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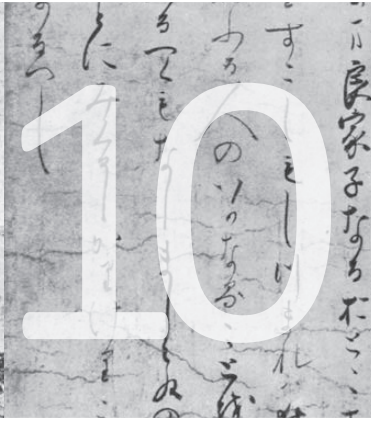


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## Summary, conclusions and future perspectives

## **GENERAL INTRODUCTION AND AIMS OF THE THESIS**

A short overview of the symptoms, pathophysiology and treatment of narcolepsy is presented in chapter 1. Narcolepsy is caused by hypocretin deficiency and characterized by the classical symptoms; excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, sleep paralysis and nocturnal sleep disruption. In addition there are numerous other signs and symptoms including increased weight and endocrine disturbances. Sodium oxybate (SXB) is a drug that may have a positive effect on all symptoms of narcolepsy. In addition it may influence endocrine function and metabolism as well as shown in Part I and II.

## **PART I ENDOCRINE STUDIES IN NARCOLEPSY**

In part I we describe a large endocrine study. Eight male hypocretin-deficient narcolepsy with cataplexy patients and eight controls matched for sex, age, body mass index, waist-to-hip ratio and fat percentage were enrolled in this study. All narcolepsy patients were free of medication for at least 2 weeks before the study. None of the controls took medication. Patients and controls underwent 24 h blood sampling at 10 minute intervals for measurement of prolactin (PRL) and growth hormone (GH) concentrations, at 20 minute intervals for leptin, and at hourly intervals for ghrelin and melatonin. This was done at baseline and on the fifth consecutive day of SXB administration. Three grams of SXB was taken two times per night for 5 consecutive nights. Subjects remained (semi)supine except for bathroom visits. Three standardized meals were served, and non-caffeinated tea and water were provided ad libitum. Daytime naps were allowed, and lights were switched off between 2300 h and 0730 h the next day. In addition to the blood samples, sleep was polygraphically recorded throughout both sampling occasionsperiod. Skin temperature was measured using I-buttons and core body temperature using the Jonah capsule as is described in Part II of this thesis.

### **Sodium oxybate increases prolactin secretion in narcolepsy patients and healthy controls**

Chapter 2 addresses the question whether plasma PRL concentrations are different between hypocretin deficient narcolepsy patients and controls. In addition we explored the effect of sodium oxybate on PRL release pattern in both groups. The PRL concentration time series was analysed with a new deconvolution programme, approximate entropy (ApEn) and with cosinor analysis. ApEn is a model-independent statistic used to quantify the regularity of a time series,

here PRL release. Basal and pulsatile PRL secretion, as well as pulse regularity and frequency, ApEn and diurnal parameters were similar in patients and controls. SXB treatment caused similar nocturnal increase in PRL secretion, advance of the acrophase and decrease in ApEn in patients and controls. Slow wave sleep was increased to a similar extent in patients and controls. This detailed study did not demonstrate altered PRL secretion in hypocretin-deficient narcolepsy patients during the basal state or during SXB administration. Therefore, hypocretin signalling is unlikely to be a regulator of the lactotrophic system. SXB administration resulted in a marked increase in PRL secretion in both narcoleptics and controls. This finding is well in line with previous reports and might be mainly due to hyperpolarisation of dopaminergic structures with a reduction in dopamine release, by neurons regulating PRL secretion.

*PRL secretion is not altered in hypocretin-deficient narcolepsy patients.*

*SXB administration increases prolactin secretion in narcolepsy patients and controls.*

### **Effect of sodium oxybate on growth hormone secretion in narcolepsy patients and healthy controls**

In chapter 3 we describe growth hormone (GH) secretion in patients and matched controls and the effect of SXB administration on GH and sleep in both groups. GH alterations may influence weight in narcolepsy. The GH concentration time series were analysed with AutoDecon and approximate entropy (ApEn). Basal and pulsatile GH secretion, pulse regularity, and frequency, as well as ApEn values, were similar in patients and controls. After SXB, slow-wave sleep (SWS) and, importantly, the cross correlation between GH levels and SWS more than doubled in both groups. In addition SXB administration caused a significant increase in total 24-h GH secretion rate in narcolepsy patients, but not in controls. GH has a potent lipolytic activity, whereas GH deficiency leads to decreases in lean body mass and increased fat mass.<sup>153;154</sup> These data suggest that SXB may alter somatotrophic tone in addition to its consolidating effect on night-time sleep in narcolepsy. Therefore, it is tempting to speculate that the putative weight-reducing effect of SXB is partly mediated by its stimulatory effect on the somatotrophic axis.

*GH secretion characteristics were not different in patients and controls.*

*SXB increases total 24-h GH secretion in narcolepsy patients only.*

**Plasma total ghrelin and leptin levels in human narcolepsy and matched healthy controls: basal concentrations and response to sodium oxybate**

In chapter 4 we describe a study in which we investigate whether total blood ghrelin or leptin levels are altered in hypocretin-deficient narcolepsy patients compared to controls, and whether ghrelin or leptin levels are influenced by sodium oxybate. No differences in mean 24-h total plasma ghrelin levels or food-induced suppression of ghrelin concentrations were found between narcolepsy patients and controls, or any influence of 5 days of sodium oxybate administration in both groups. We found that the mean 24-h total leptin concentration, and basal and pulsatile secretion rates were not significantly different between narcolepsy patients and controls. While the mean leptin pulse frequency was slightly but significantly higher in narcolepsy patients in both conditions, the clinical relevance of this finding is unclear. Mechanisms underlying increased BMI and altered ingestive behaviour in narcolepsy, and the effects of sodium oxybate administration on weight loss, are unlikely to involve changes in plasma ghrelin or leptin concentrations.

*Leptin secretion and ghrelin concentrations are not altered in narcolepsy.*

**Altered circadian rhythm of melatonin concentrations in hypocretin deficient men**

In chapter 5, we assessed whether melatonin secretion differs between narcolepsy patients and controls, and whether SXB affects melatonin secretion. Mean 24-h melatonin concentrations did not differ between narcolepsy patients and controls, either before or after SXB administration. However, the percentage of 24-h melatonin concentrations during daytime was significantly higher in narcolepsy patients, both before and after SXB administration. As a recent study demonstrated decreased responsiveness to light in hypocretin deficient mice,<sup>198</sup> it is conceivable that hypocretin signalling might be involved in the light-induced suppression of melatonin secretion. However, the effect of hypocretin on melatonin secretion in humans is likely to be modest as one might expect larger differences in the absence of hypocretin. Our findings indicate that daytime plasma melatonin concentrations (as a percentage of average 24-h concentration) are elevated in narcolepsy patients. SXB does not affect melatonin concentrations.

*Daytime plasma melatonin concentrations (as a percentage of average 24-h concentration) are elevated in narcolepsy patients.*

## PART II METABOLIC STUDIES IN NARCOLEPSY

### **Glucose and fat metabolism in narcolepsy and the effect of sodium oxybate: a hyperinsulinemic-euglycemic clamp study**

In chapter 6 we addressed the question whether narcolepsy patients are more insulin resistant than healthy controls. To investigate this we enrolled nine narcolepsy with cataplexy patients and nine, individually age, sex, BMI, fat percentage, and waist to hip ratio (WHR) matched healthy controls. All narcolepsy patients fulfilled the ICSD II criteria<sup>118</sup> and were all HLA DQB1\*06:02 positive. All patients were hypocretin deficient. None of the controls used medication and if patients were on medication, they stopped doing so at least two weeks prior to the study. All studies started at 08:30 after an overnight fast. A hyperinsulinemic-euglycemic clamp combined with stable isotopes ([6,6-2H<sub>2</sub>]-glucose and [2H<sub>5</sub>]- glycerol) was performed at baseline. In seven patients a second study was performed after three months of SXB treatment. Glucose disposal rate per unit serum insulin was significantly higher in narcolepsy patients compared to matched controls, whereas  $\beta$ -cell function was similar. Basal steady state glycerol appearance rate tended to be lower in narcolepsy patients, suggesting a lower rate of lipolysis. SXB treatment induced a trend in reduction of the GDR and a reduction in endogenous glucose production per unit serum insulin. After SXB treatment lipolysis increased, and body weight decreased in narcolepsy patients. Thus we show that narcolepsy patients are more insulin sensitive and may have a lower rate of lipolysis than matched controls. SXB stimulated lipolysis in narcolepsy patients, possibly accounting for the weight loss after treatment. While SXB tended to decrease systemic insulin sensitivity, it increased hepatic insulin sensitivity, suggesting tissue-specific effects.

*Narcolepsy patients are more insulin sensitive than healthy matched controls.*

*SXB reduces weight in narcolepsy patients.*

### **The effects of sodium oxybate on core body and skin temperature regulation in narcolepsy**

In chapter 7 we describe the study reported in part I of this thesis but now focus on its metabolic part. Sleep onset is usually preceded by an increase in (distal) skin temperature and a decline in core body temperature.<sup>97,221</sup> When compared with controls, an increased

distal skin temperature and a decreased proximal skin temperature during wake is observed in narcolepsy patients.<sup>23</sup> In this study we established the effect of short term SXB administration on skin and core temperature. Skin temperature was measured using I-buttons and core body temperature using the Jonah capsule in eight male hypocretin-deficient narcolepsy with cataplexy patients and eight matched healthy controls. At baseline, patients had significantly lower daytime core body and proximal skin temperatures compared to controls. In patients, SXB increased the nocturnal amount of slow wave sleep (SWS), increased proximal skin temperature towards normal levels, but there was no difference in core temperature found after SXB. The finding of a decreased daytime proximal skin temperature in narcolepsy patients compared to controls was previously demonstrated.<sup>23</sup> In contrast to our current findings, this previous study also revealed a higher distal skin temperature. The combination of the increased distal skin temperature and the decreased proximal skin temperature in that study led to a higher distal-proximal temperature gradient (DPG). Under controlled conditions, an increase in DPG is a reliable predictor for sleep onset in healthy controls. Furthermore, in narcolepsy, a shorter sleep onset latency was associated with an increase of proximal and distal skin temperatures and, to a lesser extent, an increase of the DPG.<sup>97</sup> However, none of these studies concerned spontaneous daytime napping in narcolepsy patients. Analysis of spontaneous naps in (semi) supine position in the present study revealed an absence of predictive value for any of the temperature measurements in narcolepsy patients at baseline. Surprisingly, during SXB administration an increase in distal skin temperature and DPG did become predictive for subsequent daytime sleep onset. In conclusion, administration of SXB normalized the sleep wake pattern as well as the skin temperature profiles in narcolepsy patients. Furthermore, SXB restored the relationship between skin temperature profile and subsequent sleep onset in patients.

*Narcolepsy patients had significantly lower daytime core body and proximal skin temperatures compared to controls.*

*With SXB administration, DPG becomes predictive for subsequent daytime sleep onset'*

*SXB normalizes proximal skin temperature.*

## PART III OTHER ASPECTS OF NARCOLEPSY

### Month of birth is not a risk factor for narcolepsy with cataplexy in the Netherlands

Chapter 9 describes changes in seasonal birth pattern of the entire Dutch population over a 79-year span and compared the monthly birth pattern of Dutch narcoleptics with the population data. Month and year of birth were noted for 307 patients with non-familial narcolepsy with cataplexy, born in the Netherlands between 1923 and 2001. The numbers of live births per month and per year from the entire Dutch population for the same period were used to calculate a virtual data set of expected births per month with exactly the number of narcolepsy with cataplexy cases, but with the birth pattern of the Dutch population. Observed and expected numbers per month were compared using the chi-square test. In contrast to earlier reports suggesting that narcolepsy patients are more often born during spring, we found a peak of narcolepsy births in August. This peak was not significantly different from expected birth numbers in the population. In addition, no other differences between observed and expected number of births was found. In conclusion: An effect of birth month on the occurrence of narcolepsy with cataplexy was not found in a study of 307 cases after adjusting for changing birth patterns in the general population. This in contrast to previous studies reporting a significantly different seasonality of birth month in narcolepsy patients compared to that of the general population, supporting the autoimmune hypothesis.<sup>248-251</sup> Our study is the first one to analyse births per year. Other studies pooled their births over ten years ignoring differences in birth patterns due to war or the introduction of birth control. In addition it was performed in a small country without large climate differences per region. In contrast to the largest study on birth months which was performed in a pooled population from the United States, France and Canada.<sup>249</sup>

*Birth month in narcolepsy can not support the autoimmune hypothesis.*

### Delusional confusion of dreaming and reality in narcolepsy

In chapter 8 we describe a study in 46 narcolepsy with cataplexy patients and age-matched controls. Participants completed a ~30-min structured telephone interview in which they were asked a series of questions pertaining to sleep, dreaming, and memory. Delusional episodes were defined as incidents in which a fully awake participant was uncertain if a



memory was dreamed or real, or was convinced that a memory was real, only later to discover that it was actually dreamed. Fleeting feelings of confusion during the transition to wakefulness were excluded because brief confusion is a well-known consequence of the hypnagogic and hypnopompic hallucinations characterizing narcolepsy. Dream delusions were extremely common in narcolepsy. Overall, 83% of narcolepsy patients reported that they had confused dreams with reality, compared to only 15% of controls. All narcolepsy patients reporting dream delusions provided multiple examples of such occurrences. Two-thirds of patients (65%) reported experiencing dream delusions at least once a week, and all but two (95%) had the experience at least once a month. In contrast, of the 6 controls who reported delusions, only 2 (5% of all controls) had experienced this more than once in their lives.

*Confusion of dreams with reality is a prevailing symptom of narcolepsy.*

## **FUTURE PERSPECTIVES**

The studies described in this thesis provide new grounds for future research and implications for clinical practice. First, as in almost any other study, these studies should be replicated, preferably in larger groups. Narcoleptic men and women are equally affected but the endocrine study was performed only in men. Future studies should focus on women and children as well. When these studies are performed in premenopausal women, the menstrual cycle should be taken into account and all women should be measured at the same day of their cycle. In children it will not be feasible to perform the same study mainly due to the amount of blood needed. However a less extensive protocol, with fewer samples and longer follow up would be quite important. Precocious puberty is highly prevalent in childhood narcolepsy,<sup>38</sup> and it is conceivable that those children have other endocrine disturbances as well. Moreover, sodium oxybate (SXB), which is prescribed off-label in narcoleptic children, is proven to increase growth hormone (GH) and prolactin (PRL) release in adult men with narcolepsy. Although, the increase of these hormones always stayed within normal limits in adult men, it might go beyond these limits in children. Moreover the effect of this increase may very well be much different in the developing child. Therefore, and for safety reasons, a trial with SXB including endocrine measurements in children is needed.<sup>268</sup>

The proportion of melatonin secreted during daytime was substantially higher in narcolepsy patients than controls. We hypothesised that reduced responsivity to light, as found in

hypocretin deficient mice<sup>198</sup> could be the cause. Since light is the major suppressor of melatonin synthesis,<sup>177</sup> it is conceivable that defects in hypocretin signalling might disrupt light-induced suppression of melatonin secretion. To test this hypothesis the protocol should be repeated in the dark. If disrupted light induced suppression is the cause of the altered pattern in narcolepsy, one should not find differences between patients and controls in a protocol performed in the dark.

The mechanism of action of sodium oxybate has not been elucidated. Apart from its effect through the GABA<sub>b</sub> receptor,<sup>76</sup> our data showed that SXB further strengthened the relation between SWS and GH secretion, so its effect may, at least in part, be mediated by an increase in GHRH activity. SXB increased the regularity of GH secretion as well. This may imply that SXB simultaneously promotes endogenous somatostatin release, since negative feedback has been shown to increase secretory regularity.<sup>148</sup> Future studies should be undertaken to test this hypothesis.

We measured total ghrelin levels and not the biologically active, octanoylated-ghrelin fraction. While there is a high correlation between the total and octanoylated fraction ghrelin level it remains possible that the active fraction is altered.<sup>174</sup> A future study measuring both total and octanoylated fraction ghrelin will have to prove that this high correlation also exists in narcolepsy. Mechanisms underlying increased BMI and altered ingestive behaviour in narcolepsy and the effects of SXB administration on GH release and weight loss are unlikely to involve changes in total plasma ghrelin or leptin concentrations. However, our study was done under strictly controlled lab circumstances with standardised meals at predetermined mealtimes. It is possible that in everyday life with different lifestyles and mealtimes alterations may be found. Therefore a study should be done under less controlled conditions with ad libitum food and bed times.

With our hyperinsulinemic-euglycemic clamp study we proved that narcolepsy patients are more insulin sensitive than matched controls. Therefore it is less conceivable that narcolepsy patients are more prone to get type 2 diabetes than controls. It would be interesting to determine the cause of our finding of increased insulin sensitivity in narcoleptics, since this finding may have implications for diabetes treatment or even prevention. There was a trend towards lower lipolysis in narcolepsy patients, which could be part of the reason why they grow obese. Future studies in larger groups with enough power to clarify if this is a real difference are needed. So far, it remains unclear why narcolepsy patients gain weight, usually shortly after disease onset. A study using a 24 h respiration chamber in combination

with doubly labelled water and tri-axial accelerometer would provide interesting information as to whether altered energy expenditure explains obesity in narcolepsy.

The temperature differences found between narcolepsy patients and controls, and the normalisation of skin temperature under SXB, were obtained at a lab under strictly controlled circumstances in which subjects remained (semi)supine for 24 h, a position known to influence skin temperature and a position that people usually do not keep for 24 h. It would be very interesting to measure skin and core temperature in an ambulatory situation in which subjects try to live their normal lives. In addition an increase in distal skin temperature and DPG became predictive for subsequent daytime sleep onset during SXB administration. If sleep onset is predictable from temperature differences in normal daily life one can think of developing practical solutions. It may be possible to prevent unwanted sleep by connecting temperature sensors to an alarm system while driving or performing monotonous tasks.

It is still unknown why people lose functioning hypocretin cells and develop narcolepsy. Although, support for an autoimmune cause is getting stronger and stronger, the modified Witebsky's postulates should be met including direct evidence from transfer of pathogenic antibody or pathogenic T cells; indirect evidence based on reproduction of the autoimmune disease in experimental animals; and circumstantial evidence from clinical clues.<sup>269</sup> Studies on birth month are an indirect way to make an autoimmune cause for the disease more likely. We could not confirm this with our study on birth months. Researchers are getting closer to the autoimmune origin of narcolepsy, but there is still a lot of work to do before the modified Witebsky's postulates<sup>269</sup> are fulfilled and autoimmunity is proven.

Last but not least, with our study on dream delusions we reveal an underappreciated and very common symptom of narcolepsy. It may be helpful to mention these dream delusions when taking a patient's history: because mentioning these delusions may help the patient to recognise this problem as a symptom of narcolepsy. Since the symptom is highly prevalent in narcolepsy patients it is important to establish the prevalence of this symptom in patients with other hypersomnias and estimate its specificity. Though the underlying mechanism of dream delusions is unknown, it is clear that many people with narcolepsy have a surprising and intense difficulty distinguishing the dreamed from the real. In concert, these patients perceive themselves as having more general difficulties with both retrospective and prospective memory. These observations highlight the possibility of source memory deficits in narcolepsy that have not yet been fully characterized and need more research.

f-MRI studies might be helpful to clarify the underlying mechanisms by finding differences in activity of hippocampal, prefrontal or other brain regions during different memory tasks. Sleep should be recorded during f-MRI studies, and advanced neuropsychological testing may help to identify and tackle the specific memory problems described in narcolepsy which so far have not been detected with formal tests. In addition, narcolepsy patients with dream delusions should be compared with patients without delusions and controls.

