Hypothalamic functions in patients with pituitary insufficiency
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Chapter 1

General Introduction
THE PITUITARY

The pituitary gland is a small, pea-sized endocrine gland weighing 0.5 gram. It is located at the base of the brain in the sella turcica, a cavity of the sphenoid bone, and is functionally connected to the hypothalamus via a slender, funnel-shaped structure: the pituitary stalk. Together with the hypothalamus it is often referred to as the ‘master glands of the body’ since these structures orchestrate the activities of peripheral endocrine glands and are able to regulate various homeostatic processes.

The gland consists of an anterior lobe (adenohypophysis) and a posterior lobe (neurohypophysis). The adenohypophysis contains five major cell types that produce six traditionally recognized hormones: corticotropes that secrete adrenocorticotropic hormone (ACTH), thyrotropes that secrete thyroid stimulating hormone (TSH), gonadotropes that secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH), somatotropes that secrete growth hormone (GH) and lactotropes that secrete prolactin (PRL). The neurohypophysis consists mainly of neuronal axons extending from the supraoptic and paraventricular nuclei of the hypothalamus. These axons are able to release the hormones oxytocin (OT) and vasopressin (AVP or ADH) into the circulation (figure 1). In addition to axons, the neurohypophysis also contains pituicytes: specialized glial cells resembling astrocytes (1).

Figure 1. The anterior pituitary produces adrenocorticotropic hormone (ACTH), thyrotropic hormone (TSH), luteinising hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), and growth hormone (GH). Their secretion is regulated by hypothalamic releasing and inhibiting factors and by negative feedback inhibition of their peripheral hormones. The posterior pituitary is a storage organ for the hypothalamic hormones antidiuretic hormone (ADH) and oxytocin.

Adapted from HJ Schneider (10).
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Pituitary insufficiency

Pituitary insufficiency, first described by the German physician Dr Morris Simmonds in 1914 (2), is the inability of the pituitary gland to provide sufficient hormone concentrations adapted to the needs of the organism (3). It is caused by either an inability of the gland itself to produce hormones or by an insufficient stimulation by hypothalamic releasing hormones (4). Pituitary insufficiency is a rare condition with an estimated incidence of 4.2 cases per 100,000 per year and a prevalence of 45.5 per 100,000 (5).

The most common cause of pituitary dysfunction are pituitary adenomas. They are classified by size (microadenomas <10mm and macroadenomas >10mm) and by their ability or inability to produce hormones (functioning and non-functioning adenomas). The secreted hormone is determined by the underlying cell type of the pituitary that has expanded to form the adenoma. In addition to pituitary adenomas, various other conditions can disrupt normal pituitary functioning. These conditions include brain damage (for instance traumatic brain injury, neurosurgery, cranial radiotherapy (CRT), stroke), non-pituitary tumours (craniopharyngioma, meningioma, glioma, chordoma, ependymoma, metastasis), infection (abscess, hypophysitis, meningitis, encephalitis), infarction or bleeding (Sheehan’s syndrome, apoplexy), autoimmune disorder (lymphocytic hypophysitis), perinatal insult, pituitary hypoplasia, and a number of genetic causes (6-10).

Pituitary insufficiency may exist on a subclinical level, revealed only by biochemical assessment of hormone concentrations. However, its clinical onset can also be acute and severe, necessitating immediate hospital admission and intensive care. Severe deficiencies of ACTH, TSH or ADH are potentially life threatening and require direct attention to warrant timely diagnosis and hormone replacement (11-13), whereas LS/FSH- or GH deficiencies cause chronic morbidity (14,15).

Signs and symptoms of the underlying disease may sometimes accompany hormonal imbalances in patients with pituitary insufficiency. Tumour masses in the sellar region with suprasellar extension can cause compression of the optic chiasm (CC), resulting in visual field defects. This visual impairment is mostly slowly progressive and has the classical presentation of bitemporal hemianopsia, but also unilateral field defects occur. Other symptoms of tumour masses are headaches and impairment of cranial nerve function (nn. III, IV and VI) in case of lateral extension, although the latter is rare (16).

Treatment of pituitary insufficiency

The most common underlying cause of pituitary insufficiency is a tumour in the sellar region, which is treated when it gives rise to specific symptoms, such as visual field defects, headaches and/or excessive hormone secretion (5). Surgery is generally the first choice, except for prolactinomas which respond very well to pharmacological treatment with a dopamine agonist (17,18). CRT can be used to prevent tumour regrowth after surgery in patients with a non-functioning macroadenoma. Although pituitary irradiation
has proven to be a useful adjunctive treatment for hormone producing tumours (i.e. TSH-, GH- and ACTH secreting pituitary adenomas and resistant prolactinomas) it is used less often nowadays with the development of effective medical therapy, including somatostatin analogues and pegvisomant for GH producing adenomas, and SOM230 for ACTH producing adenomas (19,20). Pituitary function may recover after treatment of large tumours, although it is not uncommon that surgery and CRT further impair pituitary function (6,9,21).

Once pituitary insufficiency has been diagnosed, hormonal substitution therapy is necessary. ACTH deficiency, TSH deficiency and LH/FSH deficiency can be treated by administering the (synthetic) products of their effector glands, i.e. hydrocortisone for adrenal insufficiency, levothyroxine for hypothyroidism, testosterone for male hypogonadism and estradiol (usually in combination with progestogens) for female hypogonadism. GH-deficiency can be treated with recombinant GH, and ADH deficiency with a synthetic vasopressin analogue (10).

Functional impairments in patients with pituitary insufficiency

Adequate replacement of pituitary hormones prevents previously lethal deficiencies of cortisol, thyroid hormone and ADH and is successful as a treatment of pituitary insufficiency with respect to control the biochemical balance. However, patients with pituitary insufficiency often continue to experience some degree of physical or mental impairment, indicating that adequate hormonal plasma levels are not always correlated with the patients' sense of well-being. This is reflected by several cross-sectional studies in patients with pituitary insufficiency, which point towards a decreased quality of life, even during long-term remission or cure of the underlying disease and long-term biochemical control (22-30).

To obtain more insight in the factors that contribute to this phenomenon, efforts have been made to improve quantification of these often subjective complaints and impairments. For instance, increased daytime sleepiness quantified with sleep questionnaires has been reported in hypopituitary patients with a history of hypothalamic tumour, craniopharyngioma, non-functioning macroadenoma, traumatic brain injury and subarachnoid hemorrhage (31-34). In patients treated for a non-functioning macroadenoma these complaints are accompanied by a disturbed distribution of sleep stages and a disturbed circadian activity rhythm (35). Altered sleep quality measured with polysomnography has also been reported in hypopituitary patients with a history of craniopharyngioma, prolactinoma, acromegaly and Cushing’s disease (36-39). In addition, several studies showed that substitution of recombinant human GH and other hormones is insufficient to reverse all metabolic abnormalities seen in patients with hypopituitarism, including elevated body mass index, unfavourable waist-to-hip ratio and body composition, dyslipidemia and a higher risk of hypertension (40). Moreover, it has become clear that
patients treated for pituitary adenomas display different and less effective coping strategies compared with healthy controls (41).

In addition to the abovementioned physical and mental morbidities, all-cause mortality is increased in patients with hypopituitarism when compared with age- and sex-matched controls. Meta analysis showed that the standard mortality ratio associated with hypopituitarism in men is 2.06 [95% confidence interval (CI) 1.94 – 2.20] and in women 2.80 [95% CI 2.59 – 3.02] (42).

**Imperfections in hormone replacement therapy vs. hypothalamic functions**

Why a number of problems remain after biochemical restoration of hormonal balance is largely unknown. It may be related to intrinsic imperfections in hormonal substitution strategies (43). Despite the attempts of clinicians to reproduce endogenous hormone secretion profiles of healthy individuals by adjusting time, dose and method of administration, the diurnal rhythmicity and pulsatility of hypothalamic-pituitary hormone secretion cannot be fully mimicked in patients with hypopituitarism. Moreover, when measuring hormonal concentrations in serum and plasma, we cannot be sure that this is a good reflection of the effects of hormones at the tissue level. Therefore, it is likely that imperfections in hormone replacement strategies result in subtle physiological derangements (43), but other factors may also contribute to the higher morbidity and mortality in patients with pituitary insufficiency. Notably, there is an intriguing similarity between the functions affected in hypopituitary patients on the one hand and functions of the hypothalamus on the other.

**THE HYPOTHALAMUS**

The hypothalamus is a small brain structure of 4 ml in the diencephalon that is anatomically and functionally closely connected to the pituitary (44). It contains many groups of neurons, which form distinct nuclei (figure 2). These nuclei are involved in a multitude of functions in the body. Although depicted in early brain atlases, it was the Swiss anatomist Wilhelm His who first described the hypothalamus in 1893 as a distinct neuroanatomical entity (45). Discovery of the supraoptic-hypophysial tract by Ramon y Cajal in 1894, and the subsequent discovery of the concept of neurosecretion in fish by Ernst and Berta Sharrer in 1928, formed the foundation for a vast amount of research which has greatly improved our understanding of the anatomy and function of the hypothalamus (46).

The hypothalamus secretes thyrotropin-releasing hormone, corticotropin-releasing hormone, AVP, oxytocin and other neuropeptides that regulate pituitary function. Distinct hypothalamic nuclei also play a pivotal role in eating behaviour, energy homeostasis, diurnal rhythmicity and autonomic nervous system outflow. Additionally, the hypothalamus is part of the limbic system, which controls mood and behaviour (44).
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Eating behaviour

Lesion studies in the 1940s already identified the ventromedial hypothalamus as the “satiety centre”, and the lateral hypothalamus as the “hunger centre”. Parabiosis experiments in rats in the 1950s pointed to the existence of important humoral signals in the regulation of long-term energy balance. The latter concept was confirmed by the discovery of the adipocyte-derived hormone leptin in 1994 and by the subsequent demonstration of leptin receptor expression in the hypothalamus (47). Over the past decade, several neuropeptides, receptors, and transcription factors have been identified that mediate leptin’s effects, including the hypothalamic melanocortin system which plays a crucial role in body weight regulation. The infundibular nucleus, situated in the mediobasal hypothalamus, plays a central role in various aspects of energy balance. Rodent studies demonstrated that neurons within this nucleus (called arcuate nucleus in rodents) respond rapidly to nutritional signals and form two subsets with opposite effects on feeding and energy expenditure. One set of neurons expresses proopiomelanocortin, a precursor polypeptide which gives rise to melanocyte-stimulating hormone (α- and β-MSH) and ACTH. Both α-
and β-MSH reduce food intake and increase energy expenditure (48). The other set of neurons expresses neuropeptide-Y and the agouti-related peptide and stimulates feeding, while reducing energy expenditure (47). Although much information on these hypothalamic signalling pathways has been obtained in rats and mice, the functional neuroanatomy of these neuropeptidergic systems appears to be remarkably similar in the human hypothalamus (49-51).

**Energy homeostasis**

It was Claude Bernard who first showed in 1854 that the brain is involved in the regulation of glucose metabolism when he noticed that rabbits develop diabetes following a lesion in the floor of the fourth cerebral ventricle. More recent studies showed that administration of glucose to the hypothalamus without changing plasma glucose inhibits the production of triglyceride-rich lipoproteins by the liver (52). Moreover, activation of pre-autonomic neurons in the paraventricular nucleus (PVN) increases hepatic glucose production by activation of the sympathetic signal to the liver (53). These observations confirmed that the hypothalamus has the capacity to alter peripheral energy metabolism and that the autonomic nervous system is instrumental in the hypothalamic control of hepatic glucose production.

Bruinstroop et al. demonstrated that the hypothalamus and autonomic nervous system are also necessary in controlling hepatic lipid metabolism. Specifically, the activation of neuropeptide-Y neurons in the hypothalamus during fasting has a stimulatory role on hepatic secretion of triglyceride-rich very low-density lipoproteins through the sympathetic nervous system (54). The autonomic nervous system also provides a functionally important link between pre-autonomic neurons in the hypothalamus and adipose tissue. Retrograde tracing experiments have indicated that subcutaneous and intra-abdominal adipose tissues receive autonomic projections from separate hypothalamic neurons, suggesting a potential role of the hypothalamus in body fat distribution (55,56).

**Temperature regulation**

Maintenance of body core temperature in warm-blooded species is critical to maintain physiological integrity. Warm-sensitive neurons in the medial preoptic area (POA) of the hypothalamus have a central role in the control of body core temperature (57). When body core temperature rises, the firing rate of these warm-sensitive neurons increases. This inducet a heat defence response that includes skin vasodilatation and sweating via a complex and incompletely understood pathway. Warm-sensitive POA neurons may also respond to cold by input from cold-receptors in the skin. Activation of these cold-receptors decreases their firing rate, lowering the tonic inhibition by POA neurons of cold-responsive neurons in the dorsomedial hypothalamus and raphe pallidus area, resulting in skin vasoconstriction and shivering.

The classic view of body temperature regulation presumes an integrative neuronal network that sets body core temperature by triggering cold and warm defence responses based on
input from central and peripheral thermoreceptors, but the location of this integrative network has remained unclear. Over the years, this classic view of body temperature regulation has been challenged by a model in which effector cells, i.e. neurons that trigger temperature defence responses, receive input from many sensory neurons (58). When the cumulative input reaches a certain threshold the effector cell is triggered and sets off a temperature defence response. This model does not require an integrative centre to control body core temperature and temperature sensations result from the activation of effectors and not the other way around.

Dysthermia, sometimes perceived as thermal discomfort, has been observed in patients with hypothalamic lesions. The extent and direction depend on the localization of the lesions. Temperature defence responses may be impaired and consequently patients can poorly adjust to changes in ambient temperature (59,60).

**Diurnal rhythmicity**

Many endocrine events (e.g. plasma GH secretion), behaviours (e.g. sleep/wake, feeding) and physiological phenomena (e.g. body temperature, blood pressure) display a 24-hr rhythm. The body’s master oscillator is the suprachiasmatic nucleus (SCN), a small bilateral hypothalamic nucleus located basolaterally of the third ventricle and just above the optic chiasm. It is thought that the SCN coordinates the activity of the peripheral oscillators via behavioural, neuroendocrine and autonomic pathways (61-64). The biological clock of the brain has an intrinsic rhythm of approximately 24-hours that is generated and maintained at the molecular level by transcription/translational feedback loops of several clock genes (65). This intrinsic rhythm is not exactly 24-hours. However, environmental light relayed from the retina through the retinohypothalamic tract is able to reset the master oscillator and, thus, prevents drifting out of phase with the exact 24-hour environmental rhythm. Also non-photic stimuli such as feeding, social interactions, temperature, sleep deprivation and exercise are able to entrain the SCN (66).

Lesions of the SCN of rodents result in ‘free-running’, which is characterized by disruption of the sleep-wake cycle as well as loss of predictable daily oscillations in feeding, drinking and secretion of some anterior pituitary hormones (67). The disrupted rhythm can be restored by transplanting back SCN tissue in lesioned animals (68). Human case reports also showed that lesions of the SCN lead to disruption of circadian rhythmicity (69,70).

**Autonomic nervous system outflow**

Selective lesions or electrical stimulation of different parts of the hypothalamus may elicit the well-known sympathetic “flight-or-fight” reaction with pupillary dilation, tachycardia and vasodilatation of skeletal muscle vessels, or the parasympathetic “rest-and-digest” response with pupillary constriction, increased visceral vascular flow and bradycardia (44,71). Recently, a previously unknown population of parvalbuminergic neurons in the anterior hypothalamus has been described, involved with the autonomic control of blood pressure and heart rate (72). Moreover, the autonomic outflow from the hypothalamus
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appears to be of crucial importance for the regulation of hepatic and adipose tissue metabolism. Specifically, neural connections between (pre-)autonomic neurons in the PVN that connect to the liver and white adipose tissue either via sympathetic brain stem nuclei or via the dorsomedial nucleus of the vagus, modulate peripheral carbohydrate and lipid metabolism (44,52,73).

Mood and behaviour
The hypothalamus also plays an important role in the limbic system. The limbic system represents a neuronal network that, on the functional level, regulates processes important to survival, and thus, to maintenance of homeostasis. This is reflected in a tight regulation of the stress response, and of sexual and reproductive behaviour. Besides the hypothalamus, the limbic system encompasses the prefrontal cortex, the hippocampus, the amygdala, septal- and preoptic regions, and the anterior and medial thalamus. Structurally, the hypothalamus is intimately interconnected with certain limbic structures, like the hippocampus, the amygdala, and the mediodorsal nucleus of the thalamus (74). For instance, the production and release of corticotropin releasing hormone from parvocellular neurons in the PVN is under direct control of glucocorticoid receptor expressing neurons of the hippocampus. These neurons have direct axonal projections to the PVN (75,76). Similarly, noradrenergic neurons from the amygdala and the bed nucleus of the stria terminalis project to the PVN for correct processing of arousal (77). Consequently, hypothalamic damage may affect emotional and cognitive function, and other types of behaviour, like memory, learning, and motivation.

Hypothalamic dysfunction
Inherent to the wide range of hypothalamic functions, lesions in the hypothalamus may give rise to a variety of metabolic, endocrine and behavioural abnormalities. For instance, severe obesity as a consequence of acquired structural hypothalamic damage has been described in several hundreds of individuals over the course of more than a century (78) and several cases showed anatomical hypothalamic damage in combination with poikilothermia, drowsiness, hyperphagia, depression and/or memory loss (79). These symptoms, following extensive injuries to the hypothalamus, are easily recognized by the physician and prompt for targeted therapy such as stringent food restriction or regulation of the ambient temperature to prevent dysthermia. However, less distinct symptoms including subtle disturbances of the sleep/wake cycle, slowly progressive obesity or autonomic dysregulation may easily be overlooked or unjustly attributed to suboptimal supplementation of pituitary endocrine deficiencies. This is difficult to improve upon as tests or functional imaging modalities to assess the integrity of hypothalamic functions are not available in standard clinical practice. Nonetheless, it is highly likely that expanding pituitary tumours or their treatment attribute to subtle hypothalamic injury and dysfunction.
For instance, visual disturbances caused by CC are a consequence of a large expanding tumour with suprasellar extension towards the hypothalamus (80). Despite improvement of vision regularly reported after decompression of the optic chiasm, it is conceivable that mechanic pressure of the tumour has induced permanent functional changes of adjacent tissues or structures, including the hypothalamus.

CRT has been used in the management of pituitary adenomas for more than a century (81), and has also been linked with a number of significant comorbidities (82). It is possible that CRT has permanent effects on hypothalamic neuronal function, as the hypothalamus is considered to be more vulnerable to radiation injury than the pituitary gland (83).

Inherent to the close neuroanatomical orientation of some sellar tumours, surgery may affect hypothalamic tissue not only due to the infiltrative nature of the lesion but also due to the surgical exposure itself, e.g. retractor damage (84).

AIM AND SCOPE OF THE PRESENT THESIS

Pituitary insufficiency is often associated with impairments such as fatigue, dyslipidemia, and energy loss despite adequate hormonal substitution therapy. Until now, it has remained unclear whether these symptoms are solely caused by (treated) pituitary insufficiency, or may be ascribed in part to hypothalamic injury as well, as a consequence of CC or as a consequence of treatment of the sellar tumour. Therefore, the aim of the present thesis was to increase our understanding of hypothalamic (dys)function in patients with pituitary insufficiency with a history of CC, CRT and/or pituitary surgery. As no tests or imaging modalities are available to assess the integrity of hypothalamic function, we addressed this research question in three ways.

First, we studied the effects of CRT and CC on several parameters affected by the hypothalamus, i.e. visceral and abdominal fat distribution, baroreflex sensitivity (BRS), skin temperature and sleep. Endocrine deficiencies, like GH and estrogen deficiency, are likely candidates to explain increased visceral to subcutaneous fat ratio in patients with pituitary insufficiency. However, recent reports pointed to CRT as an additional determinant of an unfavourable fat distribution. Therefore, we determined in chapter 2 the effect of CRT on abdominal fat distribution in men with treated pituitary insufficiency. CRT has also been linked to cardiovascular morbidity and mortality in patients with pituitary insufficiency. We decided to assess BRS as a marker for cardiovascular risk in patients with pituitary insufficiency. BRS is a measure of changes in heart rate in response to changes in blood pressure and can be modulated by distinct pre-autonomic neurons in the hypothalamus. In chapter 3 we investigated whether a history of CRT in patients with pituitary insufficiency affects BRS.

The SCN is crucially involved in the circadian timing of sleep-wake rhythm, and the adjacent POA accommodates the most important thermoregulatory neuronal network. As these
hypothalamic nuclei are closely situated to the optic chiasm, we aimed to elucidate in chapter 4 and chapter 5 whether CC affects skin temperature and sleep.

Second, we investigated human post-mortem hypothalamic tissue by means of immunocytochemistry. We were in the unique position to study post-mortem hypothalamic tissue of five patients with a suprasellar tumour inducing permanent visual field defects. In chapter 6 we explored whether immunoreactivity of two key neuropeptides of the SCN, i.e. AVP and vasoactive intestinal peptide, is altered in patients with a suprasellar tumour inducing permanent visual field defects as compared with control subjects.

Post-mortem hypothalamic tissue was also used to study the serotonergic system. Serotonergic signalling has been implicated in various hypothalamic functions including circadian rhythmicity, feeding behaviour and neuroendocrine regulation. Indeed, these functions show many similarities with the impairments of hypopituitary patients which raises the question whether the serotonergic system is affected in these patients. As the functional neuroanatomy underlying serotonergic signalling in the human hypothalamus is largely unknown, we studied the distribution of the serotonin transporter in human hypothalamus for the first time. These results are described and discussed in chapter 7. Finally, we explored hypothalamic serotonergic transporters with a nuclear imaging technique, i.e., single photon emission computed tomography in chapter 8. In addition, we investigated whether hypopituitary patients with a history of CC, CRT and surgery show altered serotonergic hypothalamic neurotransmission using this technique. We compared the results with hypopituitary patients without a history of CC, CRT or cranial surgery and with age- and gender matched controls.

In chapter 9 we summarized the results of this thesis and aimed to put our findings into a broader perspective.


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