)).

Just as in liver, sympathetic innervation of WAT results in secretion of lipids to be used as substrate for energy metabolism. ß2-adrenergic signaling induces phosphorylation of adipose tissue TG lipase (ATGL), hormone-sensitive lipase (HSL), and perilipins, ultimately resulting in lipolysis of stored TG and subsequent secretion of fatty acids into the circulation (reviewed in (14)). Long-term adrenergic stimulation of WAT additionally results in transdifferentiation of white adipocytes into beige/bright adipocytes (15).

In general, increased sympathetic outflow toward BAT (*e.g.*, following a cold stimulus) results in increased clearance and combustion of TG into heat (16). As BAT is an important topic of this thesis, I will provide a detailed description of BAT physiology. Upon sympathetic nerve fiber activity, NE is released and binds to

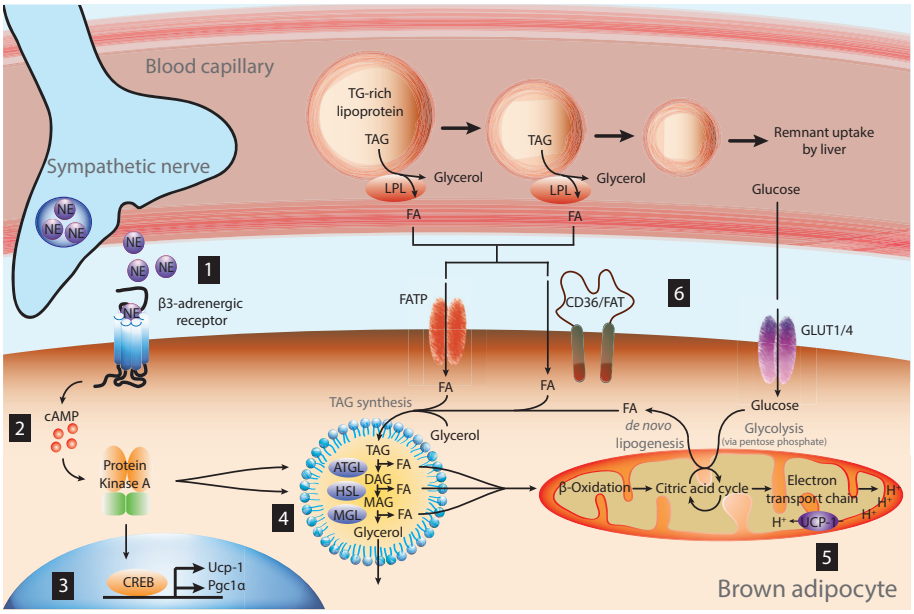

Figure 3 – Sympathetic innervation of brown adipose tissue and subsequent combustion of TG into heat. See text for details.

ß-adrenergic receptors on brown adipocytes (**Figure 3**; step 1). Of the three subtypes of ß-adrenergic receptors, the ß3-adrenergic receptor is the most significant in mature brown adipocytes from at least rodents. Binding of norepinephrine (*i.e.* noradrenalin) to the ß3-adrenergic receptor results in activation of its coupled stimulatory G-protein, after which adenylyl cyclase stimulates the formation of cyclic adenosine monophosphate (cAMP). cAMP activates protein kinase A (PKA), resulting in two important downstream effects (**Figure 3**; step 2). First, PKA stimulates phosphorylation of transcription factors that enhance expression and synthesis of uncoupling protein-1 (UCP-1) (**Figure 3**; step 3). Furthermore, PKA phosphorylates and activates intracellular HSL, resulting in increased intracellular lipolysis and, consequently, an increased flux of FAs toward the mitochondria to be combusted (**Figure 3**; step 4). In addition, FAs may bind to a hydrophobic binding pocket on the UCP-1 protein, resulting in its conformational change. This results in uncoupling between the respiratory chain and ATP synthase by proton leakage from the mitochondrial innermembrane space into the matrix leading to heat production instead of ATP (**Figure 3**; step 5). Finally, intracellular lipid stores need to be replenished by the uptake of glucose, FAs and triglyceride (TG)-derived FAs from plasma (**Figure 3**; step 6).

Because of its ability to combust large amounts of TG into heat, BAT is an appealing target for treatment of obesity, dyslipidemia and related disorders. Cold exposure and ß3-adrenergic receptor agonists were found to activate human BAT resulting in lower plasma TG and increased energy expenditure (17,18). Unfortunately, to my knowledge, until now no sympathomimetic drugs have been developed that stimulate human BAT without marked effects on the cardiovascular system, indicating the need for alternative pharmaceutical targets.

Control of inflammatory responses by reflexes of the autonomic nervous system

The hypothalamus-pituitary-adrenal (HPA) axis, which controls glucocorticoid release, is probably the most well-established neuroimmunomodulatory pathway. While the systemic control exerted by the HPA axis takes place over hours, inflammatory control by the ANS can control peripheral inflammation more rapidly and directly. Stimulation of peripheral C-afferent fibers, upon acute peripheral inflammation, releases excitatory amino acids (*e.g.* glutamate) in the spinal cord, which then binds to *N*-methyl-D-aspartate (NMDA) receptors to decrease adenosine release by efferent fibers (reviewed in (19)). Adenosine has an anti-inflammatory effect in the periphery by binding to A_{2A}-receptors on neutrophils and to A_1-receptors on nerve fibers within the spinal cord that inhibit NMDA receptor activation (20). Thus, acute inflammation results in further amplification of pro-inflammatory responses via the so-called dorsal root reflexes (**Figure 4**; item 1).

Subsequently, cytokines released at the inflammatory site may activate afferent fibers of the vagus nerve, resulting in an anti-inflammatory reflex. The afferent arm of the cholinergic reflex routes through the DMV to finally stimulate the release of ACh, the main neurotransmitter released by terminal vagal fibers, which activates nicotinic receptors composed of the α7 subunit on immune cells (reviewed in (21)). Interruption of this homeostatic mechanism in vagotomized animals aggravates inflammation (22). In animals with an intact vagal nerve, this reflex operates to reduce the release of pro-inflammatory cytokines in the periphery, thereby contributing to the homeostasis of the system by reducing inflammation (**Figure 4**; item 2).

The sympathetic nervous system (SNS) also exerts complex control on inflammation. The sympathetic neurotransmitter NE directly influences activation of $CD4^+$ T cells during initial stages of inflammation (23). In later phases, the net effect of the SNS is anti-inflammatory and can be enhanced by the ß-adrenergic agonists (24).

The development of atherosclerosis is considered as a chronic low-grade inflammatory disease. In particular serum concentrations of pro-inflammatory cytokines IL-1ß, TNFα and IL-6 have been proposed as predictors for future cardiovascular events (reviewed in (25)). Large clinical trials are now underway with

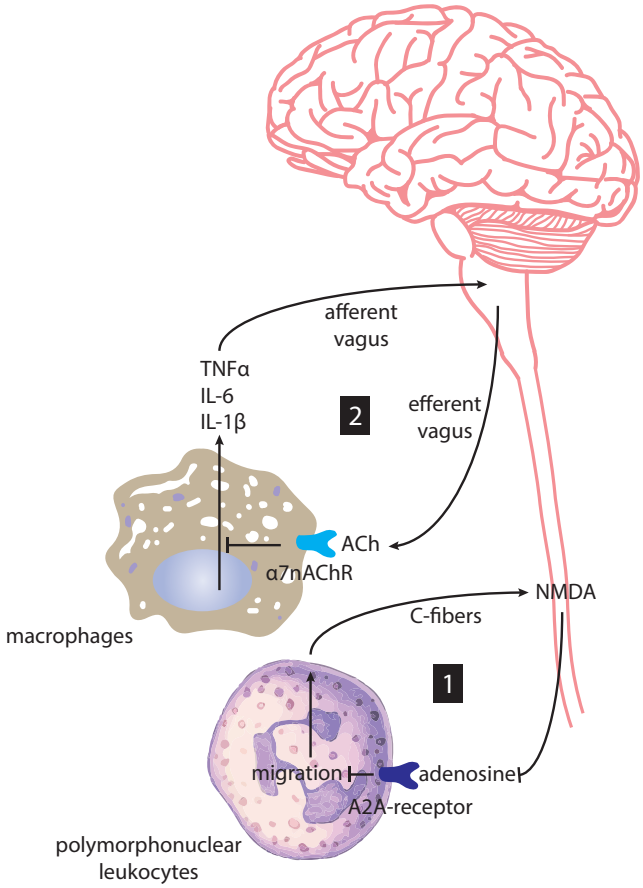

Figure 4 – Autonomic nervous system regulation of inflammatory responses; dorsal root reflex (1) and cholinergic anti-inflammatory reflex (2). See text for details.

agents that lead to reduced inflammation, investigating the effect on cardiovascular events as clinical end-point. Despite the clear primacy and big attention for the inflammatory status during atherosclerotic lesion development and cardiovascular events, little is known about the consequences of interfering with the inflammatory reflexes of the autonomic nervous system.

Interplay between lipid metabolism and inflammation; potential consequences for atherosclerosis development

Dyslipidemia is often accompanied by low-grade systemic inflammation, which is characterized by increased presence of cytokines and other markers of inflammation in the circulation. Cholesterol, fatty acids and modified lipids can directly activate inflammatory pathways. In addition, circulating (modified) lipoproteins modulate the activity of leukocytes. *Vice versa*, pro-inflammatory signaling (*i.e.* cytokines) in pre-clinical models directly affects lipid metabolism. Whereas the main lipid-lowering drugs including statins, fibrates and niacin all have potent anti-inflammatory actions, the lipid-modulating actions of anti-inflammatory agents appear to be less straightforward (reviewed in (26)).

Another compound with a dual mode of action is adenosine. As mentioned before adenosine has an anti-inflammatory effect in the periphery by binding to A_{2A}-receptors on neutrophils. Additionally, adenosine is released upon sympathetic innervation of BAT and WAT, resulting in activation of brown adipocytes and browning of white adipocytes, respectively. Treatment of mice with A_{2A} agonists prevents diet-induced obesity (DIO) and improves glucose tolerance (27). Yet, the possible beneficial effects of A_{2A} agonists on CVD remain to be determined.

Interactions between lipid metabolism and inflammatory pathways do have direct consequences for the development of atherosclerosis as seen for example with salicylates. Salicylates belong to the NSAIDs (non-steroidal anti-inflammatory drugs) and are frequently used for prevention and treatment CVD, due to their anti-inflammatory activity by inhibiting the pro-inflammatory transcription factor NF-κB function through activation of 5' AMP-activated protein kinase (AMPK) (28). Interestingly, besides its role in the control of inflammation, AMPK is crucial in maintaining cellular energy homeostasis. The net effect of AMPK activation is uptake and combustion of nutrients to increase cellular energy availability. Controversially, activation of AMPK by *e.g.* metformin (29) or salsalate (30) in BAT also enhances uncoupled respiration, resulting in a loss of energy. Taken together, salicylate drugs may potentiate a dual action in the treatment of both type II diabetes and atherosclerosis as they not only reduce inflammation (31), but also improve glucose tolerance, whole-body energy expenditure (32) and induce an anti-atherogenic lipoprotein profile (31).

Outline of this thesis

The aim of the present thesis is to gain more insight in the ANS regulation of lipid metabolism and inflammation, and the potential consequences for the development of atherosclerosis. Part I of this thesis focuses on regulation of lipid metabolism by the ANS, with special attention for BAT as an emerging pharmacological target for therapy.

Since the melanocortin system has been linked to energy expenditure in addition to the regulation of food intake, and the underlying mechanism is as yet unknown, in **Chapter 2**, the direct effects of inhibition of the central melanocortin system on lipid metabolism were evaluated. Next we determined in **Chapter 3** the potential beneficial effects of activating the central melanocortin system by intracerebroventricular (ICV) infusion of glucagon-like peptide-1 (GLP-1) on the activation of BAT, restoration of dyslipidemia and the reduction of DIO.

Interestingly, many genes involved in lipid metabolism and inflammation are expressed in a circadian manner. Disturbances in circadian rhythmicity by *e.g.* light pollution or shiftwork are associated with human obesity and CVD. The aim of the study described in **Chapter 4** was to specifically determine the potentially harming effects of prolonged daily light exposure, as perceived by the SCN, on lipid metabolism and adiposity in mice. Subsequently, in **Chapter 5**, we determined the daily rhythm in lipid metabolism and the differences that may occur between seasons considering the change in daily light exposure.

The endocannabinoid system is a group of neuromodulatory lipids and their receptors in the brain that are involved in a variety of metabolic processes including appetite and energy expenditure. Interestingly, the cannabinoid receptors are not only expressed in CNS, but also on peripheral tissues, including BAT. In **Chapter 6**, we determined the effects of cannabinoid 1 receptor (CB1R) blockade on brown fat activation, reversal of dyslipidemia and DIO. Additionally, we discriminated between direct peripheral and indirect central effects of CB1R antagonism.

Part II of this thesis describes studies on the regulation of inflammation by the ANS, with focus on the anti-inflammatory reflex. During this reflex, binding of Ach to α7nAChR and subsequent intracellular signaling results in transcriptional repression of pro-inflammatory genes. In **Chapter 7**, we investigated the consequences of hematopoietic α7nAChR deficiency on the development of inflammation, platelet reactivity and atherosclerosis. Since the nerves involved in the anti-inflammatory reflex mainly project towards the spleen, in **Chapter 8**, we investigated the effects of selective parasympathetic and sympathetic denervation of the spleen on inflammation and atherosclerotic plaque development.

Finally, the results from these studies and their therapeutic implications are discussed in **Chapter 9**.

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