Hypothalamic functions in patients with pituitary insufficiency
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Chapter 9

Summary and General Discussion
The main objective of this thesis was to increase our understanding of hypothalamic (dys)function in patients with pituitary insufficiency. This goal was driven by the clinical experience of persisting symptoms in patients adequately treated for pituitary insufficiency. We focused primarily on patients who had a history of compression of the optic chiasm (CC), cranial radiotherapy (CRT) and/or pituitary surgery to optimize the chance of the presence of functional damage to the hypothalamus.

At present, there are hardly any studies on hypothalamic post-mortem specimens from these patients. A major methodological dilemma studying hypothalamic function in vivo is the limited specificity of clinical and biochemical tests reflecting hypothalamic functions. As a consequence, disentangling hypothalamic function in patients with pituitary insufficiency is very challenging. To assess hypothalamic functions we made use of a variety of techniques focusing on 1) hypothalamic output parameters (chapters 2, 3, 4 and 5), 2) human post-mortem hypothalamic tissue specimens (chapters 6 and 7) and 3) nuclear imaging of the brain (chapter 8). This chapter provides a summary of the findings presented in this thesis, followed by a general discussion.

**SUMMARY**

**Chapter 1** provides an introduction to the anatomy of the pituitary gland and to the treatment of pituitary insufficiency. Furthermore, the hypothalamus and its main functions are briefly reviewed. We discuss the impairments perceived by patients with pituitary insufficiency and present our general hypothesis.

**The effect of cranial radiotherapy on hypothalamic output parameters**

Experimental studies suggest that neurons in the ventromedial hypothalamus regulate visceral fat content independently of food intake by modulating the sympathetic tone (1). The anatomical basis for his phenomenon is that distinct subsets of pre-autonomic neurons within the hypothalamus project to the visceral and subcutaneous fat compartments via the autonomic nervous system, representing a neuroanatomical network by which the hypothalamus may control body fat distribution (2-4). Therefore, we investigated abdominal fat distribution in chapter 2 as hypothalamic output parameter, and demonstrated that a history of CRT is associated with a higher visceral to subcutaneous fat ratio in men with pituitary insufficiency. An additional hypothalamic output parameter that we studied in the present thesis is baroreflex sensitivity (BRS). This parameter reflects the capability of the autonomic nervous system to detect and respond effectively to acute changes in blood pressure by changing heart rate (5). In chapter 3 we compared BRS in hypopituitary patients with a history of CRT to hypopituitary patients without a history of CRT. Our data indicates that a history of CRT is associated with a decreased BRS, which suggests
that CRT in patients with pituitary insufficiency causes reduced capability to increase parasympathetic activity and/or to antagonize sympathetic activity (5).

The effect of compression of the optic chiasm on hypothalamic output parameters

Suprasellar tumours frequently cause pituitary insufficiency as well as visual impairments due to CC. In close proximity to the optic chiasm is the hypothalamic suprachiasmatic nucleus (SCN). This hypothalamic nucleus has been identified as the main regulatory centre of sleep, and of circadian organization in general (6,7). Hypothetically, expanding tumours not only compress the optic chiasm, but also the SCN, which harbours the biological clock. Therefore, we hypothesized that sleep patterns are altered in hypopituitary patients with a history of CC. Indeed, in chapter 4 we demonstrated that CC is associated with permanent shorter sleep duration in hypopituitary patients, which could not be attributed to differences in hormonal replacement therapy. Another hypothalamic output parameter that we studied is skin temperature (chapter 5). The preoptic area, a hypothalamic region situated in close vicinity of the SCN, is critically involved in temperature regulation (8). Of note, several studies reported an association between sleep and fluctuations in skin temperature (9-14). For instance, skin temperature modulates the effect of early morning light on sleep (15), and mild skin warming enhances sleep propensity (16-19). Based on the observation that hypopituitary patients with a history of CC have shorter sleep duration (chapter 4), we hypothesized that thermoregulation and its association with sleep would be disturbed in this group as well. Our main finding was a lower proximal skin temperature during the day in hypopituitary patients with a history of CC. In addition, the typical correlation between sleep onset latency (i.e. the required time between lights out and the onset of sleep) and pre-bedtime distal-to-proximal skin temperature gradient (9,20) was absent in these patients, whereas it was clearly present in those without CC as well as in healthy subjects. Thus, patients with CC show impaired skin temperature regulation in association with disturbed sleep.

The effect of expanding sellar tumours on human post-mortem hypothalamic tissue

To further confirm our hypothesis that expanding suprasellar tumours impair the function of the SCN (chapter 4), we investigated the expression of two key neuropeptides in that area, i.e. arginine vasopressin (AVP) and vasoactive intestinal peptide, as assessed by quantitative immunocytochemistry in post-mortem hypothalamic tissue specimens of patients with a suprasellar tumour inducing permanent visual field defects. We showed in chapter 6 that AVP-immunoreactivity in the SCN was lower in patients with a suprasellar tumour than in controls. This raises the intriguing possibility that selective impairment of SCN neurons contributes to sleep-wake disturbances in these patients, although this is highly speculative.
Serotonin transporters in the hypothalamus

Serotonergic signalling has been implicated in various hypothalamic functions including circadian rhythmicity, feeding behaviour and neuroendocrine regulation (21-24). These functions show remarkable similarities with functional impairments in hypopituitary patients, which rises the intriguing question whether the hypothalamic serotonergic system is affected in these patients. As a first step to address this research question, we studied the functional neuroanatomy of serotonergic signalling in post-mortem hypothalamic tissue specimens in chapter 7. Serotonin transporters (SERT) were ubiquitously expressed in fibers throughout the hypothalamus, with a very dense track of fibers in the perifornical area, as a plexus along the ependyma and in close proximity to the anterior commissure. Moreover, the SCN and the infundibular nucleus showed strong SERT-immunoreactivity, suggesting a local modulatory role for serotonin in these hypothalamic nuclei. We then went on to study in chapter 8 hypothalamic specific-to-nonspecific [$^{123}$I]FP-CIT binding ratios in vivo using single photon emission computed tomography, in hypopituitary patients with a history of surgery, CRT and CC. We compared the results with similar data obtained in hypopituitary patients without hypothalamic damage and in healthy control subjects. We hypothesized that SERT expression was altered in response to CC and treatment of a sellar tumour. However, we could not detect a difference between the groups. This may be due, at least in part, to technical limitations.

GENERAL DISCUSSION

Cranial radiotherapy and hypothalamic impairment

We showed that CRT is associated with alterations in BRS and body fat distribution (chapters 2 and 3). An important question is whether these findings are related to hypothalamic impairment. It is well-known that CRT delivered to the pituitary may induce adverse effects. The development and severity of such side-effects depend on the combination of the vicinity of healthy tissue to the radiation target, the type of tissue, the dose and fractioning of CRT. Damage can induce immediate lethal effects on cells, or induce accumulating sublethal damage that limits the potential for cell replication (25). Cell death after radiation which occurs in the mitotic phase of the cell cycle (late G2-early S), may be the reason for a delayed onset of chronic complications of radiation in slowly-replicating cells, such as those present in the hypothalamic-pituitary unit (26,27). Ionising radiation is known to be able to induce degenerative changes in glial cells, leading to a lack of tropic neural support and demyelinisation, which in turn may cause subacute and chronic neural damage in the hypothalamus.

By far the most common side effect of pituitary radiation is hypopituitarism, with a five-year incidence of approximately 20% (28). Furthermore, visual complications due
to optic neuropathy (29), neurological symptoms, vascular problems resulting in stroke (30) and secondary tumours (31) are seen, although the incidence of these complications is probably less than 1% (32). Evidence for hypothalamic injury after irradiation has been provided by studies on the effects of CRT on the lactotropic axis. Lactotrophs are prolactin producing pituitary cells that receive predominantly inhibitory signals from the hypothalamus. It has been noted that prolactin levels increase after CRT, but that hyperprolactinemia does not occur when lactotrophs were directly damaged, suggesting hypothalamic injury or hypohalamus-pituitary disconnection after irradiation (33). Another clue came from studies on the differential effects of pituitary brachytherapy compared with CRT. Follow-up studies of patients treated with brachytherapy (instillation of Yttrium-90 in the sella turcica, total dose 50-150Gy) for pituitary adenomas revealed a reduced risk of hypopituitarism when compared with external CRT with a total dose of 37.5 – 45Gy (34,35). Moreover, serum prolactin levels did not show an increase after brachytherapy (36). The lower incidence of hypopituitarism after sellar brachytherapy despite higher total radiation doses when compared with CRT and the preservation of normal prolactin levels suggest a lower threshold for radiation induced damage in hypothalamic tissue.

Both BRS and abdominal fat distribution are influenced by the hypothalamus. As the hypothalamus is situated in close vicinity to the sella turcica and is susceptible to radiation induced toxicity (37), it is conceivable that CRT for sellar tumours also inflicts some degree of damage to the hypothalamus. Consequently, this may lead to changes in hypothalamic function, resulting in altered BRS and abdominal fat distribution as shown in chapters 2 and 3. In conclusion, based on our results in chapters 2 and 3 and on the observations by others we propose that hypothalamic impairment may result from CRT tumours in the sellar region.

**Expanding sellar tumours and hypothalamic impairment**

The reported alterations in sleep and skin temperature in patients with a history of CC (chapters 4, 5 and 6) contribute to our understanding of the functional impairments in patients with pituitary insufficiency. On the basis of our results, we postulate that changes in sleep and skin temperature were in essence caused by the expanding sellar tumour. Thus, growing tumours induce mechanical pressure to adjacent structures including the SCN, preoptic area and retinohypothalamic tract. This then causes impaired function of these hypothalamic nuclei, leading to alterations in circadian rhythmicity and skin temperature. In line with this assumption, we found lower AVP-immunoreactivity in the SCN in patients treated for a suprasellar tumour (chapter 6). Injury to surrounding tissue is highly conceivable, as tumour growth within the sella results in increased tissue pressure that can reach levels as high as 60mmHg (38), whereas normal intrasellar pressure is believed to be similar or less than the normal intracranial pressure of 7-15mm Hg (39,40). Additional studies have highlighted several consequences of mechanical pressure due to growing tumours. Visual field defects due to CC (41,42), mild hyperprolactinemia and
loss of pituitary hormone secretion have been demonstrated in patients with pituitary stalk compression (43,44). Also, increased intrasellar pressure is supposed to be a major mechanism involved in the pathogenesis of headaches (38). Furthermore, patients treated for a non-functioning macroadenoma, who had visual field defects preoperatively, display impaired quality of life with increased fatigue, daytime somnolence, disturbed distribution of sleep stages and disturbed circadian movement rhythm, possibly due to impaired SCN function (45-48). Together, these studies support the concept that hypothalamic function may be altered by expanding pituitary tumours giving rise to CC.

**Serotonergic neurotransmission and hypothalamic impairment**

The rationale to study the hypothalamic serotonergic neurotransmission (chapters 7 and 8) was based on the striking similarity between the functions of the hypothalamus considered to be influenced by the serotonergic system and the functions affected in patients with pituitary insufficiency, including the regulation of circadian rhythms, appetite, obesity, and neuroendocrine responses (21-24). However, very few data are available on the functional neuroanatomy underlying serotonergic signalling in the human hypothalamus. Therefore, we took a first step to explore SERT expression in post-mortem hypothalamic tissue (chapter 7). We demonstrated that SERT was abundantly expressed in fibers throughout the hypothalamus. As a next step, we confirmed this finding in the human hypothalamus in vivo, showing SERT binding selectively in the hypothalamus of healthy subjects by fusing SPECT-scan with conventional MRI (chapter 8). Once we had demonstrated SERT in the human hypothalamus, we studied SERT binding in patients treated with cranial surgery and CRT for an expanding sellar tumour inducing visual field defects. We hypothesized that subjects suffering from hypothalamic impairment may have impaired serotonergic neurotransmission. Contrary to our expectations, hypothalamic binding ratio of SERT was not altered in those patients. The obvious conclusion is that serotonergic signalling is not involved in the pathophysiology of hypothalamic impairment. However, we cannot exclude that the effect was more subtle than the current design was able to detect. Therefore, a larger study would be needed to confirm our result. Another limitation is that only one molecular component of this system, i.e. the SERT, has been studied. Although the concentration of transporters is believed to reflect the homeostatic tone of neurotransmitter systems (49), it will be interesting to study serotonin release by fenfluramine-challenge (e.g. as in (50)) as well as synaptic serotonin levels using depletion with tryptophan or para-chlorophenylalanine to get a more complete picture (51).

**Alternative hypotheses**

The results presented in this thesis provide important clues that help to disentangle the question whether the function of the hypothalamus is impaired as a consequence of an expanding sellar tumour, CRT treatment and/or CC. It should be noted, though, that our studies were all cross-sectional, which hampers the possibility to study causal relations.
Moreover, despite a large body of evidence showing a relation between (treatment of) sellar tumours and hypothalamic (dys)function, conclusive evidence of hypothalamic impairment in patients with pituitary insufficiency has not been given to date. Therefore, the hypothesized direct relations between hypothalamic dysfunction and (treatment of) expanding sellar tumours can be questioned.

If hypothalamic dysfunction is not directly related to the results found in this thesis, which other factors could be involved as underlying mechanism? Traditionally, anterior pituitary deficiencies and their hormonal treatment have been considered as likely candidates to explain increased visceral to subcutaneous fat ratio (52-55), alteration of sympathovagal balance (56-58) and sleep disturbances (59-62) in hypopituitary patients. To control for the potential confounding effect of various hormonal deficiencies, we included only patients with pituitary insufficiency in chapters 2, 3, 4, 5 and added a control group with pituitary insufficiency in chapter 8. By doing so, groups were comparable concerning biochemical parameters, hormonal treatment regimens and endocrine deficiencies. This method minimizes the possibility that differences in hormonal substitution strategy account for the results obtained.

Alternatively, rather than injury to hypothalamic structures per se, other structures that are involved in sleep, temperature regulation, BRS or abdominal body fat distribution could be impaired. For instance, the baroreceptor signal is relayed via the glossopharyngeal and vagal nerves to the nucleus tractus solitarius. Neurons of this nucleus subsequently convey this signal via the ventrolateral medulla and rostral ventrolateral medulla in the lower brainstem to the spinal cord (5). It is conceivable that CRT injures non-hypothalamic parts or branches of the baroreflex loop, resulting in altered excitatory/inhibitory input to several pre-autonomic neurons of the hypothalamus. In addition, CRT may harm forebrain regions or fibers of passage originating from these forebrain regions, as those are also able to modulate the key medullary nuclei subserving the baroreflex (63,64). Another player may be the retinohypothalamic tract, which transmits photic information from the retina to the SCN in the hypothalamus and is involved in synchronization of the SCN to the light-dark cycle (65,66). Injury to this tract by the tumour or the treatment may inhibit entrainment of the circadian clock, resulting in altered sleep characteristics.

A final possibility is melatonin secretion, which plays a role in the regulation of human sleep (67-70). The pathway involved in melatonin secretion starts in the SCN, and travels via the paraventricular nucleus and the intermediolateral nucleus of the upper thoracic spinal cord to the superior cervical ganglion and finally to the pineal gland (71,72). Injury to part of the extrahypothalamic pathway involved in melatonin excretion is a possible mechanism explaining the effects on sleep (73).

**Implications for clinical practice**

Patients as well as doctors involved in the treatment of expanding sellar tumours are frequently unprepared for the development of several long-term side effects. Often, limited
education or support is provided to patients, as physicians focus primarily on adequate hormonal replacement therapy and may be oblivious to subtle functional and poorly understood impairments that are associated with pituitary insufficiency. Consequently, subtle derangements of the sleep/wake cycle, transient hyperprolactinemia, slowly progressive obesity or autonomic dysregulation in patients with pituitary insufficiency may be easily overlooked. We recommend that physicians be aware of the impairments, even after long-term remission of the initial disease, e.g., by postgraduate training and continuous medical education programs. Physicians should inform the patients about potential long-term consequences of expanding pituitary tumours, thereby contributing to the process of disease acceptance. In addition, some of the strategies discussed below may aid to ameliorate the impairments.

In the studies presented in chapter 2 and chapter 3, patients were treated with conventional external beam radiotherapy, which is the most frequently used method of CRT to treat large remnants of pituitary adenomas with evidence of progression after surgery or if surgery is not able to normalize excess of hormones (74). Relatively novel treatment modalities for pituitary tumours are stereotactic radiosurgery techniques and intensity-modulated radiation therapy that focuses high dose radiation at precise intracranial targets (75). These modalities have the clear advantage to offer a more precise radiation delivery in one dose in combination with a reduced amount of normal tissue exposed to high radiation doses compared with conventional methods. Potentially this can reduce the risk of long-term treatment-related morbidity like pituitary insufficiency, BRS and body fat distribution. However, long-term follow-up studies are needed to demonstrate their presumed superiority and efficacy (76).

In chapters 4, 5 and 6 we demonstrated that CC is associated with altered sleep and temperature regulation. Generally, the presence of visual field defects, severe headaches or excessive hormone secretion is a clear indication for immediate treatment (77), while asymptomatic patients are candidates for conservative ‘wait-and-see’ policy. The approach for asymptomatic patients seems to be safe for microadenomas because of a low growth propensity (78,79), but is questionable for macroadenomas as tumour enlargement is expected with increasing follow up periods (78,80,81). One may consider treating asymptomatic macroadenomas before they give rise to CC, as potentially this may reduce the risk of long-term effects such as visual abnormalities, altered sleep, temperature dysregulation and pituitary insufficiency. Moreover, early surgery may increase the chances of complete tumour removal, whereas postponement may allow tumours to reach dimensions or degrees of invasiveness which will make their resection more difficult. One should keep in mind, however, that surgical interventions may be accompanied by complications, although the incidence is low (82). Therefore, future randomized studies comparing immediate treatment of asymptomatic macroadenomas with the ‘wait-and-see’ approach are highly relevant.
Another interesting therapeutic approach is to enforce circadian regulation, in order to improve sleep (chapter 4). This can be achieved by a regular schedule for going to bed and waking up, and engaging in stimulating activities in a light environment immediately after waking up (83). Also melatonin and bright light therapy are ways to entrain the circadian rhythm. Exogenous melatonin has been used in subjects over 55 years of age and was shown to be effective in restoring normal sleep (84). Bright light therapy is another method to ameliorate sleep disturbances (85). Both melatonin and light can be potential treatments of sleep impairments in hypopituitary patients with a history of CC. Inducing small temperature changes is also a potential mechanism to improve sleep (chapter 5), because changes in skin temperature could modulate sleep-regulating systems (18). Although there is currently no method to target skin temperature precisely, applying thermosuit control of skin temperature may be a potential method to enhance sleep quality (19).

Conclusions and directions for future research

In this thesis, we aimed to obtain insight in the (dys)function of the hypothalamus in patients with pituitary insufficiency with a history of CC, CRT and/or pituitary surgery. We used a variety of techniques focusing on hypothalamic output parameters (chapters 2, 3, 4 and 5), human post-mortem hypothalamic tissue specimens (chapters 6 and 7) and functional brain imaging (chapter 8). Together, our results point towards the sensitivity of the hypothalamus to expanding sellar tumours and CRT (chapters 2, 3, 4, 5 and 6). Moreover, we demonstrated that SERT is abundantly expressed in the human hypothalamus, both post-mortem and in vivo (chapters 7 and 8). However, SERT binding ratio was unaltered in patients suspect for hypothalamic impairment (chapter 8), although we do not know if this is due to methodological limitations. Obviously, one of the main goals in future studies should be the development of test to assess hypothalamic function directly. To date there are no tests or imaging modalities to assess the integrity of hypothalamic function. Conventional radiologic imaging identifies only gross anatomical abnormalities, but is not suitable to detect abnormalities at the cellular level. In recent years, neuroimaging techniques have expanded enormously. Advanced imaging protocols, development of new nuclear ligands, the use of 7-Tesla MRI and pharmacological MRI generate great opportunities for detailed in vivo studies of the human hypothalamus and pituitary. In this thesis, we reported SERT binding selectively in the hypothalamus in vivo (chapter 8). Although hypothalamic binding ratio of SERT was not altered in patients suspect for hypothalamic impairment (chapter 8), this type of research may mark the beginning of the development of diagnostic tests to identify hypothalamic impairment. Such studies can be further supported by post-mortem hypothalamic research. The use of biochemical and molecular techniques on brain tissue are likely to provide new insights or to confirm new findings from clinical studies (86). To date, it is difficult to obtain specimens
of relative rare diseases such as pituitary diseases. Over a period of 25y the Netherlands Brain Bank has been able to collect five specimens with a tumour with suprasellar extension, which we all describe in the present thesis. Potentially, patient organizations in the pituitary field may support brain donor programs by informing their members, who do not always know about the existence of human brain banks and the possibility to register as a donor. These strategies may stimulate basic research on hypothalamic impairment in patients with pathology of the pituitary and/or hypothalamus, and provide answers to clinical questions that need to be investigated in the coming years with the ultimate aim to improve pituitary patient care.
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