Autonomic nervous control of white adipose tissue: studies on the role of the brain in body fat distribution
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CHAPTER 1

Introduction

Based on:
Central nervous determination of food storage-a daily switch from conservation to expenditure: implications for the metabolic syndrome.

Felix Kreier, Andries Kalsbeek, Marieke Ruiter, Ajda Yilmaz, Johannes A. Romijn, Hans P. Sauerwein, Eric Fliers, Ruud M. Buijs


It is important to look at the integrated organism
at different times [...] and to examine the events
in different tissues, because these events
might not be similar or comparable

Per Björntorp, 1983'

'NEW' CONCEPTS
Claude Bernard, a pioneer in scientific theory and practice of modern medicine, proposed in the 1840s that nerves are either sensory or motor, that vasomotor nerves regulate blood supply by modulation of arterial tone, that the liver stores glucose and that the pancreas secretes digestive enzymes. He assumed -at that time a groundbreaking concept- that these important processes should be coordinated by the best-protected organ of the body: the brain. To test his hypothesis, Claude Bernard punctured the fourth ventricle of rabbits and found them to turn diabetic. His famous conclusion from these observations was that these mechanisms all serve one common objective: keeping the internal environment stable.

When Charles Darwin observed in the 1870s that "a hungry man, if tempting food is placed in front of him, may not show his hunger by any outward gesture, but cannot control the secretion of glands (like saliva)"; he assumed that the brain controls the body by will-dependent and will-independent nerves.

When Harvey Cushing observed in the 1920s that a fatty liver could be induced within 9 hours after peripheral injection of hypophysin (an extract of the posterior lobe of the pituitary), he hypothesized a role for the brain and the autonomic nervous system in
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This effect. Indeed, when he injected hypophysin intracerebroventricularly, the fatty liver developed already within 4 hours after injection. Moreover, he could abolish this effect by lesions to either the central nervous or the peripheral autonomic nervous system.

Interestingly, these three scientific pioneers shared the same concept to comprehend metabolism in mammals: they presumed a central role for the brain in the coordination of energy homeostasis. However, the interpretation of these experiments was extremely complex. After the discovery of various powerful hormones in the first half of the 20th century, concepts changed. The interpretation of experiments with hormones seemed less complex. In addition, the directly measurable effects were much stronger, such as the effects of intravenous injection of insulin. One could conduct an experiment easily by injecting a hormone or removing a gland. This experimental reduction was less feasible in the nervous system in vivo; it seemed a major obstacle to govern the experiment without inducing uncontrolled counterregulatory mechanisms or major side effects.

During this period a paradigm shift occurred: endocrinology grew out to an independent area of research and specialization of medicine, focused on hormones and becoming dissociated from neuroscience. Extensive endocrinological research of the 20th century revealed an amazing amount of knowledge about the role of hormones in energy homeostasis. However, the open question of what coordinates body homeostasis has concerned more and more researchers over the past decades. Today, a re-unification of neurosciences and endocrinology is starting to develop which may eventually allow an insight into the “big picture” of energy homeostasis. In this chapter, we will focus on the role of the brain as a coordinator of metabolism, using both neurons and hormones to communicate with the body. We will describe the coordinating function of the brain and provide mechanisms that maybe causing the metabolic syndrome and type 2 diabetes.

THE BIOLOGICAL CLOCK MODULATES HYPOTHALAMIC INTEGRATION

One basic principle of central nervous integration is the active filtering of noise. The brain decides which stimuli are relevant. E.g., the time of the day defines how a certain stimulus will reach the hypothalamus and even when it reaches the hypothalamic integration sites, the time will determine the level of response. The blood-brain-barrier (BBB) actively selects humoral factors that can pass to the hypothalamus. E.g. leptin and TNF-alpha get access to the brain depending on the time of the day. This circadian filter can help us to order and direct the flood of information.

Evolution forced us to develop adaptive body functions to survive a hostile world. The daily switch between light and dark and the outside temperature forced mam-
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Animals to develop a circadian rhythm generator that saves energy and avoids predation by specialization to an active and an inactive period. The central biological clock is situated in the suprachiasmatic nucleus of the hypothalamus (SCN), adjacent to the optic chiasm, from where the SCN receives light information from the eyes through the retinohypothalamic tract. Our biological clock oscillates the body between an inactive phase for regeneration and preparation and an active phase, in which energy is invested in physical activity (e.g. hunting).

Here, we will focus on the modulation of the hypothalamic output by the SCN. The central clock receives information from the external environment about the time of the day e.g. by light. Humoral input from the internal environment enables the SCN to read the internal synchronization message of the body, such as melatonin. In return, the SCN transports its time-of-the-day message throughout the body via various hormones, such as corticosterone and melatonin. The SCN communicates its state of the day selectively through the sympathetic and parasympathetic branches of the autonomic nervous system. In addition, the SCN modulates the activity of neurons within the hypothalamus itself.

SCN-lesions in animals result in the disruption of circadian behavioral and metabolic rhythms, leading to flattened hormonal rhythms and an equal distribution of locomotor activity during the day and the night. Since SCN transplants in a semi-permeable membrane, preventing neuronal sprouting, could restore locomotor activity in SCN-lesioned hamsters, one could reason that its message is broad and unspecific. However, later experiments also revealed that transplants were unable to restore hormonal rhythmicity in cortisol and gonadal function, illustrating the complexity of the regulation of hormones by the SCN. Neuroanatomical and functional studies revealed that the SCN uses different sets of hypothalamic neurons to deliver selective messages to other brain regions. The target areas can be divided into four functional groups of neurons.

1. Hypothalamic neurons projecting to the pituitary axes involved in the hormonal control of the body.
2. Hypothalamic neurons projecting to the autonomic nervous system involved in the neuronal control of the body. Note that the SCN is able to affect the sympathetic and parasympathetic branch selectively via separate projections.
3. Hypothalamic neurons of integrative centers involved in e.g., energy homeostasis and temperature regulation, such as the dorsomedial nucleus and the medial preoptic area, putatively building an intermediate step between the SCN and the hormonal or neuronal output signal via the neurons of 1. and 2.
4. Thalamic neurons in the lateral geniculate nucleus and the paraventricular nucleus, synchronizing hypothalamic-induced behavior with locomotor activity.
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CIRCADIAN OUTPUT: THE BRAIN AFFECTS BODY FUNCTIONS

The adrenal - a model for the dual control of an organ

The central clock uses hormones as an output signal to the adrenal - sufficient to predict corticosterone levels?

Corticosterone and glucose peak in the beginning of the active phase, just one or two hours before the central clock has planned to awaken us\(^1\). This so-called dawn phenomenon prepares us for the new day\(^2\). How is this rise in corticosterone accomplished? The SCN modulates the cascade of corticotrophin-releasing hormone-containing neurons that stimulate the secretion of adrenocorticotropic hormone from the pituitary, which in turn results in corticosterone secretion from the adrenals, well-known as the hypothalamus-pituitary-adrenal axis\(^3\). However, activity of the HPA-axis cannot explain the pattern of corticosterone secretion as a whole: 24-hour plotting of ACTH against corticosterone levels in plasma reveals that the sensitivity of the adrenal for ACTH is restricted to the active period\(^4\).

The parallel output signal of the central clock: neuronal projections to the adrenal

The SCN talks to the body not only via the pituitary, but also via the autonomic nervous system. With the aid of neuroanatomical tools such as retrograde tracers, central neurons controlling a particular organ can be visualized. The retrograde transneuronal tracer Pseudorabies virus travels against the direction of the neuronal signal and crosses synapses, which enables it to identify a chain of neurons in control of a particular organ. Using this technique, for the first time, a multisynaptic projection from the SCN to the adrenal by the autonomic nervous system was demonstrated\(^5\). Moreover, physiological experiments revealed that the function of this multisynaptic pathway from the SCN to the adrenal cortex is to modulate its sensitivity for ACTH\(^6\).

We propose that fat tissue is also controlled by hormones and neurons. In chapters 2 and 4 we describe the projections from the biological clock via the sympathetic and parasympathetic nervous system to fat tissue. In chapter 2 we study the physiological impact of vagal input to fat tissue.

The SCN and the oscillating body

Potentially, all cells of the body have clock genes forming an intracellular clock. Here, we review data indicating that the central clock sets the peripheral clocks on time by means of neurons and hormones\(^8\).

The neuroanatomical substrate for neuronal control was shown recently by experiments using Pseudorabies virus. The SCN has specialized neurons affecting the sympathetic or the parasympathetic branch\(^9\). Neuroanatomical tracer studies revealed a multisynaptic pathway from the SCN to various organs, e.g. heart, pancreas, liver, thyroid and pineal\(^1^{0-21}\).
The best-studied peripheral circadian oscillator is the pineal; here the SCN uses the autonomic nervous system to induce melatonin secretion. Recently, the sympathetic nervous system has been demonstrated to affect clock gene expression in the liver. Physiological studies on liver and pancreas revealed a circadian rhythm in glucose, insulin and glucagon secretion, induced by the SCN and modulated by food intake. Also daily levels of the fat-derived hormone leptin are driven by the SCN. Within the cardiovascular system, heart rate, blood pressure, QT-interval length and k+ channels show a circadian rhythm.

It is attractive to assume that the biological clock might be the basis for the observation that body performance during exercise is best at the time of the day where training was regularly performed. In order to test the hypothesis that peripheral metabolic information is communicated to the clock by neural pathways, in addition to humoral factors, we investigated the neuronal feedback from fat tissue to the brain in chapter 3.

Another group of peripheral non-circadian oscillators is illustrative of the neuronal induction of organ rhythmicity. The sympathetic nervous system has been shown to induce 10 rapid oscillations per hour in lipolysis. In a series of studies on the endocrine pancreas, precise analysis of insulin and glucagon levels in the portal vein also revealed a pulsatile secretion pattern.

**The hypothalamus-autonomic nervous system-body-axis: simply top-down?**

The central role of the hypothalamus in the control of energy homeostasis was deduced from lesion studies. Stereotactic lesions in the region of the ventral medial hypothalamic nucleus cause overfeeding and obesity, whereas lesions in the lateral hypothalamic area result in an anorexia-cachexia syndrome. These data suggest a simple one-way top-down control of the body by the hypothalamus in the control of energy homeostasis.

However, as the brain senses the body on all its integrative layers via humoral factors and afferent nerves, it also does so on the level of the autonomic motor neurons in brainstem and spinal cord. As reviewed by Grill and Kaplan, the decerebrate animal model with a hypothalamus disconnected from the brainstem sheds light on the autonomic integration of autonomic afferents and efferents. The isolated autonomic nervous system is capable of coordinating oral movements and digestion, resulting in similar weight with intra-orally placed meals as compared to intact rats. Interestingly, the isolated autonomic nervous system is not capable to compensate for food deprivation, as demonstrated by intact rats increasing their meal size but not the decerebrate rats. Thus, while the autonomic nervous system can function autonomously in the short-term control of food intake, the hypothalamus is needed for long-term coordination.
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We propose that the hypothalamic-brainstem interaction is a system in control of energy homeostasis, built up of nested autonomous units that are modulated by higher integrative layers. In chapters 2, 3 and 4 we discuss the hypothalamic network projecting to fat tissue.

The autonomic nervous system differentiates between functional body compartments

The purpose of the SCN is to anticipate fluctuations in the external environment in order to keep the internal environment stable. However, the central clock needs to activate or silence tissues, depending on their function at different time points of the day. For example, muscles work in the active phase, when the digestive tract slows down. Therefore, opposite autonomic tone on vasculature redirects blood away from the abdominal compartment towards the movement compartment, whereas cerebral blood flow is kept constant. In contrast, at night the SCN slows down heart function, resulting in a dip in blood pressure.

How can different regions of the body be controlled selectively? The brain has two avenues of communication: hormones and neurons. Hormones are present throughout the body and obtain their specificity by acting on receptors with a tissue-specific, fluctuating expression, whereas neurons deliver their message to a precisely targeted tissue in the body.

We propose that the body can be divided into different functional autonomic compartments and that at least a thoracic and movement compartment and a visceral compartment should exist. In this setting, a balanced and flexible autonomic nervous system can oscillate the activities of the organs within the compartments according to the actual needs of the body.

In chapters 2 and 3 we demonstrate that the autonomic nervous system differentiates between intra-abdominal and subcutaneous fat tissue.

In chapter 3 we reveal that the abdominal organs share autonomic control.

In chapter 5 we investigate the role of the brain in body fat distribution. We study whether estrogen and glucocorticoid receptors are expressed by pre-autonomic neurons that project to fat tissue. This might provide an anatomical basis for the effect of these hormones on fat tissue metabolism and body fat distribution.

In chapter 6 we propose that the metabolic syndrome is caused by a disturbed central clock.

In chapter 7 we speculate whether HIV-related lipodystrophy might be caused by toxicity of HIV or HIV-therapy on central (pre)autonomic neurons projecting to fat tissue.

In chapter 8 we suggest follow-up studies for the present thesis.
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