Narcolepsy beyond sleepiness:

endocrine, metabolic and other aspects

Claire E. H. M. Donjacour

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ὥρη μὲν πολέων μύθων, ὥρη δὲ καὶ ὕπνου

“There is a time for many words, and there is also a time for sleep.”

Homer, Odyssey (XI:379)

To Eveline and Julie

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General introduction and aim of the thesis

Based on 'Clinical and pathophysiological aspects of narcolepsy'.
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INTRODUCTION

Narcolepsy with cataplexy is a chronic sleep disorder caused by hypocretin deficiency, that affects approximately 25–50 per 100,000 people.[1] The first symptoms usually appear from childhood to midlife with a large peak around 15 years of age and a smaller one around 36.[2] It has a profound effect on the quality of life[3] that might exceed the burden of other serious chronic diseases like epilepsy.[4] Narcolepsy is associated with reduced vigilance,[5] severe fatigue,[6] anxiety and mood disorders[7] and a higher rate of car accidents.[8-10] In addition the unemployment rate among patients is higher than in controls.[11]

SIGNS AND SYMPTOMS OF NARCOLEPSY

Excessive daytime sleepiness

The excessive daytime sleepiness (EDS) may come in various forms, with a continuous feeling of sleepiness at one end of the spectrum, and sudden involuntary and irresistible "sleep attacks" at the other. Narcolepsy patients often experience a combination of these. Daytime sleep episodes are brief, often < 20 min, and are typically reported to be refreshing. Although sleep episodes may occur several times a day, the total amount of sleep is not or only slightly increased over a 24-h period due to nocturnal sleep fragmentation.[12]

Nocturnal sleep disturbances

Fragmented nocturnal sleep is a major and common problem in narcolepsy. While patients fall asleep very quickly numerous awakenings may follow. These awakenings are usually short but can sometimes last for hours, forcing patients to get out of bed. Importantly, fragmented night-time sleep is not the cause of EDS; while improving nocturnal sleep may sometimes alleviate daytime sleepiness, EDS will never disappear.[13]

Cataplexy

The most specific symptom of narcolepsy is cataplexy, derived from the Greek word καταπλησσω meaning "to strike down". Cataplexy is defined as a sudden bilateral loss of muscle tone with preserved consciousness, usually triggered by emotions. The combination of cataplexy and EDS is pathognomonic for narcolepsy. Cataplexy can be triggered by a

diversity of emotions. The most often reported triggers are laughter, hearing a joke, or feeling excited. Although most cataplectic attacks do have a trigger, 40% of the patients have experienced attacks without an identified trigger. Most cataplectic attacks are partial, resulting for example in sagging of the jaw with blurred speech or buckling of the knees. Partial attacks may evolve into a complete loss of skeletal muscle tone leading to a fall but, because of its gradual onset, patients can usually support themselves preventing injury.[14]

Hypnagogic hallucinations

Hypnagogic hallucinations are vivid dreamlike experiences that occur upon falling asleep. The auditory, visual, or tactile sensations are usually felt to be real, and are often bizarre and frightening. The perception of intruders, with people or animals standing over or lying under the bed is a common reported phenomenon.[15]

Sleep paralysis

Sleep paralysis is the inability to move at sleep onset or, more commonly, when awakening.[16] While the patient is awake, it is impossible for them to move their arms or legs or sometimes even open their eyes, and it can be extremely distressing, especially when occurring for the first time. It often occurs in combination with hypnagogic hallucinations. About half of the patients experience sleep paralysis. This phenomenon is much less common where it only affects 5% of the population.[17]

ADDITIONAL SIGNS AND SYMPTOMS OF NARCOLEPSY

In addition of the above mentioned symptoms, there are various other features, frequently seen in narcolepsy patients. A few of them, most relevant for this thesis will be discussed.

Obesity

It has long been known that the majority of the narcolepsy patients tend to be overweight. This was usually attributed to inactivity due to EDS and received little attention. However, more recent epidemiological studies have clearly shown that overweight is a major symptom of narcolepsy.[18-20] Obesity (body mass index [BMI] ≥ 30 kg/m^2) occurs more than twice as often in patients with narcolepsy as among the general population. The cause of weight gain

in narcolepsy is unknown. Some patients complain about a tendency to binge eat, especially during waking periods at night. However, in a study using cross-checked dietary histories, it was shown that over a period of 24 h individuals with narcolepsy tended to consume fewer calories than controls, particularly carbohydrates.[21;22] Patients with idiopathic hypersomnia have been shown to have a lower BMI than narcoleptics, indicating that inactivity due to EDS is not likely to account for the obesity.[18] It seems possible that mechanisms such as a lowered metabolic rate underlie the increase in body weight seen in subjects with narcolepsy.

Temperature differences

Narcolepsy patients have an altered thermoregulatory profile with warmer hands and feet and lower proximal skin temperature than controls, a state in which sleep propensity is high.[23] Temperature manipulation studies offsetting these temperature changes show improvement in narcolepsy symptoms.[24;25]

Memory complaints

Many narcolepsy patients have memory complaints. Interestingly, when formally tested, they do not always show objective memory deficits.[26;27] The reason for this is unclear; patients may, for example, be more vigilant in a testing situation than in everyday life.

Delusional confusion of dreaming and reality

Narcolepsy patients may report difficulty distinguishing between dreams and reality. However, although frequently heard in the consulting room, it is rarely mentioned in the literature. So far just a few case reports have described more severe examples of memory source confusion in patients suffering from narcolepsy, in which false accusations of sexual assault occurred when patients mistook a dreamed assault for the memory of an actual event.[28;29]

Endocrine alterations

The most prominent hormonal disturbances in human narcolepsy were found in leptin, and the somatotropic axis. Serum concentrations of the adipocyte-derived hormone leptin were found to be significantly lower, and the daily fluctuations in leptin levels were absent in narcolepsy.[30;31] However, others found normal leptin levels in a single sample study in a

larger group of narcolepsy patients.[32;33] For the somatotropic axis, the circadian secretion of GH appears to be altered, so that a relatively large fraction of total production occurs during daytime in narcoleptics.[34] In addition, 24-h thyrotropin (TSH) levels were reduced in narcolepsy patients. In contrast, thyroxin (T4) and triiodothyronin (T3) were normal, so the clinical relevance of these findings remains to be elucidated.[35] A blunted total and basal adrenocorticotropic hormone (ACTH) production was found in narcolepsy patients whereas the pulsatile secretion was normal[35] which seemed to indicate that hypocretins are involved in the basal but not pulsatile secretion of ACTH. The circadian rhythm of cortisol and ACTH was normal, showing that the circadian timekeeper is intact in narcolepsy. Finally, a role for hypocretin-1 in luteinizing hormone (LH) release was observed in rats.[36] Accordingly, a decreased 24-h mean LH concentration and pulsatile secretion was found in human hypocretin deficiency.[37] A high prevalence of precocious puberty is found in children with narcolepsy.[38]

Diabetes

In the sixties and eighties some reports have been published on a higher incidence of insulin resistance and type II diabetes mellitus (T2DM) in narcolepsy.[39-41] Another study confirmed a higher risk of metabolic syndrome independent of BMI by comparing narcolepsy patients to idiopathic hypersomnia patients.[22] However, in a recent study where a large group of patients were compared to healthy matched controls, no differences in insulin resistance were found by calculating pro-insulin to insulin ratio and using homeostatic model assessment method (HOMA).[42] Unfortunately in the last study, patients were medicated. This might have influenced the results. Anticataplectic drugs e.g. imipramine may produce a significant increase of fasting blood glucose levels.[43]

PATHOPHYSIOLOGY OF NARCOLEPSY

Hypocretin deficiency

The finding that hypocretin (orexin) knockout mice had symptoms of narcolepsy[44] and the nearly simultaneous report that genetically narcoleptic canine narcolepsy was caused by a mutation in the Hypocretin-2 receptor[45] led to the hypothesis that human narcolepsy might be due to a malfunction of the hypocretin system. This hypothesis was supported by the finding of greatly reduced levels of hypocretin in the cerebrospinal fluid of human narcoleptics.[46]

Two simultaneously published papers from different laboratories then reported a loss of hypocretin cells in brains of human narcolepsy patients.[47;48] Peyron et al. also reported a single case of early onset narcolepsy associated with by a hypocretin mutation. Thannickal et al. reported elevated levels of gliosis in the hypothalamic region where hypocretin cells had been lost, consistent with the autoimmune hypothesis.[48;49] Further work showed that the number of hypothalamic neurons containing dynorphin and Narp, both substances known to be localized to hypocretin cells, were reduced by 90% in narcoleptics with cataplexy.[50;51] This finding suggests that hypocretin cells are lost, rather than simply being unable to produce hypocretin.

Autoimmune hypothesis

Several lines of evidence point to an autoimmune origin for most cases of human narcolepsy. Honda discovered that nearly all Japanese narcoleptics had an HLA type (DR2, in the then standard terminology), whereas only 20–30% of the non-narcoleptic population had this haplotype.[52] Since the HLA molecules function to present antigen to the immune system, this finding suggested that the immune system was involved in the genesis of narcolepsy. A narcolepsy linkage to HLA-DQA1*01:02 and -DQB1*06:02 was demonstrated.[53] An association was demonstrated between narcolepsy and polymorphisms in the T-cell receptor alpha locus in a genome-wide association study.[54] Further evidence for the autoimmune hypothesis includes the initially disputed report that streptococcal antibodies were often elevated in narcoleptics,[55;56] with Montplaisir et al.'s initial observations recently replicated.[57] An anecdotal report of rapid onset of narcolepsy with cataplexy after an anaphylactic reaction to an insect bite[58] further supports this hypothesis. Additionally, the discovery of circulating TRIB2-specific antibodies reactive to hypocretin neurons in individuals with narcolepsy,[59-61] strongly suggest an autoimmune nature. Although this is a major breakthrough in narcolepsy research, the role of TRIB2 antibodies remains unclear and there is no direct proof that these antibodies actually damage hypocretin neurons.[62] Recent reports of an association of narcolepsy onset with immunization for the H1N1 flu[63] or H1N1 flu infection[64] supports the autoimmune hypothesis as well, although some of this evidence is disputed.[65] The latest paper demonstrates CD 4 T cell involvement and seemed to get closer to prove the autoimmune hypothesis.[66] Unfortunately the paper got retracted because the results could not get replicated. Although there are very strong indications for an autoimmune cause of narcolepsy, the exact mechanism of disease onset remains to be elucidated.

TREATMENT OF NARCOLEPSY

Every narcolepsy patient should be advised to live a regular life, trying to have similar bedtimes every day. Scheduled naps can alleviate sleepiness for a while, and may be advised.[67] Likewise, a short nap before demanding activities may be helpful. However, in the majority of patients, pharmacological treatment is necessary.[15;68] In practice, it is recommended to start treating the most disabling symptom first, and to tailor drug schedules and dosages individually. Combination therapy is often necessary.[68] It must be emphasized that drug therapy is purely symptomatic. While cataplectic attacks can often be completely abolished, EDS will never completely disappear. Drugs to treat narcolepsy are usually divided into two groups; stimulant drugs to treat EDS and anti-cataplectic drugs that also tackle dream related symptoms.

Stimulants

Examples of wake promoting agents are amphetamines, and amphetamine like drugs e.g. methylphenidate. The mode of action of these drugs is complex, but the central mechanism entails the enhancement of the release of catecholamines (dopamine and noradrenaline) as well as uptake inhibition.[69;70] A frequently prescribed non-amphetamine like substance is modafinil. Its mode of action is still a matter of debate. Several studies indicate that modafinil, like other stimulants, inhibits the reuptake of dopamine (DA) via the DA transporter (DAT).[71] However in vitro studies show that modafinil binds to the DAT with lower affinity than methylphenidate and other psychostimulants drugs.[71] Recent research identified several non-dopaminergic effects of modafinil, such as the increase of electrical neuronal coupling, or the enhancement of histamine and orexin neurotransmission that might be of primary importance to explain its efficacy as a wake-promoting and cognitive-enhancing medication.[71]

Anticataplectic drugs

In general, the amelioration of cataplexy is associated with a reduction of hypnagogic hallucinations and sleep paralysis. Tricyclic antidepressants are among the most effective agents, often in even very low doses.[68] Those most commonly used include imipramine, and clomipramine. In contrast to the tricyclics, SSRIs usually require a relatively high-dose, which can sometimes negate their more favorable side-effect profile. Venlafaxine, a noradrenalin/serotonin reuptake inhibitor, might have some beneficial effect on cataplexy too, although

publications on its efficacy are scarce. Acute withdrawal from antidepressants may severely aggravate cataplexy, sometimes leading to a status cataplecticus (sequential cataplexy attacks that may last for hours or even longer).

Sodium oxybate

A more recently approved compound to treat narcolepsy is sodium oxybate (SXB,γ-hydroxybutyrate; GHB). SXB is a hypnotic and is taken twice a night (at bedtime, and a second dose 3–4 hours later). Interestingly, despite the short half-life of the drug and its nighttime administration, the effects on daytime cataplexy are already obvious when using low dosages (3–6 gr/night).[72] Additional studies show that the use of higher doses of SXB (up to 9 gr/night) has additional positive effects on the excessive daytime sleepiness, and the quality of nocturnal sleep.[73] Moreover SXB may reduce weight, a side effect that could be beneficial to an overweight patient.[74] GHB occurs naturally in the brain, but its mechanism of action in narcolepsy is not precisely known. In healthy subjects, it was shown that administration of SXB has a slow wave sleep (SWS) promoting effects associated with an increase in GH secretion.[75] Its effects are thought to be mediated through γ-aminobutyric acid B receptor (GABAB) and has an impact on dopamine and serotonin release.[76;77] The existence of a specific GHB receptor is still under debate.

AIM OF THE THESIS

The first part of the thesis consists of a number of endocrine studies in hypocretin deficient male narcolepsy with cataplexy patients. Hypocretins are, besides their crucial function in the regulation of the sleep-wake cycle, involved in food intake, glucose sensing and homeostasis, and in virtually all endocrine axes.[78] Accordingly metabolic and hormonal disturbances are increasingly recognized as important features of narcolepsy.[22;30;35;37;38] Therefore, it may be very important to find treatment options that also deal with these aspects. SXB may very well be a suitable candidate, as the drug activates dopaminergic circuits in the brain where diminished dopamine (D2) receptor mediated signal transduction appears to induce insulin resistance, the metabolic syndrome and diabetes mellitus type 2.[79] If SXB is indeed able to reduce insulin resistance, it may also have an important impact on metabolic disturbances in obese patients without narcolepsy. In addition, more and more evidence suggests that GHRH, the hypothalamic hormone controlling the release of GH, simultaneously promotes the occurrence of non-REM sleep as well as GH release.[80] On the basis of these

data, it has been hypothesized that SXB may act by stimulating the GHRH secretion in the central nervous system and thereby promoting SWS and the release of GH.[75] When administered during nocturnal sleep in narcoleptics, it may again confine GHRH secretion to the night and decrease possible sleep inducing effects of GHRH as a consequence of daytime release.

Part I Endocrine studies in narcolepsy

The objective is to investigate whether the secretion of Prolactin (PRL) (chapter 2), Growth Hormone (GH) (chapter 3), Leptin and Ghrelin (chapter 4), and Melatonin (chapter 5) is altered in narcolepsy patients compared with individually matched healthy controls. In addition, the effect of short term administration of SXB on the aforementioned substances is described.

Earlier reports on PRL secretion in narcolepsy patients have been inconclusive, showing either increased, decreased or normal levels.[81-83] Discrepancies seemed to be due to methodological issues. The major physiological regulator of PRL release is dopamine.[84] Alterations in the dopaminergic system in the post-mortem brains of narcoleptics have been described making changes in PRL release in narcolepsy conceivable.[85] Although the exact mode of action is still unclear, SXB acts on gamma-aminobutyric acid type B (GABAB) and has an impact on dopamine and serotonin release.[77] Therefore, SXB treatment may be expected to alter PRL secretion. We hypothesised that changes in hypothalamic hypocretin signaling in narcoleptic patients may give rise to altered PRL secretion. In addition, we aimed to determine the effect of SXB administration on PRL secretion and sleep, both in a healthy and in a hypocretin-deficient state.

In healthy subjects, there is a clear association between GH secretion and SWS. This is especially clear in young males, in which the majority of 24-h GH is secreted during the first period of SWS at night.[75] It has been shown previously that GH secretion was less strictly confined to the night in narcolepsy patients.[34] As the relation between SWS and GH secretion was preserved, it was suggested that a shift of SWS episodes to the day was paralleled by a daytime shift of GH secretion. In contrast to other hypnotics, SXB is one of the few compounds that increases rather than decreases SWS. This led to the hypothesis that it may also act as a GH secretagogue. Indeed, it has been shown that single-dose SXB administration leads to an increase in GH secretion in healthy young men, paralleled by an increase in SWS.[75] Here we explore whether SXB administration leads to an increase in nocturnal GH secretion in both patients and controls, paralleled by an increase in SWS.

Leptin and ghrelin are both strong regulators of energy homeostasis, and their relationship with hypocretin has been shown to be of importance. Receptors for leptin are found on hypocretin cells,[86] and leptin can directly inhibit the expression of isolated hypocretin neurons.[87] Indirectly, leptin can affect the activity of hypocretin cells via energy-regulating neurons in the arcuate nucleus of the hypothalamus.[88] Ghrelin is an important endogenous regulator of energy balance and it excites hypocretin neurons. An interaction between these two systems has been shown to be involved in ingestive behavior.[87] Its expression is complex[89] and influenced by sympathetic nervous system activity.[90] Across the wake period, plasma concentrations wax and wane episodically, providing an appetite-stimulating signal to the brain.[91] Hypocretin neurons directly sense ghrelin and leptin but it is unknown if these connections are uni-or-bidirectional.[92] Therefore a study of hypocretin deficient narcolepsy patients provides a unique opportunity to explore the nature of these relationships. In addition we hypothesized that SXB administration would alter total ghrelin levels, which might be involved in its GH-promoting effects.

Melatonin is a modulator of sleep and high levels induce sleepiness.[93] Animal models indicate that the hypocretinergic innervation of the pineal gland could be important for the regulation of diurnal melatonin synthesis and secretion.[94] It is therefore conceivable that melatonin secretion is hampered in narcolepsy. Here we describe a study in which hourly melatonin levels are compared between narcolepsy patients and controls.

Part II Metabolic studies in narcolepsy

Hypocretins have a major role in glucose sensing and homeostasis[78] and therefore it is conceivable that hypocretin deficiency may lead to problems in glucose regulation and diabetes. Conflicting reports on the risk of diabetes in narcolepsy have been published. Several studies reported a higher prevalence of type 2 diabetes in narcolepsy.[22;39;41;95] More recent studies reported no difference in diabetes prevalence between narcolepsy patients and matched controls.[42;96] In chapter 6 we describe the first study to compare insulin sensitivity in nine narcolepsy with cataplexy patients compared with matched healthy controls, measured with the gold standard, the hyperinsulinemic euglycemic clamp technique. In addition, the effect of long term sodium oxybate treatment on insulin sensitivity is evaluated.

Sleep onset is preceded by an increase in skin temperature and a decline in core body temperature.[97] An altered thermoregulatory profile, resembling this sleep promoting pattern, has been observed in narcolepsy patients.[23] SXB is the sodium salt of GHB and may have

a positive effect on the sleep disturbances in narcolepsy.[73] The effect of both compounds are the same but the exact mechanisms are still unclear.[98] Altered thermoregulation is one of the effects of GHB described in animal studies and human case reports. Rodent studies demonstrate a slight increase in core body temperature after administration of a low dose of GHB and a clear decrease in core body temperature in higher doses.[99] In addition several studies describe hypothermia in humans with GHB intoxication.[100;101] Given the impact of GHB on temperature regulation, the altered pattern of skin temperature in narcolepsy and the positive effects of SXB on sleep in narcolepsy patients, the treatment effect of SXB may be, at least in part, mediated by restoring the physiological temperature regulation. Chapter 7 aims to determine the effect of short term Sodium Oxybate administration on the 24-hour temperature and sleep-wake profile in narcolepsy patients and controls.

Part III Other aspects of narcolepsy

Hypnagogic hallucinations are frequently described dream related experiences in narcolepsy. Confusions between dreams and reality are an often heard issue in narcolepsy, however so far no systematic studies have been published about these confusions. Here we describe an explorative study on delusional confusion of dreaming and reality in narcolepsy (chapter 8).

There are strong indications for an autoimmune cause of narcolepsy but there is no definite proof yet. One way to demonstrate an autoimmune cause involves studying the distribution of births over the months of the year. Several studies have addressed seasonal birth patterns in narcolepsy, suggesting an autoimmune involvement. Analysis methods in published studies varied, but no study corrected completely for possible changes in seasonal birth pattern over time in the appropriate control population. Therefore we describe the distribution of births over the months of the year of 307 narcolepsy patients compared to the general population in The Netherlands taking geographical and temporal criteria fully into account (chapter 9).

PART I

Endocrine studies in narcolepsy

2

Sodium oxybate increases prolactin secretion in narcolepsy patients and healthy controls

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ABSTRACT

Objective: Hypocretin deficiency causes narcolepsy and may affect neuroendocrine systems, including TSH, ACTH and LH secretion. Symptoms can be treated effectively with sodium oxybate (SXB) in many patients. This study was performed to compare prolactin (PRL) secretion in patients and matched controls and establish the effect of SXB administration on PRL and sleep in both the groups.

Design: Open label intervention. Blood was sampled before and after 5 days of SXB treatment. The study was performed at the Leiden University Medical Centre, Leiden, The Netherlands.

Methods: Subjects were admitted to the clinical research centre on both occasions.

Patients or participants: 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.

Interventions: SXB two times 3 g per night for five consecutive nights.

Results: Patients and controls underwent 24 h blood sampling at 10 min intervals for measurement of PRL concentrations. The PRL concentration time series was analysed with a new deconvolution programme, approximate entropy (ApEn) and Cosinor analysis. Sleep was polygraphically recorded. 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.

Conclusion: 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.

INTRODUCTION

Narcolepsy is a debilitating disorder characterised by excessive daytime sleepiness (EDS), cataplexy, hypnagogic hallucinations, sleep paralysis and nocturnal sleep disturbances.[102] In recent years, obesity and hormonal alterations have been recognised as additional features of the narcoleptic syndrome.[35] Interest in energy homeostasis in narcolepsy increased after the discovery of hypocretin deficiency as the cause of this disorder since hypocretins (orexins) are known to influence feeding behaviour, wakefulness and energy expenditure.[46-48;103] Hypocretins are also involved in the control of the secretion of various hormones.[104-108] Prolactin (PRL) is a polypeptide hormone, which is primarily produced in the anterior pituitary gland.[109] Apart from its role in lactation and reproduction, PRL also takes part in the regulation of body energy homeostasis.[109;110] Prolonged hyperprolactinaemia is often accompanied by weight gain in humans, which can be ameliorated by normalisation of serum PRL.[110] The major physiological regulator of PRL release is dopamine from tubero-infundibular origin. Hypothalamic dopamine inhibits the basally high secretory tone of pituitary lactotrophs by binding to D2 receptors expressed in their cell membranes.[84;111] Hence, alterations in the dopaminergic system, as described previously in the post-mortem brains of narcoleptic humans, may cause changes in PRL secretion.[85] However, earlier reports on PRL secretion in narcolepsy patients have been inconclusive, showing either increased, decreased or normal levels.[35;81-83] These discrepancies are likely due to the use of only few baseline measurements of hormone levels, small sample size, relatively poor matching or too long blood sampling intervals, which are not adequate to assess either the pulsatile nature of PRL secretion or its total daily production rate. Sodium oxybate (SXB) or gamma-hydroxybutyrate (GHB) is effective in the treatment of narcolepsy. It reduces cataplexy, improves nocturnal sleep quality and higher doses of SXB may also reduce EDS.[73] Although the exact mode of action is still unclear, SXB acts on gamma-aminobutyric acid type B ($GABA_B$) and has an impact on dopamine and serotonin release.[77;112] Therefore, SXB treatment may be expected to alter PRL secretion. Indeed, there are reports that SXB administration may increase PRL secretion.[75;113] Several reports have been published on PRL and sleep; however, the precise association between PRL release and sleep still remains to be elucidated.[75;113-117] We hypothesised that changes in hypothalamic hypocretin signalling in narcoleptic patients may give rise to altered PRL secretion. In addition, we aimed to determine the effect of SXB administration on PRL secretion and sleep, both in a healthy and in a hypocretin-deficient state. Therefore, using a combination of polysomnography and state-of-the-art endocrine techniques, we compared PRL secretion between hypocretin-deficient narcoleptic subjects and matched controls both at baseline and after five nights of SXB administration.

SUBJECTS AND METHODS

Subjects

Eight male narcolepsy patients with definite cataplexy were included, who fulfilled the diagnostic criteria of the 2nd edition of the International Classification for Sleep Disorders.[118] All narcolepsy patients were hypocretin-1 deficient and free of medication for at least 2 weeks before the study. Only one of the patients received prolonged SXB administration in advance. He did stop taking SXB 20 days prior to the study. One patient took SXB in the past for a short period of time and took stimulants on demand. Another patient was tapered off antidepressants. Five patients were not taking any drugs at the time of enrolment. All consecutive male patients eligible for the study were asked to participate. Eight healthy controls, matched for sex, age, body mass index (BMI), waist-to-hip ratio (WHR) and fat percentage, were included for comparison. Bioelectrical impedance analysis (Bodystat, Douglas, Isle of Man, UK) was used to estimate fat percentage. Subjects were eligible for study after exclusion of hypertension, any known history of pituitary, psychiatric or neurological disease (other than narcolepsy), alcohol or drug abuse, recent weight change (>3 kg weight change within the last 3 months), a sleep disorder history assessed through clinical interview (controls) and endurance sports. Routine laboratory tests were performed to rule out diabetes (fasting plasma glucose >6.9 mmol/l), anaemia, as well as hepatic and renal failure. The study was performed at Leiden University Medical Centre, Leiden, The Netherlands. The study was approved by the ethics committee of the Leiden University Medical Centre. Written informed consent was obtained from all subjects.

Protocol

Subjects were admitted to the Clinical Research Centre for 24 h blood sampling. A cannula was inserted into the antecubital vein 45 min before the start of blood sampling at 1200 h. Blood samples were collected with S-monovetten (Sarstedt, Etten-Leur, The Netherlands) from a three-way stopcock attached to a 0.9% NaCl and heparin (1 U/ml) infusion (750 ml/24 h) to keep the cannula from clotting. Sampling was performed through a long line to prevent sleep disruption by investigative manipulations. For PRL measurements, blood was collected in serum tubes at 10 min intervals. After clotting, the blood was centrifuged within 30 min of sampling (1250 ***g*** at 4 °C for 20 min). Subsequently, plasma was divided into separate aliquots in Sarstedt tubes and stored at K80 °C until assay. Three standardised meals were served at

0830, 1300 and 1800 h (Nutridrink, Nutricia, Zoetermeer, The Netherlands; 1.5 kcal/ml, 2100 kcal/day; macronutrient composition per 100 ml: protein, 6 g; fat, 5.8 g; carbohydrate, 18.4 g). Subjects remained (semi)supine except for bathroom visits. Daytime naps were allowed. Lights were switched off at 2300 h and turned on at 0730 h the next day. Twenty-four hour sampling was performed at baseline and on the 5th day of SXB administration in two night-time doses of 3 g at 2300 h and 0300 h. This starting dose, higher than the usual of 2.25 g, permitted to elicit some effect in a few days of administration. To monitor side effects, the first night of administration was done clinically.

Sleep analysis

Sleep was polygraphically recorded throughout both sampling occasions, using an Embletta X100 recorder (Embla, Broomfield, CO, USA). The recordings were scored visually by an experienced sleep technician at 30 s intervals according to the AASM criteria.[119] To allow assessment of the association between changes in serum PRL levels (measured every 10 min) and sleep stages (scored every 30 s), sleep profiles were divided into 10 min segments, separating consecutive PRL measurements as described previously.[120] These segments were condensed from the 30 s scoring intervals by calculating the percentage of time spent in stages I and II non-rapid eye movement (REM) sleep, slow wave sleep (SWS) and REM sleep.

Assays

Serum PRL concentrations were measured with a sensitive time-resolved immunofluorometric assay with a detection limit of 0.04 µg/l (Delfia, Wallac Oy, Turku, Finland). The assay was calibrated against the 3rd WHO standard 84/500, 1 ng/mlZ36 mU/l. The intra-assay coefficient of variation varied from 3.0 to 5.2%, while the inter-assay coefficient of variation was between 3.4 and 6.2% (in the 0.1–250 µg/l concentration range). In order to minimise inter-assay variability, samples from each patient and matched control were analysed in the same run.

Calculations and statistics

Deconvolution analysis

PRL concentration time series were analysed using a recently developed automated deconvolution method, which was mathematically verified by direct statistical proof and empirically validated using hypothalamopituitary sampling and simulated pulsatile time

series.[121] For PRL, the fast half-life was represented as 3.5 min constituting 37% of the decay amplitude and the slow half-life was represented as an unknown variable between 20 and 50 min.[122;123] All candidate pulse-time sets were deconvolved. Statistical model selection was then performed to distinguish among the independently framed fits of the multiple candidate pulse-time sets using the Akaike information criterion.[124] The parameters (and units) are frequency (number of bursts per total sampling period, l of the Weibull distribution), regularity of inter-pulse intervals (unitless g of Weibull), slow half-life (min), basal and pulsatile secretion rates (concentration units/session), mass secreted per burst (concentration units) and wave form shape (mode or time delay to maximal secretion after objectively estimated burst onset, min).

Approximate entropy

Approximate entropy (ApEn) (1, 20%), was used as a scale- and model-independent regularity statistic to quantify the orderliness (regularity) of PRL release. Higher ApEn denotes greater disorderliness (irregularity) of the secretion process. Mathematical models and clinical experiments have established that greater irregularity signifies decreased feedback control with high sensitivity and specificity (both >90%).[125]

Diurnal variation

The wave form of individual PRL profiles was quantified by a best-fit curve obtained using a locally weighted regression procedure with a regression window of 4 h and a Gaussian kernel.[126] The values (and timings) of the acrophase and the nadir were defined as the levels (the timings) corresponding to the maximum and the minimum of the best-fit curve respectively. The amplitude was defined as 50% of the difference between the acrophase and the nadir values.

Statistical analysis

Data are presented as mean ± SEM, unless otherwise specified. Statistical comparisons were made with either Student's t-test or repeated measures ANOVA when appropriate. Pearson's correlation coefficient was applied to assess all correlations. Cross-correlation analysis was applied to assess the association between PRL levels and the percentage of time spent in various sleep stages in the preceding 10 min sampling interval, taking into account all of the sampling intervals during sleep.[127] Statistical calculations were performed using Systat software (version 11, Systat Software, Inc., San Jose, CA, USA) and SPSS (release 17.0, SPSS, Inc., Chicago, IL, USA). All tests were two-tailed and significance level was set at 0.05.

RESULTS

Narcoleptic patients and controls did not differ with respect to gender (male), age (38.0 ± 4.7 vs 37.9 ± 4.1, $P = 0.984$ respectively), BMI (28.1 ± 1.6 vs 27.4 ± 1.4, $P = 0.742$), fat percentage (23.6 ± 2.1 vs 23.4 ± 1.7, $P = 0.946$) and WHR (0.92 ± 0.03 vs 0.90 ± 0.02, $P = 0.579$). As expected, in narcolepsy patients the mean BMI was in the overweight range. Ingestion of SXB was well tolerated and apart from mild drowsiness no other side effects were reported. The mean 24 h PRL concentration in narcolepsy patients was 5.13 ± 0.47 µg/l at baseline and 5.65 ± 0.52 µg/l during the SXB administration ($P < 0.001$, see Figure 2.1). In controls, it was 6.78 ± 1.68 and 7.40 ± 1.66 µg/l respectively ($P < 0.001$). The PRL concentration strongly increased shortly after the administration of each dose of SXB. In patients, an increase from 4.56 ± 0.26 to 12.08 ± 1.61 µg/l occurred ($P < 0.001$), and in controls PRL increased from 6.28 ± 1.72 to 14.07 ± 2.85 µg/l ($P < 0.001$) during the first SXB administration. After the second

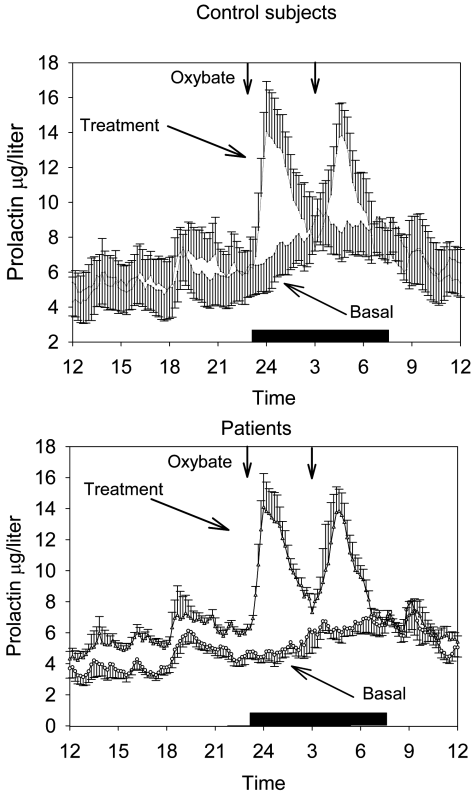

Figure 2.1 Mean 24 h plasma prolactin concentration ± SEM, before and after the SXB administration in narcolepsy patients (n = 8) and controls (n = 8). The downward arrows indicate ingestion of SXB in treatment condition. The dark horizontal bar indicates the lights off period.

Table 2.1 Deconvolution of serum prolactin concentration profiles in narcolepsy patients and healthy controls

	Narcolepsy		Controls		Narcolepsy versus Controls			
	Baseline	SXB	Baseline	SXB	Baseline	SXB	Treatment	Interaction
Pulse frequency (no/24 h)	23.5 ± 0.9	16.6 ± 1.6	21.3 ± 0.8	18.1 ± 1.8	0.08	0.74	0.006*	0.24
Half-life (min)	39 ± 4.7	33 ± 2.5	39 ± 4.1	33 ± 2.5	0.93	0.83	0.16	0.96
Basal secretion (μg/L/24 h)	91 ± 16.8	115 ± 18	144 ± 59	163 ± 62	0.40	0.43	0.04*	0.83
Pulsatile secretion (μg/L/24h)	93 ± 8.0	105 ± 17.2	105 ± 15.0	147 ± 28	0.48	0.26	0.06	0.29
Total secretion (μg/L/24 h)	184 ± 20.0	220 ± 32.0	249 ± 73	310 ± 85	0.40	0.31	0.0008*	0.83
Mean pulse mass (μg/L)	3.99 ± 0.37	6.69 ± 1.15	4.89 ± 0.55	7.87 ± 1.07	0.20	0.69	0.005*	0.25
Mode (min)	19 ± 2.0	20.6 ± 1.8	18.2 ± 1.7	18.1 ± 3.1	0.77	0.41	0.75	0.71
λ (events/ 24 h)	21.4 ± 0.7	15.1 ± 1.5	19.3 ± 0.7	16.3 ± 1.7	0.06	0.69	0.005*	0.25
γ (dimensionless)	2.19 ± 0.17	2.05 ± 0.11	2.18 ± 0.12	2.30± 0.20	0.93	0.54	0.97	0.31

Data are shown as mean ± SEM. Each group consisted of eight subjects. Comparisons were made using Student's t-test (column 8) and two-way ANOVA for repeated measurements. Secretion data are expressed in mass units per litre hormone distribution volume. * $P < 0.05$.

dose, serum PRL increased from 5.12 ± 0.36 to 12.26 ± 2.74 µg/l in patients ($P < 0.001$) and from 7.28 ± 1.94 to 13.82 ± 1.86 µg/l in controls. The effect of SXB was not statistically different between patients and controls.

Deconvolution analysis

At baseline, there were no significant differences in basal, total or pulsatile PRL secretion between narcolepsy patients and controls (Table 2.1). However, there was a clearly stimulatory effect of SXB on PRL secretion. Basal secretion increased slightly after SXB administration, whereas total PRL secretion increased with 20% in patients and nearly 25% in controls, most likely resulting from an increase of more than 60% in mean pulse mass in both the groups (Table 2.1).

Diurnal variation

The acrophase of serum PRL concentrations shifted after SXB administration to 1.5 h earlier in controls and 3 h earlier in narcolepsy patients (Table 2.2). Similarly, the nadir advanced in both the groups after the SXB administration. The amplitude increased significantly in both the groups indicating a greater degree of diurnal variation in PRL secretion after SXB treatment. Intergroup differences as well as group x treatment interaction effects were not significant, indicating a similar effect of SXB on both narcolepsy and control subjects.

Approximate entropy

The ApEn values decreased in both the groups after the SXB administration, reflecting greater regularity of PRL release. There were no differences in baseline ApEn values nor was the group x treatment interaction effect significant (Figure 2.2).

Table 2.2 Diurnal variation in prolactin concentrations

	Baseline		SXB		Treatment effect (*P*)
	Controls	Patients	Controls	Patients	
Acrophase	5:05 ± 51	5:45 ± 84	3:37 ± 43	2:34 ± 44	0.01*
Nadir	15:53 ± 48	14:06 ± 38	13:19 ± 47	13:33 ± 42	0.02*
Amplitude (µg/L)	2.19 ± 0.17	2.08 ± 0.30	3.35 ± 0.47	2.71 ± 0.49	0.015*

Data are shown as mean clock time ± SEM in minutes. Statistical comparisons were performed with ANOVA for repeated measurements. Between subjects and interaction P-values were all non-significant. * $P < 0.05$.

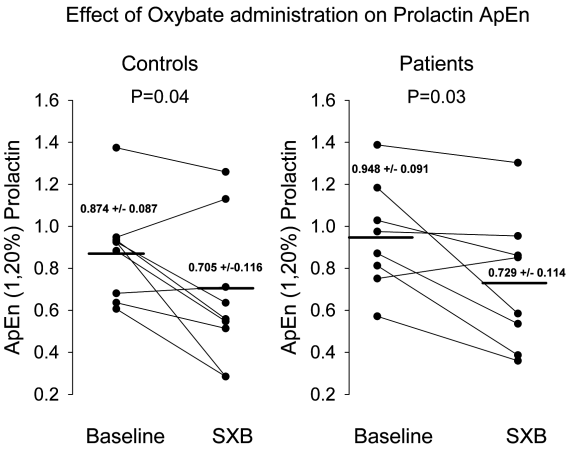

Figure 2.2 Approximate entropy of serum prolactin concentration series in patients and controls before (left column) and during the SXB (right column) administration. The horizontal black bar indicates the mean.

Sleep recordings

On average, compared with controls, narcolepsy patients spent significantly less time awake both during basal conditions and SXB treatment (Table 2.3). During the day (defined as the lights on period between 0730 and 2300 h), narcolepsy patients spent significantly less time awake, while significantly more time was spent in non-REM sleep, regardless of treatment (Table 2.3). The SXB administration resulted in a significant decrease in stages I and II non-REM and REM sleep over 24 h in both the groups (P = 0.011 and 0.009 respectively), while it significantly increased the time spent in SWS (P = 0.001). During the day, SXB treatment reduced the time spent in stages I and II non-REM and REM sleep (P = 0.038 and 0.041 respectively), while it tended to increase wakefulness (P = 0.098). The percentage of SWS during the night more than doubled in both the groups in response to SXB treatment (narcolepsy: 6.5 ± 1.9 vs 16.5 ± 3.0%; controls: 7.1 ± 1.9 vs 18.5 ± 2.4%; P = 0.001 for treatment effect), whereas there were trends for decreases in the percentages of stages I and II non-REM and REM sleep. The cross-correlation between SWS and PRL release strongly increased after SXB treatment in both narcolepsy patients (-0.03 ± 0.03 vs 0.47 ± 0.12) and controls (0.09 ± 0.06 vs 0.50 ± 0.07), $P \leq 0.001$ for treatment effect (Figure 2.3). However, the SXB administration did not significantly affect the cross-correlation between PRL release and REM sleep in either narcolepsy patients (0.12 ± 0.05 vs 0.04 ± 0.03) or controls (0.22 ± 0.04 vs 0.10 ± 0.07), P = 0.070 for treatment effect. Similarly, the cross-correlation between PRL

secretion and the percentage of time spent in stages I and II non-REM sleep did not change after SXB treatment (0.10 ± 0.09 vs 0.19 ± 0.07 in narcolepsy patients and 0.47 ± 0.06 vs 0.37 ± 0.10 in controls, *P* = 0.957 for treatment effect).

Table 2.3 Sleep patterns before and after sodium oxybate administration

	Baseline			Sodium oxybate		
	Patients	Controls	*P*	Patients	Controls	*P*
Wake total (%)	60.8 ± 2.9	68.7 ± 2.0	0.044*	60.8 ± 2.2	70.1 ± 2.4	0.013*
Wake day (%)	79.4 ± 4.2	95.6 ± 2.1	0.004*	82.9 ± 3.2	97.3 ± 1.0	0.001*
Wake night (%)	25.8 ± 5.7	18.4 ± 4.0	0.310	19.2 ± 4.3	19.2 ± 5.8	0.999
Stage I/II total (%)	29.1 ± 1.4	25.0 ± 2.4	0.155	26.3 ± 1.4	21.1 ± 2.2	0.063
Stage I/II day (%)	14.6 ± 3.0	2.5 ± 1.6	0.003*	11.1 ± 2.5	1.6 ± 1.0	0.005*
Stage I/II night (%)	55.1 ± 2.5	65.6 ± 5.7	0.114	53.5 ± 3.7	56.4 ± 5.3	0.647
SWS total (%)	3.7 ± 0.7	2.5 ± 0.7	0.239	7.6 ± 1.2	6.6 ± 0.9	0.534
SWS day (%)	2.1 ± 0.6	0.03 ± 0.03	0.005*	2.7 ± 1.1	0.05 ± 0.05	0.041*
SWS night (%)	6.5 ± 1.9	7.1 ± 1.9	0.843	16.5 ± 3.0	18.5 ± 2.4	0.611
REM total (%)	6.3 ± 1.8	3.7 ± 0.8	0.191	4.7 ± 1.0	2.1 ± 0.8	0.070
REM day (%)	2.9 ± 1.4	0.8 ± 0.5	0.203	1.2 ± 0.5	0.0 ± 0.0	0.032*
REM night (%)	12.6 ± 3.0	8.8 ± 1.8	0.305	10.8 ± 2.1	5.8 ± 2.3	0.127

Data are shown as mean ± SEM. The data are presented as percentages of sleep stages during the 24 h of study, before and after the SXB administration. Unpaired t-tests were used to assess the differences between the two groups. *$P < 0.05$.

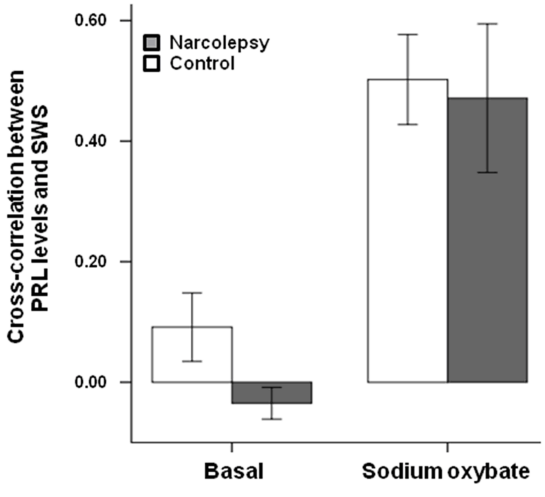

Figure 2.3 Cross-correlation coefficients between PRL levels and SWS. Sodium oxybate administration resulted in a substantial increase in the coupling between PRL release and SWS as evidenced by a significant increase in the cross-correlation ($P < 0.001$ for treatment effect).

DISCUSSION

This is the first study in which advanced endocrinological modelling has been applied to accurately assess the secretory dynamics of PRL secretion and its response to SXB challenge in narcolepsy patients. As PRL secretion dynamics was similar in hypocretin-deficient narcolepsy patients and healthy controls, our findings indicate that hypocretin is unlikely to be a major physiological regulator of PRL secretion. We showed that the SXB administration markedly increased PRL secretion and that it enhanced the association between PRL release and SWS. As SXB is known to modulate dopamine release, the major regulator of PRL secretion, these findings suggest that changes in the tubero-infundibular dopaminergic output could underlie the effect of SXB on PRL release. These effects of SXB are unlikely to involve the hypocretin system, since SXB treatment-stimulated PRL release did not significantly differ between narcolepsy patients and controls. PRL secretion is under inhibitory control of dopamine released from the tubero-infundibular dopaminergic neurons (TIDA).[109] Other inhibitors of PRL release include somatostatin and neuropeptide Y (NPY), while TRH, serotonin, oestrogen, oxytocin, as well as stress and light stimulate PRL secretion.[109] GABA has a dual effect on PRL secretion: by inhibiting TIDA neurons it stimulates PRL secretion whereas it inhibits PRL release via a non-dopaminergic pathway.[109] The effect of hypocretins on PRL release is still subject to debate. A study in male rats showed that i.c.v. administration of hypocretin-1 (orexin A) reduces plasma PRL through a pathway that appears to be partly independent of the dopaminergic system.[108] Fasting upregulates hypocretin-1 and NPY, which in turn stimulates TIDA neuronal activity and inhibits PRL secretion.[128] This effect of hypocretin-1 on dopamine, however, seems to be indirect and mediated through NPY.[78] As we found no indications for altered PRL secretion in a hypocretin-deficient state, our findings do not support a role of hypocretin in the regulation of PRL secretion. Conversely, our data also do not provide evidence for the possibility that disrupted PRL release contributes to sleep disturbance in narcolepsy.

In contrast to earlier reports in the late seventies we did not find any evidence for decreased PRL secretion in narcolepsy patients.[82;83] This discrepancy is likely due to the application of suboptimal analytical techniques in previous studies. Moreover, it is important to note in this context, that, for obvious reasons, hypocretin deficiency was not established in patients included in these early studies. A more recent study reported elevated levels of PRL in 13 narcolepsy patients (7 with typical cataplexy) compared with controls. However, in this study, which included predominantly women, the groups were not matched for BMI, and PRL levels were measured only on a single time point.[81] As PRL secretion exhibits

a marked circadian variation, is more variable in women, and is positively associated with body weight, differences in these factors may have been responsible for the differences in PRL levels between narcoleptics and controls in this study.[109] SXB administration resulted in a marked increase in PRL secretion in both narcoleptics and controls. This finding is well in line with previous reports. A nearly threefold increase in PRL within 15 min of a 2.5 g GHB injection and a fivefold increase after 1 h were reported in healthy young men.[113] Likewise, van Cauter et al. reported a dose-dependent increase in PRL secretion after the SXB administration in healthy humans.[75] The mechanism through which SXB stimulates PRL secretion is still unclear. SXB can influence dopaminergic, serotonergic and $GABA_B$ signalling, and activation of these systems could initiate PRL release.[77;112] Systemic administration with low amounts of SXB generally induces hyperpolarisation of dopaminergic structures with a reduction in dopamine release, thereby enhancing PRL secretion.[112] In addition, SXB increases the turnover of serotonin, a PRL-releasing factor, most likely due to an increase in available tryptophan, a precursor of serotonin.[84;112] Additionally, $GABA_B$ receptor stimulation may also have a stimulatory effect on serotonin and PRL release.[112] Moreover, GHRH increases GH and PRL secretion.[129] It is conceivable that SXB stimulates GHRH release and thereby promotes both GH and PRL. However, since dopamine is the major regulator of PRL secretion, the potentiating effect of SXB on PRL release is most likely due to its action on the hypothalamic TIDA neurons. In accordance with a previous study, we did not find an association between PRL release and SWS during the basal state.[114] However, after SXB administration the cross-correlation between PRL levels and SWS substantially increased. As the effect of SXB administration on PRL release is likely due to its inhibition of the activity of TIDA neurons, this finding suggests that the suppression of the hypothalamic dopaminergic system may also be responsible for the enhancing effect of SXB on SWS. Alternatively, a mechanism upstream of the TIDA system with effects on both sleep regulation and hypothalamic dopaminergic activity may be involved. In any case, this mechanism is unlikely to include the hypocretinergic system since the facilitating effect of SXB on the cross-correlation between PRL and SWS was similar in narcoleptics and controls. Further studies are needed to elucidate the exact nature of this novel regulatory circuit. A potential limitation of this study is the relatively small number of participants. However, this limitation is partly offset by the application of accurate assessment of hormone secretory dynamics, which would have been unfeasible in a larger group of subjects due to both the expensive and laborious nature of the experiments. Additionally, our subjects were very closely matched.

In conclusion, we found no evidence for altered PRL secretion in hypocretin-deficient narcolepsy patients either during the basal state or after the SXB administration. Therefore, hypocretin signalling is unlikely to be a major regulator of the lactotrophic system. Moreover, our findings suggest that the marked stimulatory effect of SXB on PRL release and SWS is mediated through its influence on the hypothalamic dopaminergic system.

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3

The effect of sodium oxybate on growth hormone secretion in narcolepsy patients and healthy controls

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ABSTRACT

Hypocretin deficiency causes narcolepsy and may affect neuroendocrine systems and body composition. Additionally growth hormone (GH) alterations may influence weight in narcolepsy. Symptoms can be treated effectively with sodium oxybate (SXB, gamma-hydroxybutyrate) in many patients. This study compared growth hormone secretion in patients and matched controls and established the effect of SXB administration on GH and sleep in both groups. Eight male hypocretin deficient narcolepsy with cataplexy and eight controls matched for sex, age, BMI, waist-to-hip ratio, and fat percentage were enrolled. Blood was sampled before and on the 5th day of SXB administration. SXB was taken 2 times 3g per night for 5 consecutive nights. Both groups underwent 24-h blood sampling at 10-min intervals for measurement of GH concentrations. The GH concentration time series were analyzed 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. Administration of SXB caused a significant increase in total 24 hour GH secretion rate in narcolepsy patients, but not in controls. After SXB, slow wave sleep (SWS) and, importantly, the cross-correlation between GH levels and SWS more than doubled in both groups. In conclusion, SXB leads to a consistent increase in nocturnal GH secretion and strengthens the temporal relation between GH secretion and SWS. These data suggest that SXB may alter somatotropic tone in addition to its consolidating effect on nighttime sleep in narcolepsy. This could explain the suggested non-sleep effects of SXB, including body weight reduction.

INTRODUCTION

Classically, narcolepsy is defined as a sleep disorder with excessive daytime sleepiness and cataplexy as the main symptoms.[130] However, in recent years, there is increasing attention to other core features of the syndrome. For example, fragmented nighttime sleep is a prominent symptom in many narcoleptic patients, and often warrants treatment.[131] In addition, patients are frequently overweight, storing excess fat in abdominal depots.[18] The increasing interest for the broad symptomatology of narcolepsy was further fuelled by new insights in the pathophysiology of the disease. In the last decade, it has been shown that deficiencies in hypothalamic hypocretin (orexin) neurotransmission are the primary cause of narcolepsy both in humans and in several animal models of the disease.[44;45;132] The hypocretin system is involved in a broad range of functions, including autonomic and hormonal regulation. Recent research therefore focused on consequences of the hypocretin deficiency in narcolepsy beyond disordered sleep regulation, such as metabolic and endocrine changes.[22;133]

Given the relation between sleep and the somatotropic axis, changes in growth hormone (GH) dynamics have received particular attention in narcolepsy.[34;106;134;135] In healthy subjects, there is a clear association between GH secretion and sleep. This is especially clear in young males, in which the majority of 24-hour GH is secreted during the first period of slow wave sleep (SWS) at night.[82;120] In a previous study, we showed that GH secretion was less strictly confined to the night in hypocretin-deficient narcolepsy.[34] As the relation between SWS and GH secretion was preserved, it was suggested that a shift of SWS episodes to the day was paralleled by a daytime shift of GH secretion.

Sodium oxybate (SXB) has evolved into a first-line treatment for narcolepsy.[72;73;136;137] SXB is a short-acting hypnotic which is dosed twice at night, at bedtime and 2.5-4 hours later. It significantly consolidates nighttime sleep, ameliorates cataplexy and in higher doses it may decrease excessive daytime sleepiness. In contrast to other hypnotics, SXB is one of the few compounds that increases rather than decreases SWS. This led to the hypothesis that it may also act as a GH-secretagogue. Indeed, it has been shown that single-dose SXB administration leads to an increase in GH secretion in healthy young men, paralleled by an increase in SWS.[75]

In the present study, we assessed the effect of repeated, twice-a-night administration of SXB on 24-hour GH secretion patterns in both patients with hypocretin-deficient narcolepsy, and matched healthy controls. GH secretion was assessed during a 24-hour sample occasion with

concomitant sleep registrations at baseline, and after 5 nights of SXB. We hypothesized that SXB administration would lead to a persistent increase in nocturnal GH secretion in both patients and controls, paralleled by an increase in SWS.

MATERIALS AND METHODS

Subjects

We included 8 male narcolepsy patients who fulfilled the diagnostic criteria for narcolepsy with cataplexy according to the 2nd edition of the International Classification for Sleep Disorders.[118] All patients were hypocretin-1 deficient, using a standardized cerebrospinal fluid assay.[132] All patients were free of medication for at least 2 weeks before study. Eight male control subjects were individually matched for age, body mass index (BMI), waist-to-hip ratio (WHR) and body fat percentage. Medical exclusion criteria were hypertension, any known (history of) pituitary, psychiatric or neurological disease, and any other chronic conditions except narcolepsy as assessed by clinical examination. Routine laboratory tests were performed to rule out diabetes (fasting plasma glucose > 6.9 mmol/L), anaemia, as well as hepatic and renal failure. Furthermore, we excluded recent weight change (> 3 kg weight gain or loss within the last 3 months), a sleep disorder history assessed through clinical interview (controls), endurance sports and alcohol or drug abuse. The study was approved by the ethics committee of the Leiden University Medical Center. All subjects provided written informed consent to participate.

Study design

All subjects underwent two 24-hour blood sampling studies, with a 5-day interval. After the baseline sampling study, subjects received SXB for 5 consecutive nights (see below). The second sampling occasion took place on the 5th day of SXB use.

Medication protocol

The first night of SXB administration took place in the hospital to provide instruction for proper usage and to monitor for possible side effects. Subjects received 3 grams of SXB in the evening (23:00 h) and during the night (3:00 h). Subjects were fasted at least 2.5 hours before drug intake, as food reduces the bioavailability of SXB. When no significant adverse

effects occurred, subjects were allowed to continue the study protocol and take SXB at home during the next three nights. The 5th night on SXB took place at the Clinical Research Center during the next sampling occasion.

Clinical protocol

Subjects were admitted to the Clinical Research Center for 24-hour blood sampling. A cannula was inserted into an antecubital vein 45 minutes before the start of blood sampling at 12:00 h. Blood samples were collected with S-Monovette (Sarstedt, Etten-Leur, The Netherlands) from a three-way stopcock attached to a 0.9% NaCl and heparin (1 U/ml) infusion (500 ml/24 h) to keep the cannula from clotting. Sampling was performed through a long line to prevent sleep disruption by investigative manipulations. Samples for IGF-1 and IGFBP-3 were both taken just before breakfast at 8:30 on each day of study. For GH measurements, blood was collected at 10-minute intervals. After clotting, the blood was centrifuged within 30 minutes of sampling (20 minutes, 1250 *g*, 4°C). Serum was then stored at -80°C until hormonal assays. Bioelectrical impedance analysis (Bodystat, Douglas, Isle of Man, UK) was used to assess lean body mass and fat percentage at 8:25 (just before breakfast).

Subjects remained sedentary except for bathroom visits. Lights were switched off at 23:00 and switched on at 07:30 the next morning. Three standardized meals were served at 08:30, 13:00, and 18:00 (Nutridrink, Nutricia, Zoetermeer, The Netherlands; 1.5 kcal/ml, 2100 kcal/d; macronutrient composition per 100 ml protein, 6 g; fat, 5.8 g; carbohydrate, 18.4 g). Subjects were asked to complete each meal provided. Water and caffeine free redbush tea were the only drinks available during the study.

Sleep analysis

Sleep was polygraphically recorded throughout both sampling occasions, using an Embletta X100 recorder (Embla, Broomfield, CO, USA). The recordings were scored visually at 30-second intervals according to the AASM criteria[119] by an experienced sleep technician. To allow assessment of the associations between changes in serum GH levels (measured every 10 minutes) and sleep stages (scored every 30 seconds), sleep profiles were divided into the 10 minute segments separating consecutive GH measurements, as described previously.[120] Every segment was condensed from the 30-second sleep epochs into percentage of time spent in wake, stage I/II non-REM sleep, stage II/IV slow wave sleep (SWS) and REM sleep.

Assays

Serum GH was measured by a time-resolved fluoroimmunoassay (DELFIA® hGH, PerkinElmer Life and Analytical Sciences, Turku, Finland). The detection limit of the assay was 0.03 mU/L, and the interassay variation ranged from 1.6 to 8.4%. Samples from each patient and matched control were handled in the same run. Total serum insulin-like growth factor IGF-1 and insulin-like growth factor binding protein IGFBP-3 concentrations were measured by radioimmunoassay (Serono, Biomedica, Milan, Italy; and Nichols, San Juan Capristano, CA, respectively). Glycosylated hemoglobin (HbA1c) levels were measured with a high performance liquid chromatography (HPLC) system (Variant, Biomed, Hercules, CA, USA). Urinary epinephrine, norepinephrine and dopamine concentrations were assessed by HPLC with electron capture detection (ESTA-Coulochem, Chelmsford, MA, USA).

Deconvolution analysis

A recently developed, fully automatic, multi-parameter deconvolution procedure, *AutoDecon*, was used to estimate various specific measures of secretion and serum disappearance rate of GH, considering all serum hormone concentrations and their dose-dependent intra-sample variance simultaneously.[138;139] The *AutoDecon* process is a statistically based algorithm to test the significance of hormone secretion events, obviating the subjective nature of previously used deconvolution methods. Apart from the initial concentration and the basal secretion rate, which both were initialized to zero, the *AutoDecon* algorithm requires only two approximations of the parameter values that are to be estimated: 1) The standard deviation of the Gaussian-shaped secretion events (Secretion*SD*) which is generally initialized as half of the data-sampling interval, and 2) a starting value for the elimination parameter, or hormone half-life. Thus, for 10-minute sampled data, the Secretion*SD* was initialized to 5-minutes together with a starting value for the GH half-life of 16-minutes. To account for intrinsic errors in the estimates of hormone secretion and clearance rates, the *AutoDecon* algorithm was then used to find the best fits for both parameters. The following parameters of the serum GH concentration time series were estimated: number of secretory bursts, secretory burst half-duration (duration at half-maximal amplitude), mean mass secreted per burst, hormone half-life, basal secretion rate, pulsatile secretion rate, and total secretion rate. Finally the first GH peak after SXB administration was defined as the first peak after medication intake detected by *AutoDecon* as described earlier.[139]

Approximate entropy (ApEn)

ApEn is a model-independent statistic used to quantify the regularity of a time series, which estimates, within a predefined tolerance *r* given a pattern of window length *m*, the likelihood of a similar pattern in the next incremental window.[140] Greater regularity yields smaller ApEn values, whereas greater independence among sequential values of a time series yields larger ApEn values. ApEn parameters of *m* = 1 and *r* = 20% of the intra-series standard deviation were used, the statistical suitability of which has been established previously.[140]

Statistical analyses

Results are expressed as mean ± standard error (SE), unless otherwise specified. Unpaired t-tests were used to assess differences in means between the two groups. In order to account for the two repeated measurements within each individual, mixed-effects models were used to assess the effects of SXB treatment and potential interaction effects. Cross-correlation analysis was applied to assess the association between serum GH concentrations and the percentage of time spent in slow wave sleep in the preceding 10 min sampling interval, taking into account all of the sampling intervals during sleep. Because of the individual matching of patients and controls and small number of subjects in each group , paired parametric (paired samples t-test) and non-parametric tests were also performed (Wilcoxon signed rank test). All tests were two-tailed and significance level was set at $P < 0.05$. Statistical calculations were performed using Systat software (version 11, Systat Software, Inc, San Jose, CA) and SPSS (release 17.0, SPSS, Inc., Chicago, IL).

RESULTS

Patients and controls were well-matched for age, BMI, waist-to-hip ratio and body fat content (Table 3.1). Serum HbA1c and glucose concentrations were similar in narcolepsy patients and controls (5.3 ± 0.08 vs. 5.3 ± 0.03, $P = 0.80$, and 4.9 ± 0.21 vs. 5.1 ± 0.12, mmol/L $P = 0.47$ respectively) SXB was well tolerated by all participants. Apart from mild drowsiness, no other side-effects were reported during the study. After 5 days of SXB, mean concentrations of IGF-1 remained similar in both patients (16.1 ± 1.4 vs. 16.5 ± 0.9 nmol/L) and controls (20.7 ± 2.5 vs. 21.7 ± 3.1 nmol/L), $P = 0.14$ and $P = 0.30$ for group and treatment effect, respectively. However, after SXB administration IGFBP-3 levels significantly decreased in both patients (4.05 ± 0.46 vs. 3.9 ± 0.3 mg/L), and controls (4.06 ± 0.31 vs. 3.84 ± 0.28 mg/L), *P* =

Table 3.1 Deconvolution of serum prolactin concentration profiles in narcolepsy patients and healthy controls

	Patients	Controls	*P*-value
Age (yrs)	38.0 ± 4.7	37.9 ± 4.1	0.98
BMI (kg/m^2)	28.1 ± 1.6	27.4 ± 1.4	0.74
Waist/hip ratio	0.92 ± 0.03	0.90 ± 0.02	0.58
Body fat (%)	23.6 ± 2.1	23.4 ± 1.7	0.95

Data are shown as mean ± SEM.

0.62 and $P = 0.035$ for group and treatment effect, respectively. (Wilcoxon signed rank test: P for intergroup difference = 0.58, P for treatment effect in patients = 0.48; P for treatment effect in controls = 0.024).

Sleep analysis

On average, compared to controls, narcolepsy patients spent significantly less time awake both during basal conditions and after SXB (Table 3.2); paired t-tests and Wilcoxon signed-rank tests yielded similar results (all $P \leq 0.039$) During the day (defined as the lights-on period between 07:30 h-23:00 h), narcolepsy patients also spent significantly less time awake, while the time spent in non-REM sleep was significantly higher regardless of treatment; paired t-tests and Wilcoxon signed-rank tests yielded similar results (all $P \leq 0.047$). SXB administration resulted in a significant decrease in stages I/II non-REM and REM sleep over 24 hours in both groups ($P = 0.011$ and $P = 0.009$, respectively), while the time spent in SWS significantly increased ($P = 0.001$). During the day, SXB administration also reduced the time spent in stages I/II non-REM and REM sleep ($P = 0.038$ and $P = 0.041$, respectively), while there was a trend for a longer period of wakefulness as well ($P = 0.098$). The percentage of SWS during the night more than doubled in both groups after SXB administration (narcolepsy: 6.5 ± 1.9 % vs.16.5 ± 3.0 %, controls: 7.1 ± 1.9 % vs. 18.5 ± 2.4 %; $P = 0.001$ for treatment effect), whereas there were trends for a decline in the percentages of stages I/II non-REM and REM sleep. During the night, SXB administration also significantly reduced the number of awakenings ($P = 0.002$), while sleep efficiency was not affected ($P = 0.082$) (Table 3.2).

Table 3.2 Sleep patterns before and after SXB administration

	Narcolepsy		Controls		Narcolepsy vs. controls (Baseline)	Narcolepsy vs. controls (SXB)	Treatment effect	Interaction (Group × Treatment)
	Baseline	SXB	Baseline	SXB				
Wake total (%)	60.8 ± 2.9	60.8 ± 2.2	68.7 ± 2.0	70.1 ± 2.4	0.044*	0.013*	0.58	0.57
Wake day (%)	79.4 ± 4.2	82.9 ± 3.2	95.6 ± 2.1	97.3 ± 1.0	0.004**	0.001**	0.098	0.60
Wake night (%)	25.8 ± 5.7	19.2 ± 4.3	18.4 ± 4.0	19.2 ± 5.8	0.31	1.00	0.40	0.087
Stage I/II total (%)	29.1 ± 1.4	26.3 ± 1.4	25.0 ± 2.4	21.1 ± 2.2	0.16	0.063	0.011*	0.62
Stage I/II day (%)	14.6 ± 3.0	11.1 ± 2.5	2.5 ± 1.6	1.6 ± 1.0	0.003**	0.005**	0.038*	0.23
Stage I/II night (%)	55.1 ± 2.5	53.5 ± 3.7	65.6 ± 5.7	56.4 ± 5.3	0.11	0.65	0.056	0.13
SWS total (%)	3.7 ± 0.7	7.6 ± 1.2	2.5 ± 0.7	6.6 ± 0.9	0.24	0.53	0.001**	0.90
SWS day (%)	2.1 ± 0.6	2.7 ± 1.1	0.03 ± 0.03	0.05 ± 0.05	0.005**	0.041*	0.49	0.56
SWS night (%)	6.5 ± 1.9	16.5 ± 3.0	7.1 ± 1.9	18.5 ± 2.4	0.84	0.61	0.001**	0.76
REM total (%)	6.3 ± 1.8	4.7 ± 1.0	3.7 ± 0.8	2.1 ± 0.8	0.19	0.070	0.009**	0.93
REM day (%)	2.9 ± 1.4	1.2 ± 0.5	0.8 ± 0.5	0.0 ± 0.0	0.20	0.032*	0.041*	0.51
REM night (%)	12.6 ± 3.0	10.8 ± 2.1	8.8 ± 1.8	5.8 ± 2.3	0.31	0.13	0.063	0.53
No. of awakenings	50.5 ± 10.5	35.0 ± 4.8	35.5 ± 7.1	15.3 ± 1.7	0.26	0.005**	0.002**	0.85
Sleep efficiency (%)	66.9 ± 7.0	81.5 ± 4.9	81.2 ± 4.0	81.9 ± 6.0	0.10	0.96	0.082	0.06

Data are shown as mean ± SEM. SXB, sodium oxybate; SWS, slow-wave sleep; REM, rapid eye movement. Percentages of sleep stages during the 24 h of study, before and after SXB administration. Unpaired t-tests were used to assess differences between the 2 groups. Mixed-effects models were applied to assess the effect of treatment and potential interaction effects between group (i.e. narcolepsy or control) and treatment. * $P < 0.05$ and ** $P < 0.01$.

Deconvolution analysis of GH time series

The deconvolution-derived GH secretory kinetics in patients and controls at baseline and after SXB are shown in Table 3.3. At baseline and after SXB administration, there were no significant differences between the groups. However, SXB resulted in a significant increase in total 24 hour GH secretion rate in narcolepsy patients (73 ± 21 vs. 112 ± 36 mU/L_{dv}), but not in controls (120 ± 19 vs. 102 ± 12 mU/L_{dv}), $P = 0.047$ for treatment × group interaction.

Regularity of serum GH concentration time series

The ApEn values of the GH time series were not significantly different between narcolepsy patients and controls, either during basal conditions (0.31 ± 0.06 vs. 0.45 ± 0.07, $P = 0.17$) or following SXB (0.23 ± 0.03 vs. 0.27 ± 0.05, $P = 0.47$). SXB administration, however, increased the regularity of GH secretion as indicated by lower ApEn values during the second study occasion in both patients and controls ($P = 0.002$ for treatment effect).

GH release and sleep association

After SXB, the ratio between GH released at night to total GH secretion significantly increased in both narcolepsy patients (0.72 ± 0.06 vs. 0.84 ± 0.03) and controls (0.55 ± 0.06 vs. 0.79 ± 0.05); $P < 0.001$ and $P = 0.456$ for treatment and group effect, respectively (Figure 3.1). We also compared the first GH secretory burst right after SXB administration (at 23:00 and 3:00 h). Compared to the baseline condition, the first dose of SXB at 23:00 h led to a significant GH secretory burst in both patients and controls ($P = 0.005$ for treatment effect; Table 3.3). However, the effect of SXB administration on the first GH secretory burst was not different between patients and controls ($P = 0.063$) (paired t-test: $P = 0.071$; Wilcoxon signed-rank test: $P = 0.093$). After the second dose the increase in GH secretion was less pronounced (Table 3.3).

During basal conditions, the mean cross-correlation between GH levels and the percentage of time spent in SWS in the previous 10 min equaled 0.24 ± 0.10 in narcolepsy patients and 0.28 ± 0.09 in controls ($P = 0.76$ for the difference in the means). SXB more than doubled the cross-correlation between GH levels and SWS in both groups (narcolepsy: 0.49 ± 0.10, controls: 0.63 ± 0.07; $P = 0.002$ for treatment effect, Figure 3.2).

Table 3.3 Deconvolution analysis of 24-hour serum GH concentrations

	Narcolepsy		Controls		Narcolepsy vs. controls (Baseline)	Narcolepsy vs. controls (SXB)	Treatment effect	Interaction (Group × Treatment)
	Basal	SXB	Basal	SXB				
Half-life (min)	13.9 ± 1.0	15.4 ± 0.8	13.1 ± 0.9	15.5 ± 0.6	0.59	0.92	0.017*	0.56
Pulse half-duration (min)	17.6 ± 2.4	17.9 ± 1.0	26.9 ± 3.6	19.8 ± 1.1	0.051	0.22	0.13	0.08
Pulse frequency (no./24 h)	20.8 ± 2.3	18.0 ± 1.5	19.0 ± 1.7	16.4 ± 1.5	0.55	0.47	0.15	0.97
Mean secreted mass/pulse (mU/L)	3.5 ± 1.0	6.7 ± 2.4	6.3 ± 1.2	6.1 ± 0.8	0.099	0.81	0.21	0.13
Mean value (mU/L)	1.0 ± 0.3	1.8 ± 0.6	1.6 ± 0.2	1.6 ± 0.2	0.19	0.75	0.12	0.091
24-h Basal production rate (mU/L_{dv})	2.4 ± 0.43	1.9 ± 0.44	5.5 ± 0.19	2.7 ± 0.66	0.13	0.32	0.09	0.23
24-h Pulsatile production rate (mU/L_{dv})	69 ± 21	109 ± 36	112 ± 18	98 ± 12	0.14	0.77	0.43	0.048*
24-h Total production rate (mU/L/L_{dv})	73 ± 21	112 ± 36	120 ± 19	102 ± 12	0.11	0.79	0.33	0.047*
Percent pulsatile (%)	93 ± 1.8	96 ± 1.0	93 ± 1.7	96 ± 0.8	0.95	0.84	0.002**	0.800
Amount of GH secreted in the 1st secretory burst, mU/ł								
After 23:00	8.3 ± 7.7	20.2 ± 7.8	12.6 ± 6.3	41.7 ± 7.3	0.673	0.063	0.005**	0.169
After 3:00	1.3 ± 0.6	6.0 ± 3.9	4.9 ± 3.5	12.5 ± 5.8	0.331	0.369	0.139	0.725

Data are shown as mean ± SEM. GH, growth hormone; L_{dv}, liter distribution volume. Unpaired t-tests were used to assess differences between the 2 groups. Mixed-effects models were applied to assess the effect of treatment and potential interaction effects between group (i.e. narcolepsy or control) and treatment. * $P < 0.05$ and ** $P < 0.01$. ł SXB was administered at 23:00 and 3:00.

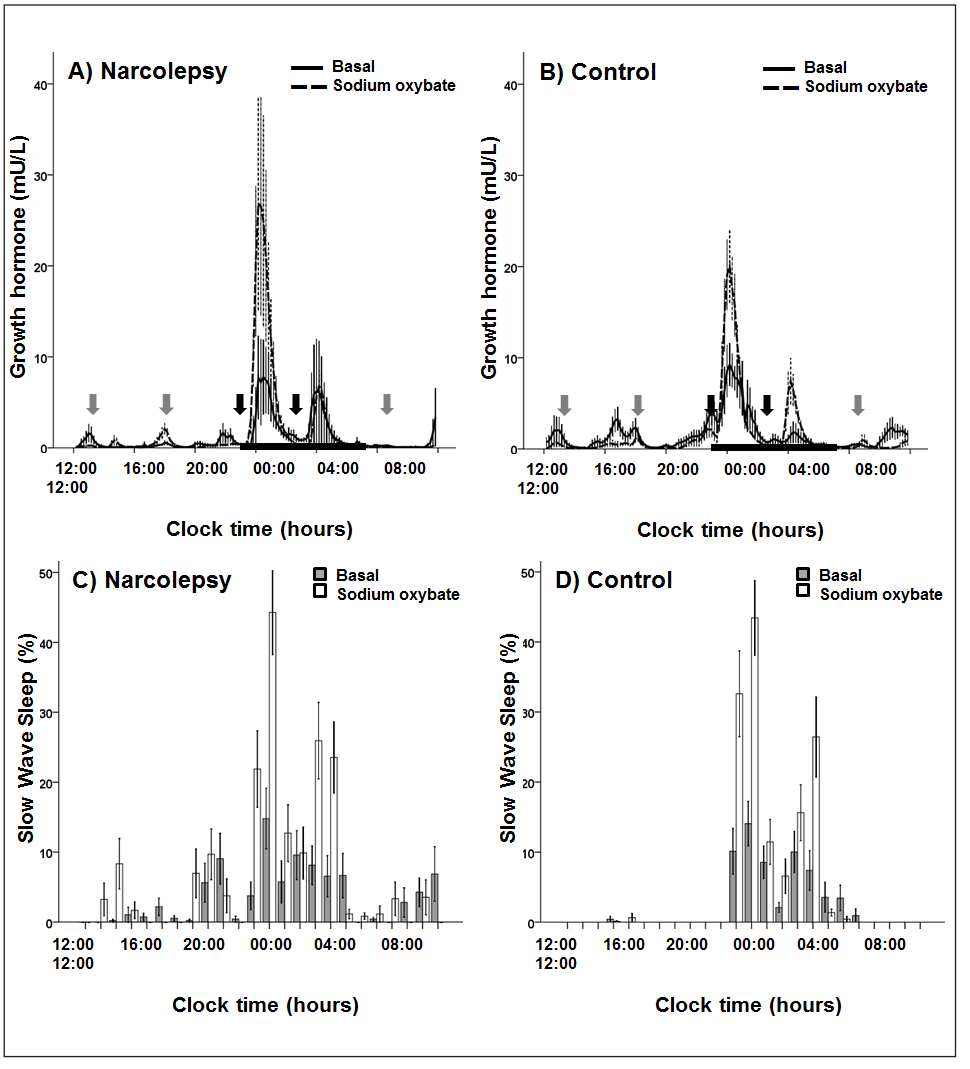

Figure 3.1 Mean serum growth hormone concentrations and slow-wave sleep in narcolepsy and matched control subjects. Blood sampling started at 1200 and was continued at 10-min intervals for 24 h, whereas sleep electroencephalogram was recorded continuously. Sodium oxybate (SXB) administration induced an immediate rise in growth hormone levels in both narcolepsy patients **(A)** and controls **(B)**. Similarly, after 5 consecutive days of SXB treatment (including the 2nd sampling occasion), the percentage of slow-wave sleep had significantly increased in both narcolepsy patients **(C)** and controls **(D)**. The black bar on the abscissa indicates the dark period (2300–0730). The gray arrows indicate the timings of the lunch, dinner, and breakfast at 1300, 1800, and 0830, respectively. The black arrows indicate the timings of SXB administration during the 2nd occasion at 2300 and 0300. Error bars show means ± SEM.

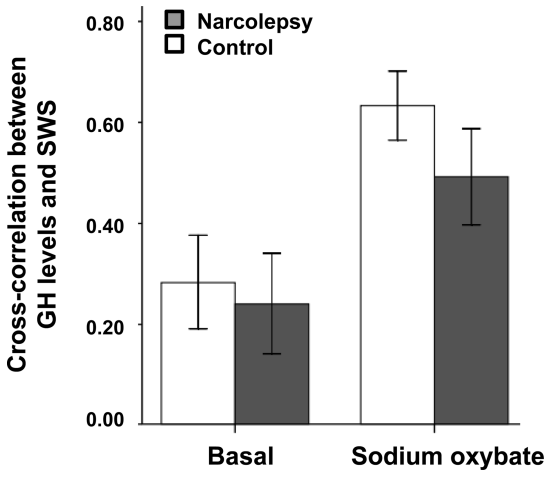

Figure 3.2 Cross-correlation coefficient between growth hormone (GH) levels and slow-wave sleep (SWS). SXB administration resulted in a substantial increase in the coupling between GH release and SWS, as evidenced by a significant increase in the cross-correlation (P = 0.002 for treatment effect). However, the effect did not differ between narcolepsy patients and controls (P = 0.282 for group effect).

DISCUSSION

We have shown that twice-a-night administration of SXB for 5 consecutive days consistently increases nocturnal GH secretion in both healthy controls and hypocretin-deficient narcolepsy patients. This was paralleled by a concomitant increase in SWS. Both the increase in GH and SWS were most prominent after the first dose of SXB at sleep onset. SXB reinforced the relation between GH and SWS, as evidenced by an almost doubled cross-correlation between the two.

GH secretion in narcolepsy has been the topic of a number of studies. Several groups found diminished or even unmeasurable GH concentrations around sleep onset.[82;83;134] In contrast, we did not find lowered total 24-hour concentrations of GH in a previous study, but rather a more dispersed pattern with a shift towards daytime secretion.[34] However, in both scenarios, SXB may partly restore the nocturnal GH 'deficit', by increasing nighttime GH secretion. The potency of SXB as a GH-secretagogue was previously shown in two daytime studies without sleep recordings,[113;141] and more recently in a controlled single-dose study using repeated sampling together with sleep registrations in healthy young males.[75] Even at the

lowest dose (2.5 grams) a twofold increase in sleep-related GH secretion was observed. We confirmed and extended these observations, showing that a second nighttime dose may further enhance GH secretion, albeit to a lesser extent than the first dose. Furthermore, stimulation of GH secretion persists after repeated use, at least after 5 consecutive days. However, in controls we did not find a difference in 24 h GH secretion before and during SXB administration. Van Cauter, Gerra, and Takahara did find an increase in GH secretion after administration of a single dose of SXB in healthy controls as well as in narcoleptic patients.[75;113;141] As a putative explanation of our findings, we believe that subchronic administration of SXB may elevate GH levels to induce feedback inhibition in controls, but not in narcoleptic patients, suggesting that narcolepsy does indeed disrupt normal control of GH release. Obviously, a single dose of SXB will not evoke such feedback inhibition, which explains the fact that other authors did not report a reduction of GH release in healthy humans. As SXB was well tolerated by subjects, this indeed suggests a potential for SXB as a strategy to counteract the relative growth hormone deficiency and sleep disturbances in the elderly, as was previously suggested.[75;80]

The close relation between sleep and the activity of the somatotropic axis has been known for a long time.[142;143] There is a wealth of data supporting the hypothesis that this relation is brought about by the simultaneous promotion of sleep and GH release by GHRH.[80;144;145] The mechanism through which SXB promotes GH secretion is unknown.[75] Some researchers claim that SXB may exert its central nervous system effects through dedicated GHB-receptors in the brain, but the existence of these receptors has been disputed.[146;147] There is clear evidence that SXB does modulate GABAergic tone through agonism of $GABA_B$ receptors, also in sleep-promoting regions of the hypothalamus.[80;146] Our data showed that SXB further strengthened the relation between SWS and GH secretion, so its effect may be mediated by an increase in GHRH activity. SXB increased the regularity of GH secretion as well. This may imply that sodium oxibate simultaneously promotes endogenous somatostatin release, as negative feedback has been shown to increase secretory regularity.[148] Although animal studies showed that hypocretin administration induced a dose-dependent reduction of GH concentrations in rats,[106] the effects of SXB on GH secretion are unlikely to be mediated by altering hypocretin tone, as results were not different between controls and hypocretin-deficient patients.

Influencing somatotropic activity in narcolepsy may have clinical relevance regarding body composition. Narcolepsy is associated with an increase in body weight. The BMI in the majority of patients is in the overweight range, as has been shown in several population based

studies.[18;19;149] In fact, there often is a clear increase in body weight around the first onset of symptoms of narcolepsy, especially excessive daytime sleepiness. Obesity in narcolepsy is not due to decreases in motor activity throughout the day.[22;150] Furthermore, the total amount of calories consumed is not increased in narcolepsy.[21] Basal metabolic rate has been studied by several groups, but inconsistent results have been reported.[151;152] The same holds true for well-known endocrine factors regulating bodyweight, such as leptin.[30-33] Obesity in narcolepsy is notoriously difficult to treat. This lends particular interest to a recent case series suggesting that SXB may decrease body weight in patients with narcolepsy.[74] In 54 treated patients, the average reduction in body weight amounted to 3.4 kg. In the patients with cataplexy, the mean weight reduction was even larger: 5.1 kg. GH has a potent lipolytic activity, while GH deficiency leads to decreases in lean body mass and an increased fat mass.[153;154] It is therefore tempting to speculate that the putative weight reducing effect of SXB is mediated by its stimulatory effect on the somatotropic axis.

We report a relatively low sleep efficiency in controls. It is conceivable that the laboratory setting disrupts sleep more than a natural environment. However the percentages of SWS and awekenings are comparable with earlier studies.[75;155]

Proper assessment of the secretion pattern of hormones that fluctuate during the day, requires repeated blood sampling over longer periods of time. Obviously, this complicates study design, and limits the number of subjects that can be included. Furthermore, five nights of SXB administration may not correctly reflect the long-term effects of SXB. Our results therefore need confirmation in future long-term studies. Nevertheless, our results suggest that future prospective long-term studies should especially focus on the effects of SXB on body weight, as this would provide a major improvement in the treatment of narcolepsy.

In conclusion, repeated administration of SXB leads to a consistent increase in nocturnal GH secretion in both healthy controls and hypocretin-deficient narcoleptic patients. SXB also strengthens the temporal relation between GH secretion and slow wave sleep. These data suggest that SXB may alter somatotropic tone in addition to its consolidating effect on nighttime sleep in hypocretin-deficient narcolepsy. This could explain the suggested non-sleep effects of SXB, including body weight reduction.

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4

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

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ABSTRACT

Study objectives: Narcolepsy is caused by a selective loss of hypocretin neurons and is associated with obesity. Ghrelin and leptin interact with hypocretin neurons to influence energy homeostasis. Here, we evaluated whether human hypocretin deficiency, or the narcolepsy therapeutic agent sodium oxybate (SXB), alter the levels of these hormones.

Methods: Eight male, medication free, hypocretin deficient, narcolepsy with cataplexy patients, and 8 healthy controls matched for age, sex, body mass index (BMI), waist-to-hip ratio, and body fat percentage were assessed. Blood samples of total ghrelin and leptin were collected over 24 hours at 60 and 20-min intervals, respectively, during 2 study occasions: baseline, and during the last night of 5 consecutive nights of SXB administration (2 × 3.0 g/night).

Results: At baseline, mean 24-h total ghrelin (936 ± 142 vs. 949 ± 175 pg/mL, $P = 0.873$) and leptin (115 ± 5.0 vs. 79.0 ± 32 mg/L, $P = 0.18$) levels were not different between hypocretin deficient narcolepsy patients and controls. Furthermore, SXB did not significantly affect the plasma concentration of either one of these hormones.

Conclusions: The increased BMI of narcolepsy patients is unlikely to be mediated by hypocretin deficiency-mediated alterations in total ghrelin or leptin levels. Thus, the effects of these hormones on hypocretin neurons may be mainly unidirectional. Although SXB may influence body weight, the underlying mechanism is unlikely to involve changes in total ghrelin or leptin secretion.

INTRODUCTION

The hypocretin system, also known as the orexin system, is of major importance in the regulation of sleep and sustained wakefulness. Moreover, hypocretin neurons are responsive to metabolites and hormones helping to translate signals of metabolic state into adaptive levels of activity and consciousness.[156;157] Hypocretin deficiency leads to narcolepsy, a sleep-wake disorder characterized by excessive daytime sleepiness, cataplexy, and disrupted nocturnal sleep. Obesity is associated with the disorder,[18] yet the cause of the increased body weight has been challenging to discern due to inconsistent findings on the hormonal and metabolic characteristics of this population.[22;30;31;33;81;151] However, altered ingestive behavior has been observed in these patients,[81;158-160] suggesting hypocretin deficiency may dysregulate feeding behavior, and possibly energy homeostasis.

Ghrelin is a peptide hormone mainly produced by endocrine cells in the stomach and gastrointestinal tract, and is an important endogenous regulator of energy balance and growth hormone (GH) secretion.[161] Its expression is complex89 and influenced by sympathetic nervous system activity.[90] Across the wake period, plasma concentrations wax and wane episodically, providing an appetite-stimulating signal to the brain.[91] During sleep, ghrelin levels rise sharply in the early part of the night and decrease gradually toward morning. During sleep deprivation, however, levels gradually rise toward a plateau in the morning.[162] Hypocretin neurons directly sense and are excited by ghrelin, and an interaction between these two systems has been shown to be involved in ingestive behavior.[87] A study by Toshinai et al. first identified this connection.[92] In that study, ghrelin-induced feeding was attenuated in rats pretreated with anti-hypocretin-1 IgG and anti-hypocretin-2 IgG, and suppressed in hypocretin knockout mice. Later, it was demonstrated that ghrelin plays a key role in the rewarding aspects of eating, but it requires the presence of intact hypocretin signaling to impart this effect.[163]

Leptin is another peptide hormone involved in energy homeostasis, the dominant role of which is to signal energy deficiency to the brain.[164] It is an adipokine produced primarily by subcutaneous white adipose tissue, and its expression is stimulated by various hormones, sympathetic outflow, energy intake, and output.[164;165] Under normal conditions, blood levels display circadian variation as levels rise across the day and peak in the middle of the night.[166] During sleep deprivation, blood leptin levels show a reduced and flattened profile.[167] Receptors for leptin are found on hypocretin cells,[86] and leptin can directly inhibit the expression of isolated hypocretin neurons.[87] Indirectly, leptin can affect the activity of hypocretin cells via energy regulating neurons in the arcuate nucleus of the

hypothalamus.[88] Conversely, because the hypocretin system greatly influences autonomic control,[156] it is plausible that hypocretin deficiency may alter leptin expression via inhibited sympathetic activity. Indeed, obese hypocretin deficient mice have lowered sympathetic vasoconstrictor outflow, while greater heart rate variability has been observed in hypocretin deficient narcolepsy patients.[168;169] Thus, leptin and hypocretin may interact to affect levels of physical activity and wakefulness in response to energy needs, and the loss of hypocretin neurons may dysregulate leptin expression and signaling. While ghrelin levels have not been previously reported in hypocretin-deficient narcoleptic patients, abnormal leptin levels have been observed.[30;31] It is unknown if the associations between hypocretin and total ghrelin or leptin are uni-or bi- directional. Because hypocretin influences sympathetic outflow and sympathetic nervous system activity affects the expression of both leptin and ghrelin, hypocretin deficiency may lead to altered levels of these hormones. This study of hypocretin deficient narcoleptic patients provides a unique opportunity to further explore the nature of these relationships. We hypothesized that both total ghrelin and leptin levels would be abnormal in hypocretin-deficient narcolepsy patients, which might help explain the increased BMI and abnormal ingestive behavior seen in this population.[22;30;81;149;170;171]

Additionally, we explored if the narcolepsy therapeutic agent, sodium oxybate (SXB), has an effect on these hormones. In a narcolepsy population, SXB improves disrupted nocturnal sleep, impaired wakefulness, and cataplexy, and promotes weight loss.[74;172] Like ghrelin, SXB administration also stimulates GH release.[75] We hypothesized that its administration would alter total ghrelin levels, the effect of which might be involved in its GH-promoting effects. Here, we investigate whether total blood ghrelin or leptin levels are altered in hypocretin-deficient narcoleptic patients compared to controls, and whether total ghrelin or leptin levels are influenced by SXB.

MATERIALS AND METHODS

Subjects

We included 8 medication-free, male hypocretin-deficient narcolepsy with cataplexy patients and 8 healthy male controls, matched for age, BMI, and body fat percentage. Hypocretin measurement was performed according to international standards.[46] Body fat percentage was measured with bioelectrical impedance analysis (Bodystat, Douglas, Isle of Man, UK). Two patients were drug naive, one patient was tapered from antidepressants ≥ 2 weeks prior

to the study, and 2 patients had prior history with SXB; however, no subject took sodium oxybate within 20 days of study initiation. The other patients did not take any medication for at least several months prior to beginning the study.

Subjects were eligible for participation after exclusion of chronic conditions, with particular attention to the absence of sleep disorders in control subjects, hypertension, pituitary disease, and weight change (> 3 kg weight gain or loss within the last 3 months) as assessed by structured clinical interview. None of the participants had previously undergone gastrectomy. Written informed consent was obtained from all subjects. The study was approved by the ethics committee of the Leiden University Medical Center.

Clinical protocol

All subjects were admitted to the Clinical Research Center for 24-h blood sampling before and after 5 days of SXB administration. A cannula was inserted into an antecubital vein ≥ 45 min before the start of blood sampling at 12:00. Blood samples were collected with S-Monovette (Sarstedt, Etten-Leur, The Netherlands) from a 3-way stopcock that was attached to a 0.9% NaCl and heparin (1 U/mL) infusion (750 mL/24 h) to keep the cannula from clotting. For total ghrelin measurements, blood was collected in EDTA tubes at 60-min intervals, and these tubes were immediately put on ice. Ghrelin samples were acidified with 50 µL of 1 N HCL. Within 5 min of sampling, tubes were centrifuged at 1,250 *g* at 4°C for 20 minutes. For leptin measurements, blood was collected at 20-min intervals. After clotting, the blood was centrifuged within 30 min of sampling (20 min, 1,250 *g*, 4°C). Serum was then stored at -80°C until hormonal assays. Three standardized meals were served at 08:30, 13:00, and 18:00 (Nutridrink, 1.5 kcal/mL, 2,100 kcal/d; macronutrient composition per 100 mL: protein, 6 g; fat, 5.8 g; carbohydrate, 18.4 g; Nutricia, Zoetermeer, The Netherlands). Subjects were asked to complete each meal provided. Food-induced suppression of total ghrelin release was defined as the ratio between total ghrelin levels one hour post- prandially to the levels immediately before the meal (lunch and dinner) or 30 min postprandially to 30 min before the meal (breakfast). Subjects remained sedentary except for bathroom visits. In both study occasions, lights were switched off (dark period) at 23:00 and then switched on at 07:30.

Sodium oxybate

In the drug-intervention study occasion, SXB was administered in a total nightly dose of 6 grams/night for 5 consecutive nights in both the narcoleptic patients and the controls. Each

night, 3 grams of SXB were administered orally at 23:00 and 03:00. Lights were turned off after ingestion of the first dose.

Assays

Plasma total ghrelin and leptin levels were measured by radioimmunoassay (LINCO Research, St. Charles, MO, USA) with a detection limit of 93 pg/mL, and an interassay variation ranging from 14.7% to 17.8% for total ghrelin and a detection limit of 0.5 μg/L and an interassay variation ranging from 3.0% to 5.1% for leptin. Samples from each patient and matched control were handled in the same run.

Deconvolution analysis

Leptin concentration time series were analyzed via a recently developed automated deconvolution method, empirically validated using hypothalamo-pituitary sampling and simulated pulsatile time series.[121] The Matlabbased algorithm first detrends the data and normalizes concentrations to the unit interval [0, 1]. Second, the program creates multiple successive potential pulse-time sets, each containing one fewer burst via a smoothing process (a nonlinear adaptation of the heat-diffusion equation). Third, a maximum-likelihood expectation estimation method computes all secretion and elimination parameters simultaneously conditional on each of the multiple candidate pulse-time sets. The fast half-life was represented as 3.4 min constituting 19% of the decay amplitude. The slow half-life was estimated as an unknown variable between 6 and 70 min. Here we present only results for pulse frequency (pulses per 24 h), basal secretion, pulsatile secretion, and total secretion per 24 h, all expressed as μg per liter distribution volume.

Data analysis and statistics

Results are expressed as mean ± SD unless otherwise specified. Unpaired t-tests were used to assess differences in means between the 2 groups, while paired t-tests were applied to assess changes in means within each group. All tests were 2-tailed, and significance level was set at $P < 0.05$. Statistical analyses were performed using SPSS for Windows (release 17.0, SPSS, Inc., Chicago, IL).

RESULTS

Subjects

Patients and controls did not differ with respect to age, BMI, waist-to-hip ratio, and body fat percentage (Table 4.1). SXB was well tolerated by all participants. Apart from mild drowsiness, no other side effects were reported during the study.

Sleep and wakefulness differences

When compared to controls, during baseline conditions and after SXB administration, narcolepsy patients spent significantly less time awake across a 24 h period, and during the day (defined as the lights-on period between 07:30-23:00) they spent less time awake and more time in slow wave sleep ([SWS] $P = 0.004$ and $P = 0.005$, respectively; Table 4.2).

Effect of sodium oxybate administration on sleep and wakefulness

In both groups, administration of SXB resulted in a significant decrease in stages I/II NREM and REM sleep over 24 h ($P = 0.011$ and $P = 0.009$, respectively), while at night, awakenings were significantly reduced ($P = 0.002$) and the percentage of SWS more than doubled (narcolepsy: 6.5% ± 5.5% vs.16.5% ± 8.4%, controls: 7.1% ± 5.5% vs. 18.5% ± 6.4%; $P = 0.001$ for administration effect). During the day, time spent in stages I/II NREM and REM sleep ($P = 0.038$ and $P = 0.041$, respectively) was reduced, while there was a trend towards longer periods of wakefulness ($P = 0.098$).

Table 4.1 Demographics, body composition, baseline parameters

	Patients	Controls	*P*-value
Age (yrs)	38.0 ± 4.7	37.9 ± 4.1	0.98
BMI (kg/m^2)	28.1 ± 1.6	27.4 ± 1.4	0.74
Waist/hip ratio	0.92 ± 0.03	0.90 ± 0.02	0.58
Body fat (%)	23.6 ± 2.1	23.4 ± 1.7	0.95

Data are shown as mean ± SEM.

Table 4.2 Sleep patterns before and after SXB administration

	Narcolepsy		Controls		Narcolepsy vs. controls (Baseline)	Narcolepsy vs. controls (SXB)	Treatment effect	Interaction (Group × Treatment)
	Baseline	SXB	Baseline	SXB				
Wake total (%)	60.8 ± 2.9	60.8 ± 2.2	68.7 ± 2.0	70.1 ± 2.4	0.044*	0.013*	0.58	0.57
Wake day (%)	79.4 ± 4.2	82.9 ± 3.2	95.6 ± 2.1	97.3 ± 1.0	0.004**	0.001**	0.098	0.60
Wake night (%)	25.8 ± 5.7	19.2 ± 4.3	18.4 ± 4.0	19.2 ± 5.8	0.31	1.00	0.40	0.087
Stage I/II total (%)	29.1 ± 1.4	26.3 ± 1.4	25.0 ± 2.4	21.1 ± 2.2	0.16	0.063	0.011*	0.62
Stage I/II day (%)	14.6 ± 3.0	11.1 ± 2.5	2.5 ± 1.6	1.6 ± 1.0	0.003**	0.005**	0.038*	0.23
Stage I/II night (%)	55.1 ± 2.5	53.5 ± 3.7	65.6 ± 5.7	56.4 ± 5.3	0.11	0.65	0.056	0.13
SWS total (%)	3.7 ± 0.7	7.6 ± 1.2	2.5 ± 0.7	6.6 ± 0.9	0.24	0.53	0.001**	0.90
SWS day (%)	2.1 ± 0.6	2.7 ± 1.1	0.03 ± 0.03	0.05 ± 0.05	0.005**	0.041*	0.49	0.56
SWS night (%)	6.5 ± 1.9	16.5 ± 3.0	7.1 ± 1.9	18.5 ± 2.4	0.84	0.61	0.001**	0.76
REM total (%)	6.3 ± 1.8	4.7 ± 1.0	3.7 ± 0.8	2.1 ± 0.8	0.19	0.070	0.009**	0.93
REM day (%)	2.9 ± 1.4	1.2 ± 0.5	0.8 ± 0.5	0.0 ± 0.0	0.20	0.032*	0.041*	0.51
REM night (%)	12.6 ± 3.0	10.8 ± 2.1	8.8 ± 1.8	5.8 ± 2.3	0.31	0.13	0.063	0.53
No. of awakenings	50.5 ± 10.5	35.0 ± 4.8	35.5 ± 7.1	15.3 ± 1.7	0.26	0.005**	0.002**	0.85
Sleep efficiency (%)	66.9 ± 7.0	81.5 ± 4.9	81.2 ± 4.0	81.9 ± 6.0	0.10	0.96	0.082	0.06

Data are shown as mean ± SEM. Percentages of sleep stages during the 24 h of study, before and after SXB administration. Unpaired t- tests were used to assess differences between the 2 groups. Mixed-effects models were applied to assess the effect of treatment and potential interaction effects between group (i.e. narcolepsy or control) and treatment. * $P < 0.05$ and ** $P < 0.01$.

Baseline total ghrelin levels

Mean 24-h total ghrelin levels at baseline were virtually identical between narcolepsy patients and controls (P = 0.873; Figure 4.1A). Mean total ghrelin levels were also not different between the 2 groups when the analyses were restricted to the dark period (P = 0.973). In fact, at no single time-point an intergroup difference could be detected (all $P \geq 0.232$). Food induced suppression of total ghrelin concentration (expressed as the ratio between postprandial to preprandial total ghrelin concentration) was similar in the 2 groups (lunch: P = 0.413, dinner: P = 0.301, breakfast: P = 0.437, and mean postprandial total ghrelin levels averaged over the 3 occasions [P = 0.540]) (Table 4.3).

Effect of sodium oxybate on total ghrelin levels

Twenty-four hour mean total ghrelin levels during SXB administration were not different between narcolepsy patients and controls (P = 0.642; Figure 4.1B). Similar to baseline, mean

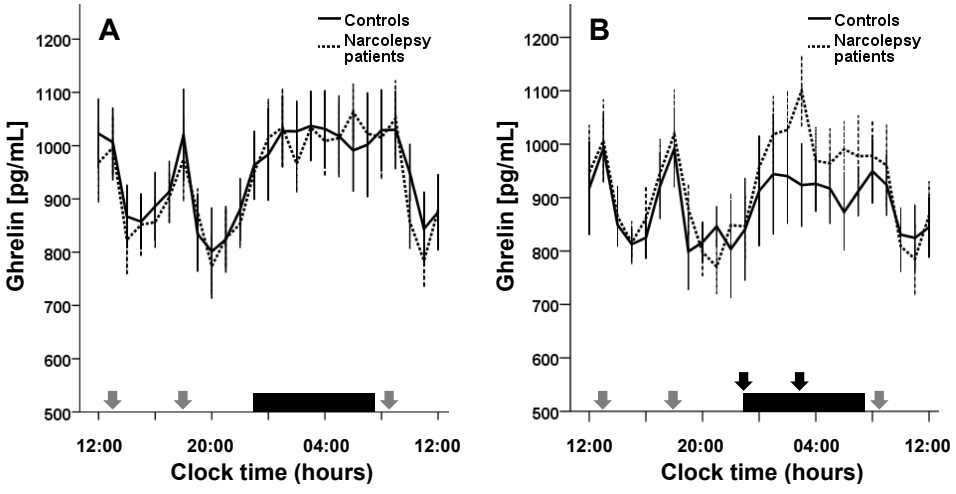

Figure 4.1 Mean 24-h ghrelin levels in narcolepsy patients and matched controls. The diurnal plasma ghrelin levels, as well as food induced suppression of ghrelin release were not significantly different between narcolepsy patients and matched controls, either during basal conditions **(A)** or after five days of sodium oxybate administration **(B)**. Hourly blood sampling started at noon and continued for 24 hours. The black bar on the abscissa indicates the dark period (23:00-7:30 h). The grey arrows indicate the timings of the lunch, dinner and breakfast at 13:00 h, 18:00 h and 08:30 h, respectively. The black arrows indicate the timings of sodium oxybate administrations during the second occasion at 23:00 h and 03:00 h. Error bars show the means ± SD.

Table 4.3 Plasma ghrelin concentrations and deconvolution of leptin levels before and after administration of sodium oxybate in both narcoleptic patients and controls

	Baseline			Sodium oxybate		
	Patients	Controls	*P*	Patients	Controls	*P*
Ghrelin						
24-h total integrated concentration (pg/mL)	936 ± 142	949 ± 175	0.873	920 ± 142	886 ± 150	0.642
Dark period[a] (pg/mL)	1012 ± 156	1009 ± 196	0.973	983 ± 163	910 ± 211	0.449
Food induced suppression of ghrelin concentration[b] (pg/mL)						
Lunch	0.83 ± 0.10	0.86 ± 0.09	0.413	0.87 ± 0.08	0.88 ± 0.16	0.920
Dinner	0.93 ± 0.16	0.83 ± 0.17	0.301	0.89 ± 0.20	0.80 ± 0.10	0.261
Breakfast	1.05 ± 0.10	1.01 ± 0.09	0.437	0.98 ± 0.12	0.98 ± 0.06	0.880
Postprandial total ghrelin[c] (pg/mL)	0.93 ± 0.08	0.90 ± 0.11	0.540	0.91 ± 0.08	0.88 ± 0.06	0.428
Leptin						
Total 24-h secretion (µg/Lx24h)	115 ± 98	79.0 ± 88	0.18	100 ± 113	64.0 ± 35	0.58
Basal 24-h secretion (µg/Lx24h)	64.7 ± 63	37.9 ± 30	0.96	56.0 ± 70	47.6 ± 63	0.94
Pulsatile 24-h secretion (µg/Lx24h)	50.3 ± 36	25.6 ± 11	0.11	43.8 ± 46	31.0 ± 27	0.29
Pulse frequency (no/24h)	18.5 ± 2.7	15.3 ± 4.8	0.04	19.8 ± 2.4	19.0 ± 3.0	0.04

[a] In both study occasions, lights were switched off (dark period) at 2300 h and then switched on at 0730 h.
[b] Expressed as the ratio between post- to preprandial ghrelin concentration.
[c] Averaged over three occasions.

total ghrelin levels during the dark period did not differ between the 2 groups ($P = 0.449$), and at no single time-point a difference could be detected between groups (all $P \geq 0.05$). Postprandial total ghrelin suppression, as defined above, was also similar between the 2 groups after SXB administration: lunch ($P = 0.920$), dinner ($P = 0.261$), and breakfast ($P = 0.880$); mean postprandial total ghrelin levels averaged over the 3 occasions ($P = 0.428$) (Table 4.3). The average change in 24-h total ghrelin levels between the second and first occasion amounted to -15 ± 72 pg/mL in narcolepsy patients and -63 ± 87 pg/mL in controls, but was not significantly different from zero in either group (paired t-tests: $P = 0.56$ and $P = 0.078$, respectively).

Baseline leptin levels

Mean 24-h total leptin levels at baseline were not significantly different between narcolepsy patients and controls ($P = 0.18$; Figure 4.2A). Mean pulse frequency was different between the 2 groups ($P = 0.04$), but mean 24-h basal and pulsatile secretion levels were not different ($P = 0.96$; $P = 0.11$, respectively; Table 4.3).

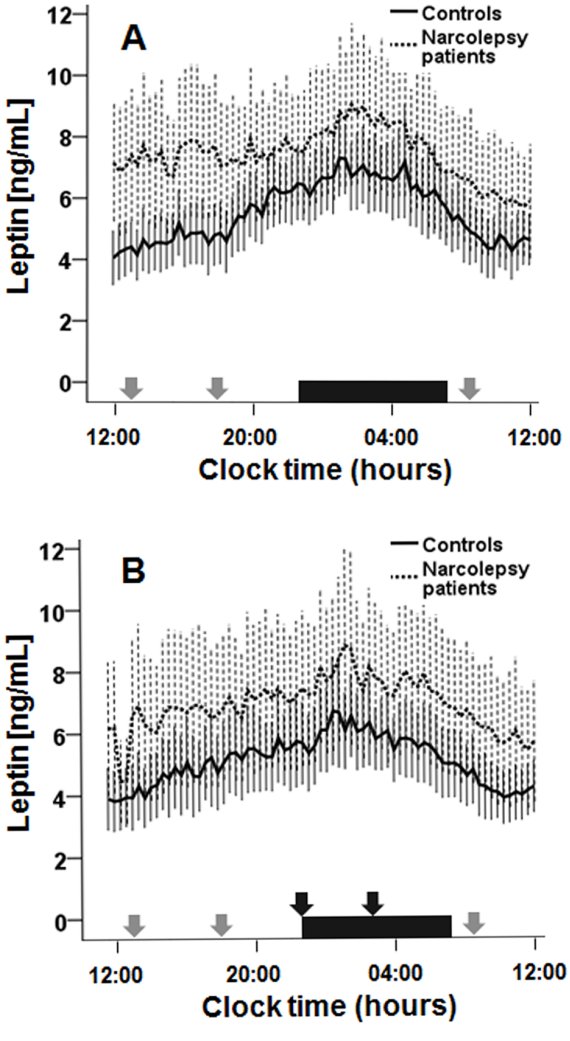

Figure 4.2 Mean 24-h plasma leptin concentration ± SD, before **(A)** and during sodium oxybate administration **(B)** in narcolepsy patients and matched controls. The black horizontal bar on the abscissa indicates the lights off period. The grey arrows indicate the timing of meals and the black arrows indicate timing of sodium oxybate administration **(B)**.

Effect of sodium oxybate on leptin levels

Mean 24-h total leptin levels during SXB treatment were not significantly different between narcolepsy patients and controls ($P = 0.58$; Figure 4.2B); neither were mean 24-h basal and pulsatile secretion rates ($P = 0.94$; $P = 0.29$, respectively). Mean pulse frequency was different between the 2 groups ($P = 0.04$; Table 4.3).

DISCUSSION

We found no differences in mean 24-h total plasma ghrelin levels or food-induced suppression of ghrelin concentrations between narcolepsy patients and controls, nor any influence of 5 days of SXB administration in both groups. In view of the capacity of ghrelin to stimulate growth hormone secretion, it is worth noting that a report from this same research protocol showed no differences in mean hourly GH levels between patients and controls, supporting our conclusion that total ghrelin levels are not altered with hypocretin deficiency.[173]

Despite the excitatory influence of ghrelin on hypocretin neurons and the interaction of the ghrelin-hypocretin systems to influence food reinforcement, our finding did not show the total ghrelin level to be influenced by hypocretin deficiency, suggesting a unidirectional relationship. These findings also suggest that disturbed ingestive behavior is unlikely mediated by an altered total ghrelin level in narcolepsy patients. Notably, 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,[174] it remains possible that the active fraction may be altered in this population.

In contrast to earlier reports,[30;31] more recent, larger, controlled studies have not demonstrated an abnormal leptin level in humans with hypocretin deficiency.[32;33] Similar to the recent research on this subject, we found that the mean 24-h total leptin level, and basal and pulsatile secretion levels were not significantly different between narcolepsy patients and controls. The mean leptin pulse frequency was slightly but significantly higher in narcolepsy patients in both conditions, but the clinical relevance of this finding is unclear. Because sleep disruption and insulin resistance have been shown to affect leptin levels,[175] it is plausible that previous investigations showing decreased leptin in narcolepsy may have resulted from a study sample of narcoleptic patients with relatively poor sleep or a difference in insulin sensitivity compared to the control group.

There were several limitations to the study. The small number of patients and controls raise the possibility of a type II statistical error. However, the intergroup differences were very small; therefore, a large sample size would be needed to detect a difference if present. As with many chronic diseases, compensatory mechanisms are likely involved in narcolepsy as the condition progresses from onset into the chronic stage. Studying narcoleptic patients only during the chronic stage challenges the interpretation that loss of hypocretin cells in the hypothalamus does not alter leptin and ghrelin levels since compensatory adaptation may have already taken place. However, alterations in appetite and weight regulation remain present and clinically relevant in the chronic stage of the disease, and therefore our findings remain relevant despite putative compensations. Additionally, since sleep-wake state instability is intrinsic to hypocretin deficiency, standardizing research parameters such as study environment, meal timing and composition, and predefined bedtimes may have created a setting not representative of real-life conditions for these patients. Therefore, although we did not find alterations in total ghrelin and leptin concentrations in this controlled and standardized environment, it remains possible that the release of these hormones is affected by the altered sleep, wake, and eating patterns described in this population.

As expected, in both groups nighttime administration of SXB increased SWS and reduced awakenings, and the narcoleptic patient group showed a trend towards increased wakefulness the following day. As demonstrated in other studies, acute SXB administration corresponds with a significant increase in GH release.[75;173] However, we found no evidence that the GH-elevating effect is mediated through an influence on total ghrelin secretion. Various treatment effects of SXB exhibit discrete temporal dynamics with some effects occurring acutely and other effects taking place only after chronic exposure. Although the difference in total ghrelin levels between patients and controls after SXB administration was not significant, it is possible that significant differences would be seen with higher doses, prolonged periods of nightly administration, or in a larger group of subjects. Lastly, we did not see an effect of SXB on the leptin level, and to our knowledge, an interaction between this drug and hormone has not been reported elsewhere. Therefore, mechanisms underlying increased BMI and altered ingestive behavior 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. Future investigations should further evaluate if the sleep-wake instability intrinsic to hypocretin deficient narcolepsy promotes ingestive and activity patterns that promote positive energy balance.

5

Altered circadian rhythm of melatonin concentrations in hypocretin-deficient men

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ABSTRACT

Hypocretin deficiency causes narcolepsy. It is unknown whether melatonin secretion is affected in this sleep disorder. Therefore, in both narcolepsy patients and matched controls we measured plasma melatonin levels hourly for 24 h before and after five days of sodium oxybate (SXB) administration. Although mean melatonin concentrations were similar between patients and controls, in narcoleptics the percentage of 24-h melatonin secreted during the daytime was significantly higher, and melatonin secretion exhibited a weaker coupling to sleep. SXB did not affect melatonin secretion. These findings suggest that hypocretin deficiency might disturb both the circadian control of melatonin release and its temporal association with sleep.

INTRODUCTION

Narcolepsy in humans is caused by hypocretin deficiency due to loss of hypocretin-producing neurons in the hypothalamus by an as yet unknown mechanism.[47;48] An autoimmune cause is assumed but has not been proven yet.[62] Hypocretins play an important role in the regulation of various homeostatic functions, most notably the consolidation of the sleep/wake cycle.[176] Therefore, it is not surprising that the inability to stay awake during the day and asleep during the night are among the major symptoms of narcolepsy.[102] Another potential modulator of sleep is melatonin, high levels of which have been associated with sleepiness.[93] The synthesis and secretion of melatonin in the pineal gland is under the influence of the circadian clock genes in the suprachiasmatic nucleus (SCN) of the hypothalamus.[93] Apart from its circadian control, melatonin production is also regulated by the light/dark cycle and primarily occurs at night.[93] Specialized melanopsin-containing cells in the retina detect a narrow band of blue wavelengths and subsequently signal the SCN, resulting in melatonin suppression during exposure to (day)light.[177] Furthermore, accumulating evidence derived from experiments on various animal models indicates that the hypocretinergic innervation of the pineal gland could also be important for the regulation of diurnal melatonin synthesis and secretion.[94;178-181]

Sodium oxybate (SXB), or γ-hydroxybutyrate, has been shown to be highly effective in the treatment of narcolepsy-associated sleep disturbances.[73] It reduces cataplexy, improves nocturnal sleep fragmentation, and, at higher doses, it may also reduce excessive daytime sleepiness (EDS). The mechanisms of action of SXB are poorly understood. It is known to act on the GABAB receptor, whereas the existence of a special GHB receptor is still a matter of debate. SXB might act via modulation of melatonin release, which is known to affect sleep. Indeed, previously we have shown that GABAergic mechanisms affect melatonin release.[182-184] Several papers have delineated melatonin secretion in narcolepsy patients, primarily focusing on whether altered melatonin secretion could contribute to EDS and nocturnal sleep disturbances.[185-187] Although most of these studies found melatonin levels within the normal range in narcolepsy patients, in a more recent study Blazejova et al. discovered elevated saliva melatonin levels during the day in almost half of the patients.[188] These patients had a shorter sleep onset on the Multiple Sleep Latency Test (MSLT) compared to those without elevated daytime melatonin levels. However, the MSLT and melatonin sampling were performed on separate occasions. Therefore, as comparison of findings from these previous studies is hampered by significant methodological differences, it remains to be elucidated whether, how, and to which extent melatonin secretion is altered in narcolepsy patients.

Given the recent indications for a hypocretinergic innervation of the pineal gland, we hypothesized that melatonin secretion and/or its circadian rhythmicity might be altered in hypocretin-deficient narcolepsy patients, and that SXB might reverse these alterations.[94;178-181] To evaluate this hypothesis we performed 24-h blood sampling studies in combination with polysomnography on a group of hypocretin-deficient narcolepsy patients and rigorously matched healthy controls, both before and after SXB administration.

SUBJECTS AND METHODS

Subjects

Seven male narcolepsy patients with definite cataplexy, who fulfilled the diagnostic criteria of the second edition of the International Classification for Sleep Disorders were included.[118] All narcolepsy patients had hypocretin levels under the detection limit using a standardized cerebrospinal fluid assay[132] and were free of medication for at least 2 weeks before study. Seven healthy controls, matched for sex, age, body mass index (BMI), waist-to-hip ratio (WHR) and fat percentage were included for comparison (see Table 5.1 for subjects' characteristics). Subjects were eligible for study after exclusion of hypertension, any known (history of) pituitary, psychiatric or neurological disease (other than narcolepsy), alcohol or drug abuse, recent weight change (> 3 kg weight change within the last 3 months), a sleep disorder history (controls), and endurance sports. Routine laboratory tests were performed to rule out diabetes, anaemia, hepatic, and/or renal disease. The study was approved by the ethics committee of the Leiden University Medical Centre and adhered to the ethical standards outlined in the Helsinki Declaration.[189] Written informed consent was obtained from all subjects.

Table 5.1 Group characteristics

	Narcolepsy	Controls	*P*-value
Age (yrs)	34.3 ± 3.4	34.7 ± 3.0	0.93
BMI	28.2 ± 1.8	27.8 ± 1.6	0.89
Fat %	22.9 ± 2.3	23.9 ± 1.9	0.76
WHR	0.90 ± 0.04	0.90 ± 0.01	0.99

Data are shown as mean ± SEM. Comparisons were made using independent t-test.

Protocol

Subjects were admitted to the Clinical Research Centre for 24-h blood sampling. A cannula was inserted into an antecubital vein 45 min before the start of blood sampling at 12:00 h. Blood samples were collected with S-Monovette (Sarstedt, Etten-Leur, The Netherlands) from a three-way stopcock attached to a 0.9% NaCl and heparin (1 U/ml) infusion (750 ml/24 h) to keep the cannula from clotting. Sampling was performed through a long line to prevent sleep disruption by investigative manipulations. For melatonin measurements, blood was collected in EDTA tubes at 60-min intervals, except the sample at 23:30 h which was taken 30 min after the previous sample. Blood was put on ice immediately after sampling and centrifuged within 5 min (1250 *g* at 4°C for 20 min). Subsequently, plasma was divided into separate aliquots in Sarstedt tubes and stored at -80°C until assay. Three standardized meals were served at 08:30, 13:00, and 18:00 h (Nutridrink, Nutricia, Zoetermeer, The Netherlands; 1.5 kcal/ml, 2100 kcal/d; macronutrient composition/100 ml: protein, 6 g; fat, 5.8 g; carbohydrate, 18.4 g). A 24-h urine sample was collected for the determination of catecholamine concentrations. Patients and controls were always studied in the same room, mostly in parallel to keep circumstances equal and rule out seasonal differences. Subjects remained (semi)supine except for bathroom visits. Daytime naps were allowed. Light intensity was between 300 and 400 lux during L and < 10 lux during D. Lights were switched off at 23:00 h and turned on at 07:30 h the next day. Twenty-four hour sampling was performed at baseline and on the fifth day of SXB administration.

Sodium oxybate

SXB was administered in two nighttime doses of three grams each, at 23:00 h and 03:00 h. To monitor for possible side effects, the first night of administration was done on the neurology ward.

Sleep analysis

Sleep was polygraphically recorded throughout both sampling occasions, using an Embletta X100 recorder (Embla, Broomfield, CO, USA). The recordings were scored visually by an experienced sleep technician at 30-s intervals according to AASM criteria.[190] To allow assessment of the association between changes in plasma melatonin levels (measured every 60 min) and sleep stages (scored every 30 s), sleep profiles were divided into 30-min segments as described previously.[120] These segments were condensed from the 30-s scoring intervals by calculating the percentage of time spent in stages I and II non-REM sleep, slow

wave sleep (SWS), and REM sleep in the 30 min immediately preceding each melatonin measurement. The temporal relation between melatonin levels and the conjoining sleep segments was subsequently assessed by cross-correlation analysis.

Melatonin assay

Melatonin levels were measured by radioimmunoassay after extraction from plasma with dichloromethane.[191] Duplicate aliquots each containing 100 µl of extracted plasma were assayed by adding 100 µl of a specific rabbit antiserum (R19540, INRA, Nouzilly, France) and 300 µl of labelled [125I]-2-iodomelatonin. The mixture solution was incubated overnight at 4°C. Then 800 µl of an anti-rabbit gamma globulin was added, and the tubes were put on ice for 1 h. Tubes were then centrifuged and the supernatant discarded. The pellet was counted using a γ-ray counter. The detection limit of the assay was 1 pg/tube (10 pg/ml plasma).

Statistical analysis

Results are expressed as mean ± standard error (SEM), unless otherwise specified. Statistical comparisons were made with either the Student's t-test or repeated-measures ANOVA, as appropriate. The area under the curve (AUC) of the melatonin versus time plots was calculated as a measure of the total 24-h melatonin secretion. As there was considerable interindividual heterogeneity in absolute melatonin concentrations, diurnal variations in melatonin secretion were assessed by calculating the percentage of the 24-h AUC during the lights-on period (i.e., 07:30 - 23:00 h). Cross-correlation analysis was applied to assess the association between melatonin levels and the percentage of time spent in various sleep stages in the preceding 30 min.[127] Cross-correlation analysis is a standard method of assessing the degree of correlation between two time series, and was defined here as:

$$r = \frac{\sum_{t=0}(m_t - \bar{m})(s_t - \bar{s})}{\sqrt{\sum_{t=0}(m_t - \bar{m})^2}\sqrt{\sum_{t=0}(s_t - \bar{s})^2}}$$

Where *t = 0,1,2,...,24*, and m_t = the melatonin concentration at each time point, $\bar{m}$ = the mean 24-h melatonin concentration, s_t = the percentage of time spent in various sleep stages in the preceding 30 min at each time point, and $\bar{s}$ = the 24-h mean of each s_t. For cross-correlations during nighttime only, *t* ranged from 0 to 9. Statistical calculations were performed using SPSS (release 17.0, SPSS, Inc., Chicago, IL). All tests were two-tailed, and the significance level was set at 0.05.

RESULTS

Subjects

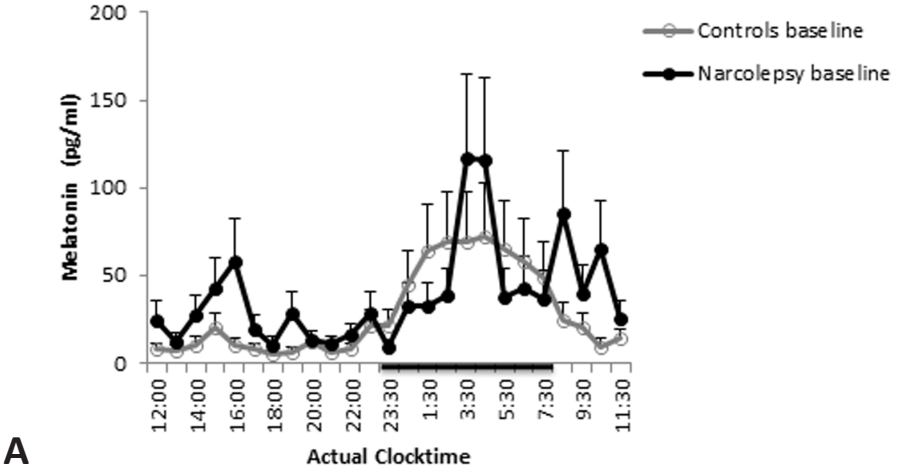
Narcolepsy patients and controls did not differ with respect to age, BMI, fat percentage, and WHR (all $P \geq 0.76$, Table 5.1). There were also no differences in urinary epinephrine, norepinephrine, and dopamine levels either at baseline (all $P \geq 0.70$) or after SXB administration (all $P \geq 0.16$). Ingestion of SXB was well-tolerated; apart from mild drowsiness, no other side-effects were reported.

Melatonin profiles

Mean 24-h melatonin concentrations did not differ between narcolepsy patients and controls, either before (39.2 ± 16.4 vs. 28.6 ± 4.6 pg/mL, $P = 0.56$) or after SXB administration (39.8 ± 9.2 vs. 31.0 ± 5.1 pg/mL, $P = 0.43$) (Figure 5.1). Similarly, differences in mean melatonin levels were not significant between the two groups when analyzed for day- and nighttime separately, either during the basal condition or after SXB treatment (all $P \geq 0.11$). As expected, with repeated-measures ANOVA, we found a significant effect of *Circadian Time* on melatonin levels in both groups ($P < 0.005$). However, the effect of *Circadian Time* on melatonin levels differed between narcolepsy patients and controls ($P = 0.042$ for *Time × Group* interaction effect). The percentage of total 24-h melatonin secreted during the day was significantly higher in narcolepsy patients compared to controls, both during the basal condition (46.6 ± 4.1 vs. 32.5 ± 5.8%) and after SXB intake (51.5 ± 3.5 vs. 34.4 ± 4.0%), ($P = 0.007$ for *Group* effect; Figure 5.2). Administration of SXB did not significantly affect the percentage of melatonin secreted during the daytime ($P = 0.415$ for the *Treatment* effect and $P = 0.718$ for *Group × Treatment* interaction effect).

Sleep recordings

Compared to controls, narcolepsy patients spent significantly less time awake both during basal conditions and SXB treatment (Table 5.2). During the day (defined as the lights-on period between 07:30 - 23:00 h), narcolepsy patients spent significantly less time awake, while significantly more time was spent in non-REM sleep, regardless of treatment (Table 5.2). SXB administration resulted in a significant decrease in stages I/II non-REM and REM sleep over the 24 h in both groups ($P = 0.011$ and $P = 0.009$, respectively), while it significantly



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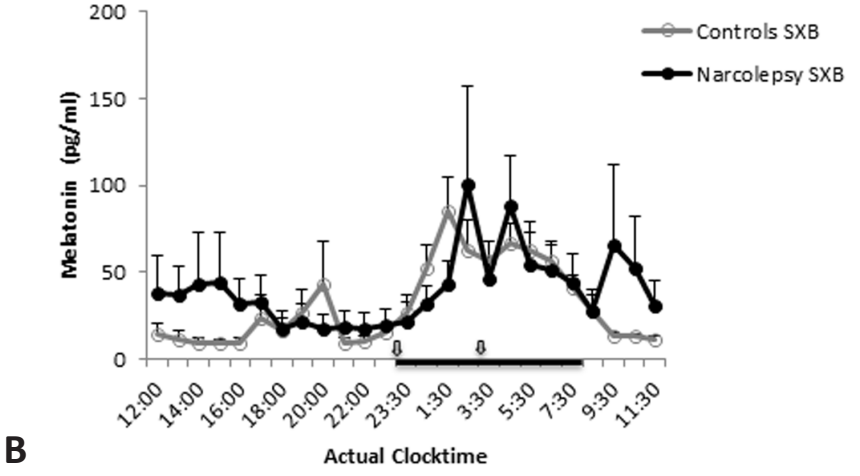

B

Figure 5.1 Mean melatonin concentrations plus standard error of the mean in narcolepsy patients and controls at baseline **(A)** and during SXB administration **(B)**. No difference in mean concentrations was found between groups in both conditions. Black bar on the abscissa indicates the dark period (23:00–07:30 h). Downward arrows **(B)**) indicate ingestion of SXB (23:00 h and 03:00 h). Time is displayed in actual clock time.

increased the time spent in SWS ($P = 0.001$). During the day, SXB treatment reduced the time spent in stages I/II non-REM and REM sleep ($P = 0.038$ and $P = 0.041$, respectively), while it tended to increase wakefulness ($P = 0.098$). The percentage of SWS during the night more than doubled in both groups in response to SXB treatment (narcolepsy: 6.5 ± 1.9 vs.16.5 ± 3.0%, controls: 7.1 ± 1.9 vs. 18.5 ± 2.4%; $P = 0.001$ for treatment effect), whereas there were trends for decreases in the percentages of stages I/II non-REM and REM sleep.

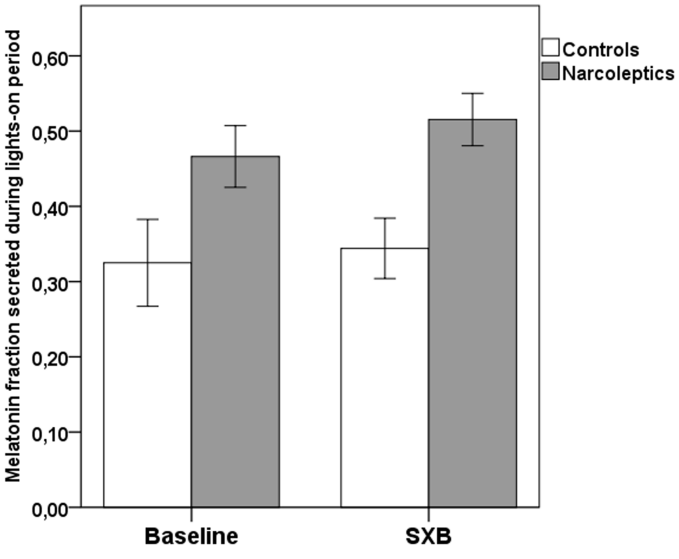

Figure 5.2 Fraction of melatonin secreted during the day in narcolepsy patients and controls, both at baseline and during sodium oxybate administration (lights-on period 07:30-23:00 h). Fraction of melatonin secreted during daytime is higher in narcolepsy patients compared to matched controls (P = 0.007 for group effect, P = 0.415 for treatment effect, and P = 0.718 for group × treatment interaction effect). Bars represent standard errors of the mean.

Table 5.2 Sleep patterns before and after sodium oxybate administration

	Baseline			Sodium oxybate		
	Patients	Controls	*P*	Patients	Controls	*P*
Wake total (%)	60.8 ± 2.9	68.7 ± 2.0	0.044*	60.8 ± 2.2	70.1 ± 2.4	0.013*
Wake day (%)	79.4 ± 4.2	95.6 ± 2.1	0.004*	82.9 ± 3.2	97.3 ± 1.0	0.001*
Wake night (%)	25.8 ± 5.7	18.4 ± 4.0	0.310	19.2 ± 4.3	19.2 ± 5.8	0.999
Stage I/II total (%)	29.1 ± 1.4	25.0 ± 2.4	0.155	26.3 ± 1.4	21.1 ± 2.2	0.063
Stage I/II day (%)	14.6 ± 3.0	2.5 ± 1.6	0.003*	11.1 ± 2.5	1.6 ± 1.0	0.005*
Stage I/II night (%)	55.1 ± 2.5	65.6 ± 5.7	0.114	53.5 ± 3.7	56.4 ± 5.3	0.647
SWS total (%)	3.7 ± 0.7	2.5 ± 0.7	0.239	7.6 ± 1.2	6.6 ± 0.9	0.534
SWS day (%)	2.1 ± 0.6	0.03 ± 0.03	0.005*	2.7 ± 1.1	0.05 ± 0.05	0.041*
SWS night (%)	6.5 ± 1.9	7.1 ± 1.9	0.843	16.5 ± 3.0	18.5 ± 2.4	0.611
REM total (%)	6.3 ± 1.8	3.7 ± 0.8	0.191	4.7 ± 1.0	2.1 ± 0.8	0.070
REM day (%)	2.9 ± 1.4	0.8 ± 0.5	0.203	1.2 ± 0.5	0.0 ± 0.0	0.032*
REM night (%)	12.6 ± 3.0	8.8 ± 1.8	0.305	10.8 ± 2.1	5.8 ± 2.3	0.127

* $P < 0.05$. Percentages of sleep stages during the 24 h of study, before and after SXB administration. Data are shown as mean ± SEM. Unpaired t-tests were used to assess differences between the two groups.

Table 5.3 Cross-correlations between melatonin concentrations and percentages of time spent in each sleeping stage in the preceding 30 min during the total 24-h period or during the nighttime (23:00-07:30) only

	Patient baseline	Patient SXB	Control baseline	Control SXB	P for group effect	P for treatment effect	P for group x treatment interaction
SWS (24 h)	0.04 ± 0.09	0.00 ± 0.07	0.33 ± 0.10	0.25 ± 0.07	0.023*	0.264	0.561
I/II NREM sleep (24 h)	0.24 ± 0.07	0.22 ± 0.09	0.53 ± 0.13	0.52 ± 0.04	0.007**	0.857	0.959
REM sleep (24 h)	0.19 ± 0.11	0.07 ± 0.07	0.34 ± 0.09	0.18 ± 0.07	0.349	0.106	0.979
Awake (24 h)	-0.31 ± 0.05	-0.36 ± 0.09	-0.73 ± 0.13	-0.63 ± 0.08	0.007*	0.709	0.376
SWS (night)	0.12 ± 0.16	-0.15 ± 0.10	-0.01 ± 0.11	-0.04 ± 0.10	0.517	0.266	0.634
I/II NREM sleep (night)	0.17 ± 0.10	0.23 ± 0.12	0.46 ± 0.15	0.35 ± 0.12	0.105	0.877	0.524
REM sleep	0.10 ± 0.15	-0.29 ± 0.09	0.21 ± 0.09	-0.02 ± 0.11	0.221	0.030*	0.538
Awake (night)	-0.32 ± 0.07	0.08 ± 0.08	-0.60 ± 0.08	-0.35 ± 0.11	0.003**	0.006**	0.457

Data are shown as mean ± SEM. Comparisons were made using repeated measurements ANOVA. * $P < 0.05$. ** $P < 0.01$.

Melatonin and sleep

The cross-correlations between melatonin levels and the percentages of time spent in either SWS, phase I/II non-REM sleep, or time awake were significantly weaker in narcolepsy patients compared to controls during the 24-h period (all $P < 0.023$; Table 5.3). Although SXB treatment did not influence the association between melatonin levels and sleep during the total 24-h period, restriction of the analyses to the lights-off period showed that, in both groups, SXB treatment inverted the association between melatonin concentrations and REM-sleep, while it attenuated the negative association between melatonin concentrations and time spent awake (Table 5.3).

DISCUSSION

Recent publications have suggested an association between altered temporal patterning of melatonin secretion in persons complaining of poor sleep and medical conditions linked with disordered sleep.[192-194] However, to our knowledge, this is the first study which combines measurements of diurnal plasma melatonin concentration profiles and polysomnography in hypocretin-deficient narcolepsy patients and matched controls. Although mean 24-h levels of plasma melatonin were similar between narcolepsy patients and controls, the circadian distribution of melatonin release was altered in narcolepsy, since the proportion of melatonin secreted during daytime was substantially higher in narcolepsy patients. Moreover, our findings indicate that the temporal coupling between sleep and plasma melatonin levels is diminished in narcolepsy patients, suggesting that hypocretin deficiency differently affects the circadian distribution of sleep and melatonin release because otherwise, despite an altered diurnal pattern of sleep and melatonin secretion, their cross-correlation would have been expected to remain unchanged.

In agreement with earlier reports, we did not find differences in mean melatonin levels between narcolepsy patients and controls.[185;188;195] Moreover, we were able to detect daytime melatonin secretion in all our patient and control subjects. This is in line with an earlier study in which daytime melatonin secretion was established in less than half of the narcolepsy patients.[188] Two reasons are likely to account for the fact that we did find an increase in all patients and Blazejova in less than half of them: first, we measured (daytime) melatonin levels much more frequently, and, second, we assessed melatonin levels in plasma instead of saliva in which melatonin concentration is estimated to be only 24% of that in plasma.[196]

The proportion of total 24-h melatonin secreted during the daytime was substantially higher in narcolepsy patients. This finding may reflect disruption of the circadian control of melatonin secretion as a consequence of hypocretin deficiency. Several studies – on sheep,[181] pig,[179] rat,[180] and zebrafish[94] – have found evidence for hypocretinergic innervation of the pineal gland as indicated by the presence of hypocretin fibers and/or receptors in this region. Functional evidence for the connection between the hypocretin and melatonin system was recently provided in a study on zebrafish by Appelbaum et al.[94] Zebrafish have only one hypocretin receptor gene (humans have two), and serve as a simplified model for the study of the hypocretin system.[197] Hcrtr-/- null-mutant fish display the disturbed nocturnal sleep phenotype, but not the other symptoms of narcolepsy.[94] The mutant fish exhibit reduced mRNA expression of arylalkylamine-N-acetyltransferase, a key enzyme in the melatonin production pathway, during the night,[94] suggesting that melatonin secretion may be stimulated by hypocretin signalling during the night. As our data indicate that hypocretin deficiency results in a smaller amount of the total diurnal melatonin being released during the nighttime, our findings are in line with those of Appelbaum et al. and suggest that hypocretin is mainly involved in the consolidation of the diurnal rhythm of melatonin release, confining it mainly to the dark phase of the circadian cycle. Indeed, another study on zebrafish demonstrated synaptic plasticity of the hypocretinergic projections to the pineal gland that exhibited marked circadian rhythmicity, again suggesting that hypocretin signalling may modulate circadian variations in melatonin secretion. A recent study byMcGregor et al. provides further clues as how hypocretin might be involved in the modulation of circadian rhythms.[198] Although these authors did not assess melatonin release, they found decreased responsivity to light in hypocretin deficient mice. Since light is the major suppressor of melatonin synthesis, it is conceivable that defects in hypocretin signalling might disrupt light-induced suppression of melatonin secretion, resulting in relatively higher daytime concentrations as described in the present study. 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.

A potential limitation of this study is the relatively small number of participants. However, this limitation is partly offset by the large number of melatonin samples and the standardized conditions under which the sampling was performed. All subjects were assessed at the same location, during the same time of year, and under the same lighting conditions. This is essential because these factors have been shown to be major modulators of melatonin secretion.[93] Furthermore, our subjects were closely matched and all narcolepsy patients were proven to be hypocretin deficient.

In summary, in narcolepsy patients, melatonin secretion was relatively higher during the day and exhibited a weaker coupling to sleep, suggesting that hypocretin deficiency might disturb both the circadian control of melatonin release and its temporal association with various stages of sleep. Furthermore our findings indicate that SXB does not affect melatonin secretion per se but might act to modulate its temporal coupling with sleep.

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PART II

Metabolic studies in narcolepsy

6

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

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ABSTRACT

Introduction: Narcolepsy is associated with obesity though it is uncertain whether this is caused by changes in glucose and fat metabolism. Therefore, we performed a detailed analysis of systemic energy homeostasis in narcolepsy patients, and additionally, investigated whether it was affected by three months of sodium oxybate (SXB) treatment.

Methods: Nine hypocretin deficient patients with narcolepsy-cataplexy, and nine healthy sex, age, and BMI matched controls were enrolled. A hyperinsulinemic-euglycemic clamp combined with stable isotopes ([6,6-$^{2}H_{2}$]-glucose and [$^{2}H_{5}$]- glycerol) was performed at baseline. In seven patients a second study was performed after three months of SXB treatment.

Results: Glucose disposal rate (GDR) per unit serum insulin was significantly higher in narcolepsy patients compared to matched controls (1.6 ± 0.2 vs. 1.1 ± 0.3 µmol/ kgFFM/min/ mU×L; *P* = 0.024), whereas β-cell function was similar (*P* = 0.50). Basal steady state glycerol appearance rate tended to be lower in narcolepsy patients (5.2 ± 0.4 vs. 7.5 ± 1.3 µmol/kgFM/ min; *P* = 0.058), suggesting a lower rate of lipolysis. SXB treatment induced a trend in reduction of the GDR (1.4 ± 0.1 vs. 1.1 ± 0.2 µmol/ kgFFM/ min/ mU×L; *P* = 0.063) and a reduction in endogenous glucose production (0.24 ± 0.03 vs. 0.16 ± 0.03 µmol/ kgFFM/ min/ mU×L: *P* = 0.028) per unit serum insulin. After SXB treatment lipolysis increased (4.9 ± 0.4 vs. 6.5 ± 0.6 µmol/kgFM/ min; *P* = 0.018), and body weight decreased in narcolepsy patients (99.2 ± 6.0 vs. 94.0 ± 5.4 kg; *P* = 0.044).

Conclusion: 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.

INTRODUCTION

Narcolepsy is a sleep disorder that is characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, sleep paralysis, disturbed nocturnal sleep and obesity.[1] Both obesity and disturbed nocturnal sleep are important risk factors for the development of type 2 diabetes mellitus (T2DM).[199] Narcolepsy is caused by a loss of hypocretin (orexin) neurons in the hypothalamus.[47;48] Hypocretins are neuropeptides known to be involved in sleep-wake regulation, feeding behavior as well as body weight and temperature regulation.[103;176] Furthermore hypocretins are important hypothalamic regulators of glucose homeostasis. Disturbed activation of hypocretin neurons during sleep deprivation may lead to increased basal endogenous glucose production and a decreased insulin sensitivity in rats.[200] Moreover, hypocretin deficiency leads to an age-related defective glucose tolerance and insulin resistance.[201] Intracerebroventricular infusions of hypocretin-1 increase hepatic glucose production in rats,[202;203] but intracerebral injections of hypocretin-1 in the ventromedial hypothalamus increase glucose uptake and promote insulin induced glucose uptake and glycogen synthesis in skeletal muscle of mice.[204] Taken together, these data imply that the absence of hypocretin in human narcolepsy may disturb glucose homeostasis.

In the sixties and eighties of the previous century, some reports suggested a higher incidence of T2DM in narcolepsy patients.[39;41;95] Another study confirmed a higher risk of metabolic syndrome independent of body mass index (BMI) when comparing patients with narcolepsy to those with idiopathic hypersomnia.[22] However, in a recent study comparing narcolepsy patients with healthy controls matched for BMI, no differences in insulin resistance could be detected using the homeostatic model assessment (HOMA),[42] a method which estimates β-cell function and insulin resistance from a single pair of fasting glucose and insulin measurements.[205] Similarly, another more recent study could not find differences in glucose tolerance and β-cell function between 17 narcolepsy patients and healthy controls.[96] So far, there have been no studies using a hyperinsulinemic-euglycemic clamp to estimate insulin sensitivity in narcolepsy patients.

Metabolic syndrome and insulin resistance may lead to T2DM within several years if lifestyle is not adapted.[206] Under normal circumstances pancreatic islet β-cells compensate for insulin resistance by increasing insulin release. However, when these compensatory mechanisms fail T2DM will develop.[207] The most accurate method available for measuring insulin sensitivity is the hyperinsulinemic-euglycemic clamp technique.[208] During a clamp a fixed dose of insulin is continuously infused along with a variable amount of glucose so as to maintain

euglycemia, i.e., the plasma glucose levels are “clamped” at a predefined level. Thus, the amount of glucose infused necessary to keep plasma glucose levels constant can be used as a measure of peripheral insulin sensitivity since relatively lower amounts of glucose will be needed in case of insulin resistance.[208]

Sodium oxybate (SXB), also known as γ-hydroxybutyrate (GHB), is an effective treatment of narcolepsy. It reduces cataplexy, improves nocturnal sleep fragmentation and in higher doses it may also reduce excessive daytime sleepiness.[73] SXB activates dopaminergic circuits in the brain.[77] As diminished dopamine (D2) receptor mediated signal transduction appears to induce insulin resistance, the metabolic syndrome and T2DM,[79] it is conceivable that SXB might be protective against developing T2DM. Additionally, there are indications that SXB might reduce body weight,[74] which could also decrease the risk of T2DM.

The abovementioned discrepancies between the findings from earlier reports regarding potential disturbances in glucose metabolism in narcolepsy patients may be due to suboptimal assessment of insulin sensitivity. Moreover, to our knowledge, fat metabolism, which is obviously key to systemic energy homeostasis, has not been scrutinized before in narcolepsy patients. Therefore, in the present study we applied the gold standard for measuring insulin sensitivity, i.e., the hyperinsulinemic-euglycemic clamp, complemented with a stable isotope technique to assess both glucose and fat metabolism in a group of 9 hypocretin deficient narcolepsy with cataplexy patients and 9 individually matched healthy controls. In addition, we assessed the effect of three months of treatment with SXB on glucose and fat metabolism in narcolepsy patients.

METHODS

Subjects

Nine narcolepsy with cataplexy patients and nine, individually age, sex, BMI, fat percentage, and waist-to-hip ratio (WHR) matched healthy controls were enrolled. All patients fulfilled the ICSD-2 criteria for narcolepsy with cataplexy.[118] All were HLA DQB1*06:02positive, and CSF hypocretin-1 deficient. None of the controls used medication. Seven patients were drug naive, one discontinued methylphenidate 2 weeks prior to the study. The last patient was tapered off antidepressants and methylphenidate and did not take any drugs in the 2 weeks prior to the start of the study. None of the patients used any other medication. Subjects were eligible for study after exclusion of hypertension, any known (history of) pituitary,

psychiatric, or neurological disease (other than narcolepsy), alcohol or drug abuse, recent weight change (> 3 kg weight change within the last 3 months), or a sleep disorder history (controls). Routine laboratory tests were performed to rule out overt diabetes, thyroid disease, anemia, and hepatic and/ or renal disease. The study was approved by the ethics committee of the Leiden University Medical Centre. Written informed consent was obtained from all subjects.

Clinical protocol

All studies started at 08:30 after an overnight fast. Alcohol consumption and excessive exercise on the day preceding the study was not allowed. Caffeinated drinks were also not allowed from 18:00 prior to and on the day of study, and smoking was prohibited until the end of the measurements. Height, weight, BMI, and waist-to-hip ratio were measured according to the WHO recommendations.[209] Bioelectrical impedance analysis (Bodystat, Douglas, Isle of Man, UK) was used to estimate lean body mass and fat percentage. Subjects were requested to lie down on a bed in a semi-supine position. A polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was inserted into a contralateral dorsal hand vein for blood sampling; this hand was kept in a heated box (60°C) throughout the test to obtain arterialized blood.[210] Samples were taken for measurement of basal levels of glucose, insulin, total cholesterol, LDL cholesterol, HDL-cholesterol, triglycerides, glycerol and background enrichment of [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol. At 08:30 (t = 0 min), an adjusted primed (17.6 µmol/kg × actual plasma glucose concentration (mmol/L)) continuous (0.33 µmol/kg/min) infusion of [6,6-$^2H_2$]-glucose (enrichment 99.9%; Cambridge Isotopes, Cambridge, Massachusetts, USA) was started and continued throughout the study. After 60 min, a primed (1.6 µmol/kg) continuous (0.11 µmol/kg/min) infusion of [$^2H_5$]-glycerol (Cambridge Isotopes) was started and continued throughout the study. Subsequently, a primed continuous infusion of insulin (Actrapid, Novo Nordisk Pharma BV, Alphen aan de Rijn, The Netherlands; 40 mU/m^2/min) was started (t = 120 min). Exogenous glucose 20% enriched with 3% [6,6-$^2H_2$]-glucose was infused at a variable rate to maintain the plasma glucose level at 5.0 mmol/ L. From t = 210 to 240 min, blood was drawn every 10 min for determination of [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol specific activities, glucose, insulin, and glycerol. While blood in the serum samples was allowed to clot, plasma samples were immediately put on ice. Within 60 min of sampling, all samples were centrifuged at 1610 *g* at 4°C for 20 min and then stored at -80°C until assay.

Sodium oxybate

Narcolepsy patients started SXB treatment after completion of the first clamp session. SXB was given in a regular starting dose of 2 nighttime doses of 2.25 grams. Subsequently, the dose was titrated up to an optimum for each individual patient, although never exceeded the maximum dose of 9 grams per night. When there was satisfactory symptom control for 3 months, according to the patient and the treating neurologist, patients were allowed to embark on the second clamp session. The same neurologist (GJL) evaluated all cases. No other drugs were allowed during these 3 months.

Assays

Serum insulin concentrations were measured by enzyme-labelled chemiluminescent immunometric assay (Immulite 2500; Siemens, Munich, Germany) with an intra-assay coefficient of variation (CV) of 4%. Enrichment of plasma [6,6-$^{2}H_{2}$]-glucose was determined in a single analytical run using gas chromatography coupled to mass spectrometry, as described previously.[211] All isotope enrichments were measured on a gas chromatograph mass spectrometer (model 6890/5973; Hewlett-Packard, Palo Alto, CA). Serum cholesterol, high-density lipoprotein (HDL) and triglycerides (TG) were measured with a fully automated P-800 module (Roche, Almere, The Netherlands). For both TG and total cholesterol (TC) the CV was less than 2%. For HDL the CV was less than 3%. Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald equation.[212]

Calculations

In all clamp sessions a physiological steady state was achieved during the last 30 min of both the basal as well as the hyperinsulinemic period; therefore, the rates of appearance and disappearance for glucose and glycerol were calculated as the tracer infusion rate divided by the tracer to tracee ratio.[213] Under steady-state conditions the rate of appearance equals the rate of disappearance, or the disposal rate. Glucose flux rates were expressed per kg fat free mass (FFM), whereas glycerol flux rates were normalized per kg fat mass (FM). Endogenous glucose production (EGP) during the basal steady state is equal to the rate of appearance of glucose whereas EGP during the clamp was calculated as the difference between the rates of glucose appearance and infusion. Since the fasting plasma insulin concentration is a strong inhibitory stimulus for EGP, the basal hepatic insulin resistance index (µmol/ min/ kgFFM/ pmol×L) was calculated as the product of fasting EGP and fasting

plasma insulin concentration.[208] The metabolic clearance rate of insulin was calculated as the constant infusion rate of insulin divided by the steady state serum insulin concentration corrected for endogenous insulin secretion. β-cell function was estimated using the HOMA as described previously.[205]

Statistical analysis

Results are expressed as mean ± standard error (SEM) unless otherwise specified. The nonparametric Mann-Whitney *U* test was used to assess differences in medians between patients and controls, whereas the Wilcoxon signed ranks test was applied to assess differences between baseline and SXB conditions. The nonparametric Spearman rank correlation coefficient was used to assess correlations between changes in weight and other metabolic parameters. A paired-samples t-test was used if applicable. All tests were two-tailed, and significance level was set at $P < 0.05$. Statistical analyses were performed using SPSS for Windows (release 18.0, SPSS, Inc.).

RESULTS

Subjects

Narcolepsy patients did not differ from controls with respect to sex, age, BMI, WHR, lean body mass, or fat percentage (all $P \geq 0.33$, Table 6.1). Moreover baseline glucose, insulin, total cholesterol (4.7 ± 0.23 vs. 4.7 ± 0.26 mmol/L), LDL cholesterol (3.1 ± 0.18 vs. 3.0 ± 0.23 mmol/L), HDL-cholesterol (0.98 ± 0.06 vs. 1.2 ± 0.11 mmol/L), and triglycerides (1.4 ±

Table 6.1 Demographics and body composition

	Narcolepsy patients	Controls	*P*
Male/Female	7/2	7/2	
Age (Y)	35.7 (0.9)	37.1 (1.5)	0.33
BMI	29.1 (1.3)	28.3 (1.1)	0.60
Fat (%)	25.0 (2.6)	24.4 (2.8)	0.95
Lean body mass (kg)	71.4 (3.6)	73.4 (3.6)	0.57
WHR	0.89 (0.2)	0.90 (0.2)	0.57

Data are means ± SEM. Intergroup differences were assessed by the nonparametric Mann-Whitney *U* test. WHR, waist-to-hip ratio.

0.26 vs. 1.0 ± 0.14 mmol/L) did not differ between the 2 groups (all $P \geq 0.10$). SXB was well tolerated except in one patient who discontinued due to an unacceptable increase in sleep paralysis. Another patient with excessive daytime sleepiness as major complaint could not be assessed after treatment due to the fact that he insisted on taking stimulants. Thus 9 patients and controls were measured at baseline, and 7 patients were assessed for a second time. Narcolepsy patients lost an average amount of 5.2 kg of their body weight after 3 months of treatment with SXB (99.2 ± 6.0 vs. 94.0 ± 5.4 kg; $P = 0.044$).

Glucose metabolism in narcolepsy patients versus controls

An overview of all metabolic parameters in narcolepsy patients and controls during both the basal steady state and the hyperinsulinemic steady state is presented in Table 6.2. During the basal steady state there were no significant differences in glucose disposal rate (Glucose Rd) between patients and controls. In addition, during the hyperinsulinemic steady state the glucose disposal rate was similar in narcolepsy patients and controls (55.9 ± 7.9 vs. 40.2 ± 5.3 µmol/kgFFM/min, $P = 0.122$), despite significantly lower steady state plasma insulin levels (33.9 ± 2.9 vs. 42.6 ± 3.5 mU/L, $P = 0.047$). When adjusted for the differences in plasma insulin levels, the glucose disposal rate (1.6 ± 0.2 vs. 1.1 ± 0.3 µmol/kgFFM/min/mU × L, $P = 0.024$) was significantly higher in patients, whereas the rate of endogenous glucose production (0.26 ± 0.03 vs. 0.24 ± 0.07 µmol/kgFFM/min/mU × L, $P = 0.085$) did not differ between both groups. In addition, β-cell function as assessed using the baseline glucose and insulin levels was equal in the two groups (8.7 vs. 10.3 Mann-Whitney $U = 33$, $Z = -0.66$, $P = 0.50$).

Lipid metabolism in narcolepsy patients versus controls

During the basal steady state plasma glycerol levels (46.7 ± 2.8 vs. 67.2 ± 11.3 µmol/L, $P = 0.070$), as well as the rate of glycerol appearance (5.2 ± 0.4 vs. 7.5 ± 1.3 µmol/kgFM/min, $P = 0.058$) tended to be lower in narcolepsy patients compared to controls, suggesting a lower rate of lipolysis in narcolepsy patients. However, these trends disappeared during the hyperinsulinemic steady state, indicating an intact insulin mediated suppression of lipolysis in narcolepsy patients. These findings remained unchanged when adjusted for plasma insulin levels (Table 6.2).

Table 6.2 Metabolic parameters in nine patients with narcolepsy and nine matched controls during the basal steady state and during the hyperinsulinemic steady state

	Basal steady state			Hyperinsulinemic steady state		
	Narcolepsy	Controls	*P*	Narcolepsy	Controls	*P*
Glucose (mmol/L)	5.2 ± 0.1	5.4 ± 0.2	0.965	5.1 ± 0.1	4.8 ± 0.1	0.038*
Glucose R_d (µmol/kgFFM/min)	19.0 ± 0.7	18.9 ± 0.4	0.757	55.9 ± 7.9	40.2 ± 5.3	0.122
M (µmol/kgFFM/min)	-	-	-	46.8 ± 7.6	31.4 ± 4.6	0.190
EGP (µmol/kgFFM/min)	19.0 ± 0.7	18.9 ± 0.4	0.757	8.3 ± 0.7	8.3 ± 0.9	0.627
Plasma insulin (mU/L)	6.2 ± 0.7	5.9 ± 1.0	0.796	33.9 ± 2.9	42.6 ± 3.5	0.047*
HIR (µmol kgFFM/min/pmol × L)	806.5 ± 91.2	773.7 ± 127.4	0.566	1976.3 ± 242.1	2299.9 ± 104.9	0.310
MCR_l (mL/m^2/min)	-	-	-	0.11 ± 0.02	0.07 ± 0.01	0.085
Glucose R_d per unit serum insulin (µmol/kgFFM/min/mU × L)	3.6 ± 0.6	4.2 ± 0.9	0.825	1.6 ± 0.2	1.1 ± 0.3	0.024*
M per unit serum insulin (µmol/kgFFM/min/mU × L)	-	-	-	1.4 ± 0.2	0.9 ± 0.3	0.031*
EGP per unit serum insulin (µmol/kgFFM/min/mU × L)	3.6 ± 0.6	4.2 ± 0.9	0.825	0.26 ± 0.03	0.24 ± 0.07	0.085
Glycerol (µmol/L)	46.7 ± 2.8	67.2 ± 11.3	0.070	18.0 ± 1.4	20.8 ± 3.3	0.691
Glycerol R_a (µmol/kgFM/min)	5.2 ± 0.4	7.5 ± 1.3	0.058	2.2 ± 0.2	2.0 ± 0.2	0.965
Glycerol R_a per unit serum insulin (µmol/kgFM/min)	$9.7 \times 10^{-1} \pm 1.5 \times 10^{-1}$	$1.4 \pm 2.1 \times 10^{-1}$	0.102	$6.7 \times 10^{-2} \pm 8.1 \times 10^{-3}$	$4.9 \times 10^{-2} \pm 5.5 \times 10^{-3}$	0.102

Data are means ± SEM. Intergroup differences were assessed by the nonparametric Mann-Whitney *U* test, * $P < 0.05$.
Abbreviations: EGP = endogenous glucose production; FFM = fat free mass; FM = fat mass; HIR = hepatic insulin resistance; M = glucose metabolized; MCR_l = metabolic clearance rate of insulin; R_d = rate of disappearance. During basal steady state glucose R_d equals EGP. During steady state conditions the rates of appearance (R_a) of glucose and glycerol is equal to their respective rates of disappearance (R_d).

Glucose metabolism in narcolepsy patients before and after SXB

β-cell function did not change after SXB treatment (*P* = 0.61). SXB had no obvious effects on the basal steady state glucose metabolism (Table 6.3). After SXB treatment the steady state plasma insulin levels were significantly higher during the hyperinsulinemic conditions (33.8 ± 3.8 vs. 48.9 ± 4.8 mU/L, *P* = 0.018), perhaps due to a significantly lower metabolic clearance rate of insulin (0.11 ± 0.02 vs. 0.06 ± 0.01 mL/m^2/min, *P* = 0.018). When adjusted for these differences in plasma insulin levels, SXB treatment induced a trend towards a reduced rate of glucose disposal (1.4 ± 0.1 vs. 1.1 ± 0.2 µmol/kgFFM/min/mU × L, *P* = 0.063). In addition there was a significant reduction in endogenous glucose production (0.24 ± 0.03 vs. 0.16 ± 0.03 µmol/kgFFM/min/mU × L, *P* = 0.028) during the hyperinsulinemic condition. Thus, SXB had a tendency to decrease peripheral (primarily muscle) insulin sensitivity, while it increased hepatic insulin sensitivity (Table 6.3).

Lipid metabolism in narcolepsy patients before and after SXB

SXB treatment significantly increased both basal levels of glycerol (47.3 ± 3.7 vs. 62.4 ± 5.3 µmol/L, *P* = 0.043) and the rate of glycerol appearance (4.9 ± 0.4 vs. 6.5 ± 0.6 µmol/kgFM/min, *P* = 0.018), indicating that SXB treatment increases lipolysis in narcolepsy patients (Table 6.3). After SXB treatment the rate of lipolysis became lower during hyperinsulinemic conditions as evidenced by a trend towards lower plasma glycerol levels (17.8 ± 1.6 vs. 14.6 ± 1.9 µmol/L, *P* = 0.063) and a lower rate of glycerol appearance (1.9 ± 0.2 vs. 1.4 ± 0.2 µmol/kgFM/min, *P* = 0.018), suggesting that SXB increases the sensitivity of fat tissue to the inhibitory effects of insulin on lipolysis (Table 6.3). These findings remained largely unchanged when adjusted for plasma insulin levels (Table 6.3).

Metabolic parameters in relation to weight change

The change in body weight after SXB treatment was strongly associated with alterations in glucose disposal rate per unit serum insulin (r = -0.93, *P* = 0.003; Figure 6.1), but not with changes in endogenous glucose production per unit serum insulin (r = 0.29, *P* = 0.535).

Table 6.3 Metabolic parameters in seven patients with narcolepsy before and after sodium oxybate (SXB) treatment

	Basal steady state			Hyperinsulinemic steady state		
	Before SXB	After SXB	*P*	Before SXB	After SXB	*P*
Glucose (mmol/L)	5.2 ± 0.1	5.1 ± 0.1	0.063	5.1 ± 0.1	5.0 ± 0.1	0.028*
Glucose R_d (µmol/kgFFM/min)	18.6 ± 0.7	18.0 ± 0.8	0.128	49.4 ± 7.8	47.5 ± 5.1	0.866
M (µmol/kgFFM/min)	-	-	-	41.0 ± 7.6	41.1 ± 5.1	1.000
EGP (µmol/kgFFM/min)	18.6 ± 0.7	18.0 ± 0.8	0.128	4.8 ± 0.47	7.2 ± 0.76	0.612
Plasma insulin (mU/L)	6.6 ± 0.9	7.0 ± 1.4	0.735	33.8 ± 3.8	48.9 ± 4.8	0.018*
HIR (µmol/kgFFM/min/pmol × L)	847.9 ± 113.9	847.1 ± 151.1	0.499	1819.6 ± 277.4	2379.7 ± 246.5	0.063
MCR_I (ml/m^2/min)	-	-	-	0.11 ± 0.02	0.06 ± 0.01	0.018*
Glucose R_d per unit serum insulin (µmol/kgFFM/min/mU × L)	3.3 ± 0.7	3.7 ± 1.2	0.612	1.4 ± 0.1	1.1 ± 0.2	0.063
M per unit serum insulin (µmol/kgFFM/min/mU × L)	-	-	-	1.2 ± 0.1	0.9 ± 0.2	0.091
EGP per unit serum insulin (µmol/kgFFM/min/mU × L)	3.3 ± 0.7	3.7 ± 1.2	0.612	0.24 ± 0.03	0.16 ± 0.03	0.028*
Glycerol (µmol/L)	47.3 ± 3.7	62.4 ± 5.3	0.043*	17.8 ± 1.6	14.6 ± 1.9	0.063
Glycerol R_a (µmol/kgFM/min)	4.9 ± 0.4	6.5 ± 0.6	0.018*	1.9 ± 0.2	1.4 ± 0.2	0.018*
Glycerol R_a per unit serum insulin (µmol/kgFM/min)	$8.6 \times 10^{-1} \pm 1.6 \times 10^{-1}$	1.2 ± 0.3	0.063	$6.2 \times 10^{-2} \pm 8.8 \times 10^{-3}$	$2.9 \times 10^{-2} \pm 2.8 \times 10^{-3}$	0.018*

Data are means ± SEM. Intergroup differences were assessed by the nonparametric Wilcoxon signed ranks test, * $P < 0.05$. Abbreviations: EGP = endogenous glucose production; FFM = fat free mass; FM = fat mass; HIR = hepatic insulin resistance; *M* = glucose metabolized; MCR_I = metabolic clearance rate of insulin; R_d = rate of disappearance. During basal steady state, glucose R_d equals EGP. During steady state conditions the rates of appearance (R_a) of glucose and glycerol is equal to their respective rates of disappearance (R_d).

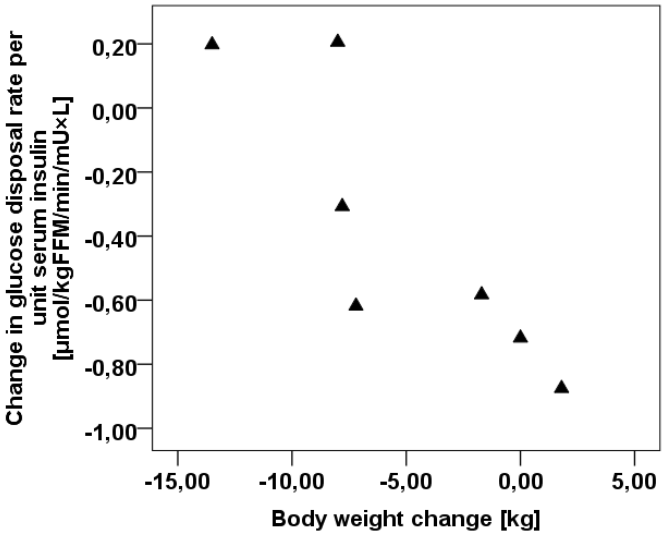

Figure 6.1 Correlations in body weight change and glucose disposal rate.

DISCUSSION

The aim of this study was to accurately assess both glucose and fat metabolism in narcolepsy patients, as well as the metabolic effects of SXB treatment using the most accurate method available, i.e. a combination of a hyperinsulinemic-euglycemic clamp and stable isotope techniques. Surprisingly, we found increased peripheral insulin sensitivity in narcolepsy patients, whereas hepatic insulin sensitivity and β-cell function were not different from matched healthy controls. The higher insulin sensitivity in narcolepsy patients was reflected by a higher rate of insulin-mediated glucose uptake in peripheral tissues, of which skeletal muscle is the most important. Lipolysis tended to be lower in narcolepsy patients, which could be due to insulin sensitivity of adipose tissue. This finding may at least partly account for comorbid overweight in narcolepsy, which is seen in two thirds of the patients.[18] Also the higher insulin sensitivity itself might contribute.

Furthermore, we found that SXB treatment increased hepatic but tended to decrease whole body insulin sensitivity. Moreover, SXB stimulated lipolysis, which might be one of the reasons why patients lose weight while on this drug. In our study, patients lost on average 5.2 kg of weight after 3 months of SXB treatment which is in line with an earlier report that SXB treatment is associated with weight loss.[74] The mechanisms through which hypocretin-

deficiency and SXB treatment affect glucose and fat metabolism might involve modulation of autonomic nervous system activity, which recently has been demonstrated to be critically implicated in the regulation of energy homeostasis.[214] SXB appears to hamper insulin mediated glucose disposal in patients who maintain a stable body weight during treatment, but it simultaneously induces weight loss in other patients, which clearly compensates for the reduction in insulin sensitivity (Figure 6.1). On average, glucose disposal per unit of circulating insulin was reduced by SXB (Table 6.3). The impact of SXB on insulin action appears to be tissue specific: while glucose disposal is diminished, the capacity of insulin to suppress glucose production is reinforced by SXB (Table 6.3). The mechanism underlying the effects of SXB is unknown. As glucose disposal rate is primarily determined by glucose uptake in muscle tissue, and given the fact that the capacity of insulin to suppress lipolysis was not affected by SXB treatment, our findings suggest that the systemic effects of SXB on insulin sensitivity are chiefly mediated by changes in muscle, rather than fat, insulin sensitivity.

Since peripheral insulin sensitivity is increased and pancreatic β-cell function is normal in narcolepsy patients, our data do not support the notion that the risk of T2DM is increased in narcolepsy, which is in contrast to some earlier reports.[22;39;41;95] We found no differences in pancreatic β-cell function, which is in line with previous observations.[42;96] However, increased insulin sensitivity has not been previously reported in narcolepsy. Several explanations might account for these differences. Firstly, to our knowledge we are the first to apply the hyperinsulinemic-euglycemic clamp to assess insulin sensitivity, whereas previous studies used indirect and derived measures of insulin sensitivity such as the homeostasis model assessment and minimal model analysis.[96] Although the method used by Beitinger et al. highly correlates with the clamp technique, it is not the same and less accurate.[215] Secondly, in both earlier studies patients continued to be on their usual medication which might have confounded their results. For example, antidepressants, which are used as anticataplectic drugs, may induce insulin resistance.[216] Thirdly, all our patients were proven to be hypocretin deficient and HLA-DQB1*06:02 positive which makes our group more homogeneous than the ones in earlier reports. There is a growing body of evidence that hypocretin plays a fundamental role in glucose metabolism.[200;214;217] However, the precise role of hypocretin in systemic glucose homeostasis is likely to be complex and seems even paradoxical since it can have both hyperglycemic[202] as well as hypoglycemic effects.[214] This dual effect of hypocretin in glucose homeostasis is probably related to its decisive role in the control of sleep/ wake and circadian rhythms, i.e., at awakening hypocretin not only stimulates arousal but also glucose production as well as glucose uptake.[218]

One limitation of this study is the relatively small number of subjects. However, this limitation is partly offset by the application of very sensitive techniques, which would have been unfeasible in larger groups of subjects due to the expensive and arduous nature of the experiments.

In conclusion, our findings show that narcolepsy patients are more insulin sensitive and tend to have a lower rate of lipolysis than weight matched controls. SXB stimulated lipolysis in narcolepsy patients, possibly accounting for the observed weight loss after treatment. While SXB had a tendency to decrease systemic insulin sensitivity, it increased hepatic insulin sensitivity, suggesting tissue-specific effects.

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7

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

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ABSTRACT

Study objectives: Patients suffering from narcolepsy with cataplexy show altered skin temperatures, resembling the profile that is conductive to sleep onset in healthy controls. Temperature manipulation counteracting the altered temperature profile improves both daytime and nocturnal narcoleptic sleep-wake disturbances. The aim of the present study is to investigate the effects of Sodium Oxybate, a widely used drug to treat narcolepsy, on the 24-hour profiles of temperature and sleep-wakefulness in narcolepsy patients and controls.

Design: Prospective case-control study.

Setting: Tertiary narcolepsy referral center in a university hospital.

Patients or participants: Eight hypocretin-deficient, male narcolepsy with cataplexy patients and eight healthy matched controls.

Interventions: Temperatures of the core body and proximal and distal skin areas, as well as the sleep-wake state were measured twice for 24 hours while participants maintained a supine posture. After the baseline assessment, 2 x 3 grams of Sodium Oxybate was administered for five nights, immediately followed by the second assessment.

Measurements and results: At baseline, daytime core body temperature and proximal skin temperature were significantly lower in narcolepsy patients compared to controls (core: 36.78 ± 0.05°C vs. 36.97 ± 0.05°C, F = 8.31, $P = 0.01$; proximal: 33.4 ± 0.26°C vs. 34.32 ± 0.26°C, F = 5.66, $P = 0.03$). In patients, Sodium Oxybate administration increased proximal skin temperature during the day (F = 6.46, $P = 0.04$) to a level similar as measured in controls, but did not affect core body temperature, distal temperature or distal-proximal temperature gradient (DPG). Sodium Oxybate administration normalized the predictive value of DPG for the onset of daytime naps ($P < 0.01$).

Conclusions: Sodium Oxybate improved the symptoms of narcolepsy in concert with a partial normalization of the skin temperature profile, by increasing daytime proximal skin temperature and by restoring the known relationship between skin temperature and daytime sleep propensity.

INTRODUCTION

Narcolepsy with cataplexy is a sleep disorder characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, sleep paralysis and impaired maintenance of nocturnal sleep.[131] A decreased level of hypocretin-1 (orexin-A) in the CSF is the hallmark of the disease and is considered to explain all narcolepsy symptoms.[46]

Skin and core body temperature play an important role in sleep and wake regulation.[97;219;220] Wake is associated with a relatively low skin temperature and a relatively high core body temperature, while sleep is associated with a higher skin temperature and a lower core body temperature. Sleep onset is preceded by a decline in core body temperature and an increase in skin temperature. The decrease in core body temperature is mediated through increased skin perfusion, which consequently leads to the increase in skin temperature, and facilitates cooling of the body.[221;222] These changes are facilitated in part by the postural change from an upright to a supine position that commonly occurs during sleep.[223]

Previous studies demonstrated an altered diurnal profile of skin temperature in narcolepsy. Compared with controls, patients with narcolepsy show an increased distal skin temperature and a decreased proximal skin temperature in the waking state.[23;25;224] This pattern may be considered as characteristic of lowered vigilance[225;226] or even 'sleep promoting', since it is also seen in controls immediately before sleep onset.[97;219] Indeed, temperature manipulation studies in narcoleptic patients counteracting these changes have shown to improve nocturnal sleep and excessive daytime sleepiness.[24;25] All together, these findings suggest a relationship between hypocretin function, temperature and sleep regulation.

Gammahydroxybutyrate (GHB) is a hypnotic used to improve nocturnal sleep and EDS in narcolepsy.[227] GHB has a wide range of effects, but the exact mechanisms are still unclear. Altered thermoregulation is one of the effects described in animal studies and human case reports. Rodent studies demonstrate a slight increase in core body temperature after administration of a low dose of GHB (5-10 mg/kg) and a clear decrease in core body temperature in higher doses (< 500 mg/kg).[99] Several studies describe hypothermia in humans with GHB intoxication.[100;101]

Sodium Oxybate (SXB) is the sodium salt of GHB and is registered for the treatment of narcolepsy. Its effects are comparable to the effects of GHB. Given the impact of GHB on temperature regulation, the altered pattern of skin temperature in narcolepsy and the positive effects of SXB on sleep in narcolepsy patients, it may be hypothesized that

the treatment effect of SXB may in part be mediated by its possible restorative effect on temperature regulation. The aim of the present study is to investigate the effect of SXB on core body and skin temperature in relation to its effects on sleep. Therefore, we continuously measured sleep, core body temperature and skin temperature for 24 hours in narcolepsy patients and controls, before and after five days of treatment with SXB during a constant routine protocol.

METHODS

Subjects

8 male narcolepsy patients (18-65 years of age) were included after informed consent. They all fulfilled the criteria for narcolepsy with cataplexy according to the International Classification of Sleep Disorders-2 (ICDS-2),[118] suffered clear-cut cataplexy and were hypocretin-1 deficient. Two patients were drug naive, one patient was tapered from antidepressants ≥ 2 weeks prior to the study, and 2 patients had prior history with SXB; however, no subject took SXB within 20 days of study initiation. The other patients did not take any medication for at least several months prior to beginning the study. Eight healthy male controls, free of any neurologic, endocrine or psychiatric disease, were individually matched for age and body mass index (BMI).

Study design (Figure 7.1)

The results of this study originate from an extensive protocol that was described previously.[133;173;228] All subjects stayed overnight in the hospital and underwent a baseline 24h temperature measurement and polysomnography. During this measurement subjects remained (semi)supine except for bathroom visits. Lights were switched off at 23:00h and switched on at 7:30h, and daytime naps were allowed. At 8:30h, 13:00h and 18:00h a standardized cold meal was served and during the whole day water and tea (caffeine free) were available. Following the baseline study, subjects were treated with SXB for five consecutive days, the first and the 5th day in the hospital. A second 24h temperature measurement and polysomnography was performed on the 5th day of SXB use.

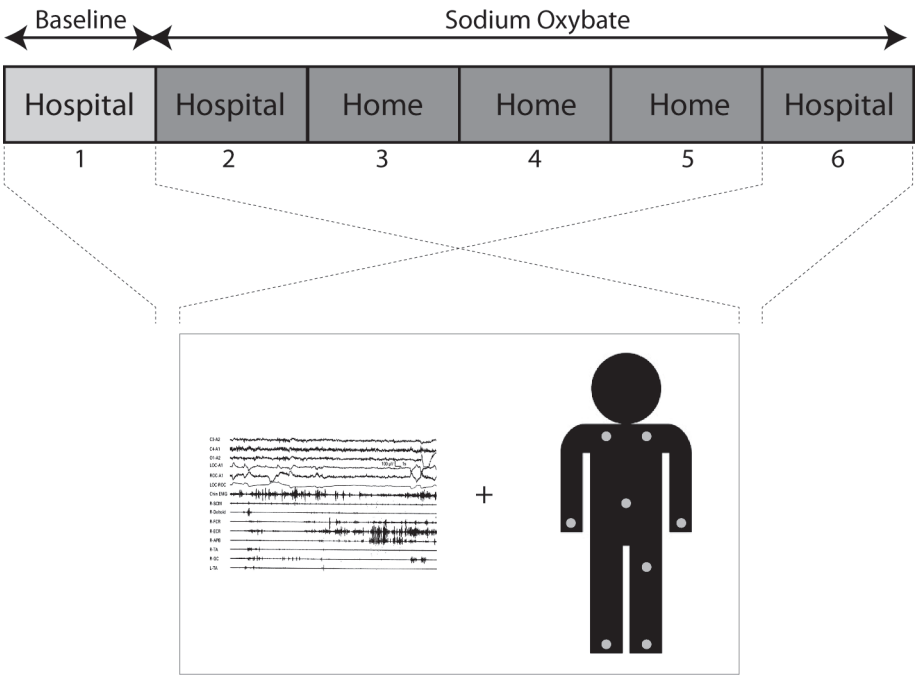

Figure 7.1 At day 1 subjects underwent a 24h temperature measurement and polysomnography without any treatment. Following this baseline study, subjects were treated with SXB for five consecutive days, the first and the 5th day in the hospital. A second 24h temperature measurement was performed on the 5th day of SXB use. The dots on the man indicate the location of the iButtons for skin temperature measurement.

Medication protocol

To monitor possible side effects, the first SXB administration was done in the hospital. Since food reduces the bioavailability of the drug, patients were not allowed to eat for at least 2.5 hours prior to drug administration. Subjects received 3 grams SXB at 23:00h and 3:00h. When no adverse-effects were experienced, subjects were allowed to continue the study and used this dosage of SXB for the next 4 nights. The 5th night the subjects spent in the hospital again for the second measurement.

Temperature measurement

During the baseline measurement and 5th night of SXB use, subjects stayed overnight in the hospital and a 24h temperature measurement was performed.

Core body temperature was measured with a wireless monitoring system: an ingestible and biocompatible capsule with Vitalsense monitor (Mini Mitter Company Inc., A Respironics, Inc. Company Bend, Oregon, USA).[229]

Skin temperature was measured with wireless monitoring systems: Thermochron iButtons (type DS1921H-F50; Maxim Integrated products, Inc., Sunnyvale, CA, USA)[230] Skin temperature was measured at 7 locations: left infraclavicular area, both hands, abdomen (1cm above the umbilicus), left midthigh (musculus rectus femoris) and both feet. Distal skin temperature was obtained from the temperatures at the thenar area at the palmar side of both hands and medial metatarsal area at the plantar sides of both feet.[231] Proximal skin temperature contained the infraclavicular, the thigh and abdominal temperature. Additionally, the distal-proximal temperature gradient (distal minus proximal skin temperature, DPG) was calculated.

Both core body temperature and skin temperature were sampled once per minute with a temperature resolution of 0.125°C.

Sleep analysis

Polysomnographic sleep recordings were performed with a portable, Embletta X100 recorder (Embla Broomfield, CO, USA) and scored by an experienced sleep technician according to the American Academy of Sleep Medicine criteria.[119]

Daytime naps were defined as naps if they fulfilled the following criteria: (1) a period of any sleep stage (I, II, III or REM) during the 'lights on' period (between 7:30h and 23:00h), (2) for at least two consecutive minutes, (3) no sleep was registered at least 10 minutes prior to the nap.

Data analysis and statistics

To compare sleep characteristics between patients and controls unpaired t-tests were used. Paired t-tests were used to analyze sleep characteristics before and during SXb administration. Analysis of differences for the number of daytime naps between patients and controls was performed with the Mann Whitney *U* test and the Related-Samples Wilcoxon Signed Rank test because of small group size and skewed distribution.

To evaluate group differences, group by time of day differences and treatment by time of day effects on temperature, the mean temperature of each episode of 30 minutes was

calculated. With these data Generalized Linear Model for repeated measures with Huynh-Feldt corrections were run using IBM SPSS 20 (Illinois) with between factor narcolepsy and within factors SXB and time of day. This analysis was performed on the 24 hours data, and separately for daytime (7:30h-23:00h) and night time (23:00h-7:00h). Posthoc t-tests were used to evaluate the times of day where narcolepsy or SXB related differences reached significance.

To evaluate the effect of temperature on nap probability in patients at baseline and during SXB administration, mixed effect logistic regression analysis (MlwiN, Center for Multilevel Modeling, Institute of Education, London, UK) was applied to account for the 2-level hierarchical dependency of the data structure: temperatures measured each minute (time of day), nested within subjects. For all analysis the outcome variable was sleep onset, which was binomially coded for every 1 minute epoch (since temperature was measured once per minute) as wake = 0 and sleep onset = 1. The predictive value of temperature for the odds of sleep onset was evaluated using logistic regression. The equation evaluated was as follows: $\text{logit}(P_{ij}) = \beta 0ij + \beta 1 \times Xij$ (subscripts indicate time of day i for subject j), with P representing the sleep onset probability and X representing either proximal skin temperature, distal skin temperature, distal-proximal temperature gradient (DPG) or core body temperature. For each of these temperatures, this analysis was performed with three different regressors. The first regressor evaluated was the temperature during the minute prior to the 1-minute epoch. The second and third regressor rather evaluated the predictive value of monotonic changes in temperature prior to sleep onset. To this end, the second regressor was the difference between the temperature immediately prior to the 1-minute epoch and the temperature 5 minutes before. The third regressor was the difference between the temperature immediately prior to the 1-minute epoch and the temperature 15 minutes before.

RESULTS

Subjects

Eight patients (mean age 38.0 ± 4.7 years) and eight controls (mean age 37.9 ± 4.1 years) were included after informed consent. Mean BMI was 28.1 ± 1.6 kg/m^2 for patients and 27.4 ± 1.4k g/m^2 for controls.

Sleep

Sleep characteristics are given in Table 7.1. During the day, patients were significantly less awake compared to controls ($P = 0.004$). SXB administration, results in significantly less stage I/II sleep during the day ($P = 0.049$), and a trend towards more wake ($P = 0.052$) was seen. SXB intake demonstrated a significantly higher percentage slow wave sleep during the night in patients ($P = 0.014$) and in controls ($P = 0.045$).

Daytime napping occurred in all patients at baseline and during SXB administration, varying from 3 to 16 naps per patient at baseline and from 3 to11 naps per patient during treatment. At baseline three controls took 2 or 3 daytime naps per person, while during SXB administration five controls took one nap. Both at baseline and during SXB administration, patients had significantly more daytime naps then controls (baseline number of naps for patients and controls, respectively: N = 57 and N = 8, $P < 0.01$; treatment number of naps for patients and controls, respectively: N = 46 and N = 5, $P < 0.01$). No significant improvement in the number of daytime naps was seen in controls ($P = 0.334$) or in patients ($P = 0.248$) during SXB administration.

Temperature in narcolepsy patients vs. controls at baseline

Temperature profiles are demonstrated in Figure 7.2 and the results of statistical analysis in Table 7.2. Patients had a significantly lower core body temperature. Proximal skin temperature showed a trend to be lower in patients (F = 4.13, df = 1, $P = 0.06$), while in distal skin temperature and in distal-proximal temperature gradient (DPG) no significant differences were found. Analysis of the effect of group by time of day showed a nearly significant effect of narcolepsy by time of day for proximal skin temperature (F = 2.24, df = 5.49, $P = 0.05$).

Separate analysis of daytime and night time temperatures demonstrated a significantly lower proximal skin temperature (F = 5.66, df = 1, $P = 0.03$) and core body temperature (F = 8.31, df = 1, $P = 0.01$) in patients during daytime. Furthermore, a significant effect of group by time of day was seen for core body temperature during daytime (F = 2.82, df = 7.11, $P = 0.01$) and for distal skin temperature during nighttime (F = 4.34, df = 2, $P = 0.02$).

Post-hoc tests indicated a significantly ($P < 0.05$) lower core body temperature in narcolepsy between 16:30 and midnight (00:00) and between 10:00 and 12:00 the next morning. The same was found in proximal skin temperature between 15:30 and 23:00 and between 11:00 and 12:00 the next morning.

Table 7.1 Sleep variables before and after SXB administration

	Patients (N = 8)			Controls (N = 8)			Patients vs. controls (baseline)
	Baseline	SXB	*P*	Baseline	SXB	*P*	*P*
Wake total (%)	61.0 ± 2.9	61.6 ± 2.2	0.787	68.9 ± 2.1	70.4 ± 2.4	0.284	0.045*
Wake day (%)	80.4 ± 4.1	84.9 ± 3.3	0.052	96 .5 ± 2.2	98.4 ± 1.0	0.333	0.004**
Wake night (%)	25.8 ± 5.7	19.2 ± 4.3	0.064	18.5 ± 4.0	19.2 ± 5.8	0.484	0.316
Stage I/II total (%)	29.0 ± 1.4	26.2 ± 1.4	0.070	24.9 ± 2.4	21.0 ± 2.2	0.092	0.156
Stage I/II day (%)	14.7 ± 2.9	11.2 ± 2.6	0.049*	2.6 ± 1.7	1.6 ± 1.0	0.463	0.003**
Stage I/II night (%)	55.1 ± 2.5	53.4 ± 3.7	0.497	65.5 ± 5.7	56.4 ± 5.2	0.078	0.117
SWS total (%)	3.7 ± 0.7	7.6 ± 1.2	0.018*	2.6 ± 0.7	6.6 ± 0.9	0.045*	0.263
SWS day (%)	2.1 ± 0.6	2.7 ± 1.1	0.526	0.05 ± 0.05	0.05 ± 0.05	0.356	0.013*
SWS night (%)	6.5 ± 1.9	16.5 ± 3.0	0.014*	7.2 ± 2.0	18.5 ± 2.4	0.045*	0.818
REM total (%)	6.3 ± 1.8	4.6 ± 1.0	0.112	3.7 ± 0.8	2.1 ± 0.8	0.055	0191
REM day (%)	4.3 ± 1.7	1.2 ± 0.5	0.050	0.8 ± 0.5	0.0 ± 0.0	0.175	0.077
REM night (%)	12.6 ± 3.0	10.8 ± 2.1	0.309	8.8 ± 1.8	5.8 ± 2.3	0.133	0.305
Sleep time total (min.)	561.1 ± 41.9	552.8 ± 31.9	0.787	447.9 ± 29.9	426.9 ± 34.0	0.284	0.045*
Sleep time day (min.)	254.1 ± 64.4	140.9 ± 30.8	0.117	32.4 ± 20.7	15.0 ± 9.3	0.326	0.010*
Sleep time night (min.)	378.4 ± 29.2	411.9 ± 22.0	0.064	415.6 ± 19.7	411.9 ± 29.5	0.484	0.316

Percentages of sleep stages during the 24 hours of study, before and during SXB administration. Data are shown as mean ± SEM.

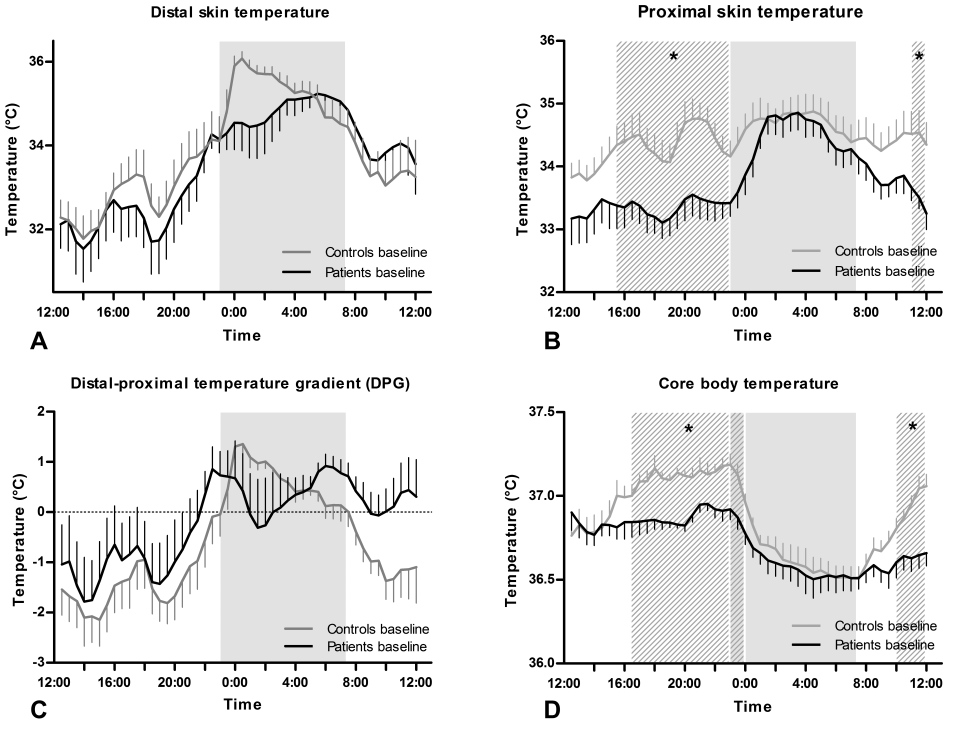

Figure 7.2 Temperature profiles of narcolepsy patients vs. controls.

Table 7.2 Temperatures of controls vs. patients at baseline

	df	F	*P*-value
Group effect			
Proximal skin temperature	1	4.13	0.06
Distal skin temperature	1	0.17	0.69
DPG	1	0.52	0.48
Core body temperature	1	6.46	0.02*
Group by time of day effect			
Proximal skin temperature	5.49	2.24	0.05
Distal skin temperature	7.46	1.25	0.28
DPG	8.91	1.75	0.09
Core body temperature	4.83	1.96	0.10

* $P < 0.05$.

Temperature in narcolepsy patients: baseline vs. SXB (Table 7.3, Figure 7.3)

In patients, a significant main effect of SXB on proximal skin temperature (F = 6.41, df = 1, *P* = 0.04) as well as a nearly significant SXB by time of day effect (F = 2.22, df = 4.80, *P* = 0.08)

Table 7.3 Temperatures at baseline vs. during SXB administration

	Controls			Patients		
	df	F	*P*-value	df	F	*P*-value
Treatment effect						
Proximal skin temperature	1	0.03	0.88	1	6.41	0.04*
Distal skin temperature	1	0.48	0.51	1	2.11	0.19
DPG	1	1.41	0.27	1	0.46	0.52
Core body temperature	1	0.01	0.91	1	2.07	0.19
Treatment by time of day effect						
Proximal skin temperature	5.06	0.61	0.70	4.80	2.22	0.08
Distal skin temperature	6.48	0.37	0.91	11.05	1.36	0.21
DPG	6.74	0.33	0.93	9.44	1.56	0.14
Core body temperature	5.74	1.65	0.16	7.58	1.56	0.16

* $P < 0.05$.

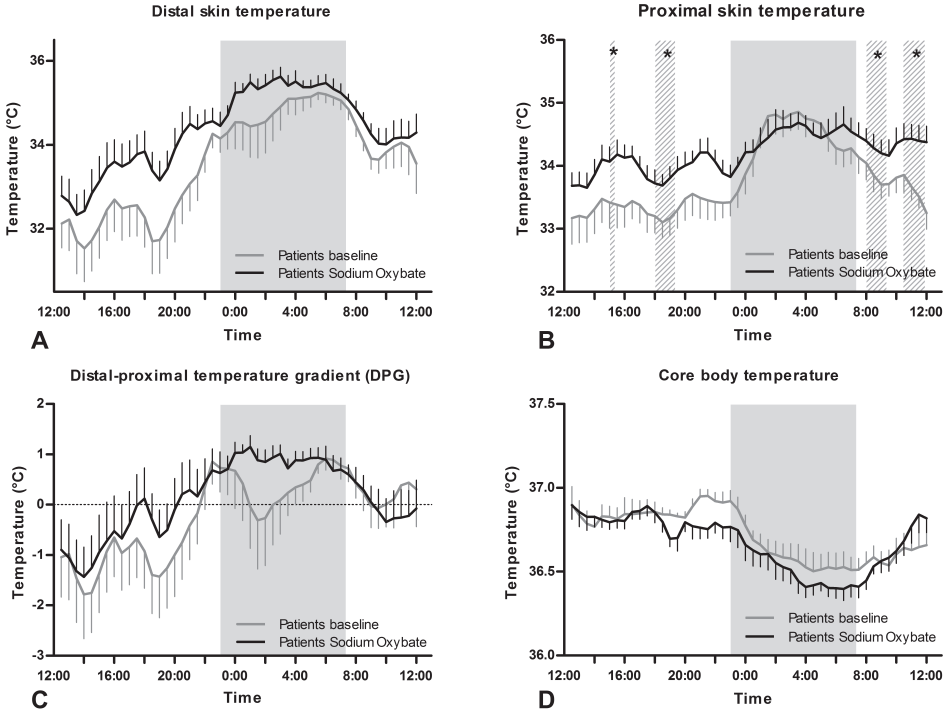

Figure 7.3 Temperature profiles of narcolepsy patients at baseline and during SXB administration. **(A)** distal skin temperature in patients at baseline and during treatment with SXB, **(B)** proximal skin temperature in patients at baseline and during treatment with SXB, **(C)** distal-proximal temperature gradient (DPG) in patients at baseline and during treatment with SXB **(D)** core body temperature in narcolepsy patients at baseline and during treatment with SXB. The gray area indicates the lights off period and the striped area the period during which the temperature significantly differed according the post-hoc tests (* $P < 0.05$). Data are expressed as mean ± SEM.

was seen. Additional separate daytime and night time analysis demonstrated that proximal skin temperature was higher during the day in the SXB condition (F = 6.46, df = 1, *P* = 0.04), but no difference was found during night time (F = 0.08, df = 1, *P* = 0.79). In post-hoc tests significance (*P* < 0.05) was reached from 15:00 to 16:00, from 18:00 to 19:30, and from 8:00 to 9:30 and 10:30 to 12:00 the next morning.

For core body temperature, distal skin temperature and DPG, no main significant effect was found for SXB administration.

Summarizing, SXB administration in patients increased proximal skin temperature at several time points during daytime. There was no effect on core body temperature, distal skin temperature and DPG.

Temperature in controls: SXB vs. baseline (Table 7.3)

In controls, no significant effect of SXB or SXB by time of day was found on core body temperature, skin temperatures and DPG.

The predictive value of temperature changes in the onset of daytime naps (Table 7.4).

Since daytime napping was rare in controls, only daytime naps in patients were analyzed. Mixed effect logistic regression analysis of sleep onset in patients at baseline revealed no predictive value of the four temperature variables for daytime sleep onset. However, during SXB administration changes in DPG significantly predicted sleep onset. Change in DPG 15 minutes prior to sleep onset, 5 minutes prior to sleep onset and DPG one minute prior falling asleep significantly predicted daytime napping (respectively O.R. 1.99 (*P* = 0.009), O.R. 2.23 (*P* = 0.042) and O.R. 1.33 (*P* = 0.029) per degree Celsius change in temperature).

DISCUSSION

The aim of this study was to investigate the effects of SXB on core body and skin temperature in relation to its effects on sleep in patients suffering from narcolepsy with cataplexy. This is the first study in which both core body and skin temperature were measured in combination with continuous sleep registration in narcolepsy. At baseline, patients had significantly lower daytime core body and proximal skin temperatures compared to controls. In patients, SXB increased nocturnal slow wave sleep (SWS), normalized proximal skin temperature, and strengthened the relationship between changes in skin temperature and subsequent daytime sleep onset.

Table 7.4 Effect of temperature on daytime lapse probability

	Baseline				Treatment SXB			
	O.R.	95% CI	Z	*P*	O.R.	95% CI	Z	*P*
Proximal skin temperature								
1 minute prior to sleep onset	1.32	0.79-2.29	0.97	0.331	0.81	0.44-1.48	-0.69	0.489
5 minutes prior to sleep onset	1.27	0.22-7.20	0.27	0.784	0.31	0.05-2.02	-1.22	0.221
15 minutes prior to sleep onset	0.96	0.36-2.54	-0.08	0.934	0.39	0.12-1.23	-1.60	0.110
Distal skin temperature								
1 minute prior to sleep onset	0.96	0.79-1.16	-0.46	0.642	1.24	0.98-1.57	1.77	0.077
5 minutes prior to sleep onset	1.51	0.89-2.58	1.52	0.129	2.48	0.77-8.01	1.51	0.130
15 minutes prior to sleep onset	1.35	0.90-2.02	1.41	0.159	1.90	0.95-3.78	1.82	0.069
DPG								
1 minute prior to sleep onset	0.91	0.73-1.13	-0.85	0.393	1.33	1.03-1.71	2.18	0.029*
5 minutes prior to sleep onset	1.45	0.83-2.53	1.32	0.186	2.23	1.03-4.82	2.03	0.042*
15 minutes prior to sleep onset	1.33	0.89-1.97	1.41	0.159	1.99	1.18-3.36	2.59	0.009**
Core body temperature								
1 minute prior to sleep onset	0.59	0.16-2.21	-0.78	0.437	1.23	0.37-4.05	0.34	0.732
5 minutes prior to sleep onset	1.51	0.01-160.25	0.17	0.863	4,29	0.01-1798.59	0.47	0.637
15 minutes prior to sleep onset	0.20	0.01-4.40	-1.01	0.311	16.68	1.01-276.09	1.97	0.049

Results of mixed effect logistic regression analysis for patients without SXB and patients during SXB administration (nights were excluded), indicating effects of temperature fluctuations as regressor for fluctuations in lapse probability (odds ratio per degree Celcius change in temperature). The logistic regression model was as follows: $\text{logit}(P_{ij}) = 0_{ij} + \beta 1 \times X_{ij}$ (subscripts indicate time of day i for subject j), with P representing the lapse probability and X representing either proximal skin temperature, distal skin temperature, distal-proximal temperature gradient (DPG) or core body temperature at the moments: difference between the temperature during the 1-minute epoch prior to sleep onset and 15 minutes prior to sleep onset, difference between the temperature during the 1-minute epoch prior to sleep onset and 5 minutes prior to sleep onset or the temperature during the 1-minute epoch prior to sleep onset. * $P < 0.05$; ** $P < 0.01$.

An altered thermoregulatory profile in narcolepsy

In the present study, core body temperature and proximal skin temperature were lower in narcolepsy, mainly caused by significant differences during daytime. No significant differences in distal skin temperature were found, although the nocturnal time course of distal skin temperature significantly differed between patients and controls.

The finding of a decreased daytime proximal skin temperature in narcolepsy patients compared to controls was previously demonstrated as well.[23] In contrast to our current findings, this previous work also reported a higher distal skin temperature. The combination of the increased distal skin temperature and the decreased proximal skin temperature in that study resulted a higher distal-proximal temperature gradient (DPG). Comparison of the present study with the previous one indicates that the absence of a higher distal skin temperature in patients, and subsequently the absence of a higher DPG, is mainly due to a higher distal skin temperature in controls in the current study. Since a higher distal skin temperature can be a direct consequence of a supine position,[232] maintaining this position throughout our study can be the explanation of the higher distal skin temperature found in controls. Concertedly, these results indicate that narcolepsy patients are likely to attain, even in an upright or sitting position, the high distal skin temperature that healthy controls reach only when remaining in a supine position.

We found a lower core body temperature during the day in patients. In the past, core body temperature has been more extensively studied than skin temperature. Unfortunately, previous studies in narcolepsy are not conclusive at this point; results vary from an elevated core body temperature to a lowered core body temperature.[25;233-236] Manipulation studies demonstrate a minimal effect of manipulation of core body temperature on sleep propensity,[25;221;231] however, a high core body temperature is associated with higher vigilance.[25;222]

SXB normalizes temperature profiles in narcolepsy

In patients, SXB administration significantly increased daytime proximal skin temperature, reaching levels comparable with healthy controls. Paradoxically, based on previous temperature manipulation studies proximal skin warming would result in increased sleepiness.[24;231;237] This finding is in contradiction with the knowledge that SXB reduces the amount of daytime sleep attacks.[238] However, warming up the skin by direct manipulation as performed in this previous study, may represent a different physiological mechanism compared with the intrinsic skin warming resulting from SXB administration.

In healthy subjects, sleep onset is preceded by a decline in core body temperature and an increase in distal skin temperature.[222] In healthy controls, an increased DPG is associated with a lower vigilance[225;226] and an accelerated sleep onset.[97;219] In narcolepsy, a shorter sleep onset latency was found to be associated with an increase of proximal and distal skin temperatures and, to a lesser extent, an increase of the DPG.[23] 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 DPG did become predictive for subsequent daytime sleep onset. The relationship between skin temperature and sleep onset that is known to exist in controls thus seemed to be restored by the administration of SXB.

Does altered thermoregulation play a role in SXB's effects on sleep?

An increase in nocturnal SWS, previously reported to be one of the principal effects of SXB on sleep,[238-241] was confirmed in this study. If this is mediated by an altered temperature regulation is questionable, since there were no nocturnal temperature effects seen during SXB intake in this study. The relatively high percentage of wake during the night in controls is probably due to the laboratory settings.

Study limitations

Since body position directly affects skin temperature, the major limitation of this study is the setting during which patients were in (semi) supine position for 24 hours. This body position differs from the situation in normal daily life, and the setting in previous studies. Moreover, the clinical effects of SXB on nocturnal sleep can already be experienced with the dose we have used in the first night of its use, but it usually takes several weeks and a higher dose to obtain significant improvement of EDS. Subsequently, it is presumable that there are some long-term effects, particularly during daytime that may have been missed in this study. Furthermore, the present study only included male subjects, while men and women are equally affected with narcolepsy.

Conclusion

In conclusion, during a constant routine protocol a decreased daytime core body and proximal skin temperature were observed in narcolepsy patients compared to controls. Administration of SXB normalized the sleep wake pattern as well as the temperature profiles in narcolepsy patients. Furthermore, SXB restored the relationship between skin temperature and subsequent sleep onset – that is known to exist in controls – in patients. To further explore the role of SXB in temperature regulation and sleep in narcolepsy, studies with patients and controls of both sexes have to be performed in normal daily life.

Acknowledgements

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PART III

Other aspects of narcolepsy

8

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

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ABSTRACT

The month of birth has been proposed as a risk factor for narcolepsy, suggesting a harmful influence during early development. Several authors have described an excess of births in March in those developing narcolepsy later. Analysis methods in published studies varied, but no study corrected completely for possible changes in seasonal birth pattern over time in the appropriate population. The present study 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 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 the 1970s the peak of births shifted from spring to autumn, confirming the need to correct for changing seasonal patterns. There was no significant difference between observed and expected birth numbers per month. 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.

INTRODUCTION

Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness, cataplexy and nocturnal sleep disturbances.[102] Narcolepsy with cataplexy is associated strongly with a loss of hypocretin-producing neurones in the hypothalamus.[47] The exact aetiology of this cell loss is unknown.[242] The strong association of narcolepsy with cataplexy with several human leucocyte antigen (HLA) subtypes suggested that autoimmune processes play a role in the pathogenesis of narcolepsy.[243] However, searches for evidence to support the autoimmune hypothesis, for instance the detection of autoantibodies or inoculation with immunoglobulins, have failed.[242;244] The recent discovery of circulating anti-Tribbles homologue 2 (TRIB2) antibodies reactive with hypocretin neurones strongly supports the autoimmune nature of narcolepsy.[59] Another approach to demonstrate autoimmune involvement involves studying the distribution of births over the months of the year. For some disorders with a presumed autoimmune origin (multiple sclerosis, type I diabetes and inflammatory bowel disease), seasonal birth patterns were found to differ from those of the population as a whole.[245;246] The underlying concept is that exposure to various environmental factors such as viruses may vary during the course of the year. An exposure in early life may later set events in motion leading to the development of a specific disease. Several studies have addressed seasonal birth patterns in narcolepsy.[170;247-251] A relative abundance from March to June as well as a relative paucity in September has been described in a study of 555 German cases.[248] A similar pattern was also described for 886 cases, from French, Canadian and Californian sources combined.[249] This type of study is not without potential problems. First, birth data from the narcolepsy population under study should ideally be compared with an appropriate control population, as the putative environmental risk factors might depend upon the geographical origin of the populations, as well as on cultural habits or racial susceptibility. Secondly, seasonal birth patterns in a population may well differ over time under the influence of factors such as changes in contraceptive use or in cultural attitudes towards procreation, as these may affect a priori chances of risk exposure. Hence, any comparison should correct for changes in birth patterns over years or decades. None of the above-mentioned studies corrected completely for both sources of potential error. In the present study we first investigated if and how the distribution of births over the year changed between 1923 and 2001 in the entire population of the Netherlands. Then, we compared the birth pattern of Dutch narcoleptic patients with that of the entire Dutch population, taking account of changes in birth pattern over time in the general population.

METHODS

Patient data

Data from 337 Dutch narcolepsy patients with cataplexy were available from The Leiden University Medical Centre narcolepsy database. Diagnostic criteria were those of the International Classification of Sleep Disorders.[118] Thirty cases were excluded to improve the homogeneity of the study group; reasons for exclusion were being born outside the Netherlands (n = 9), atypical cataplexy (n = 10) and familial cases (n = 8). Three cases, born before 1923, were excluded because complete birth data were not available for the Dutch population prior to that year.

Statistical analysis

The month and year of birth were noted for 307 (146 men) patients with narcolepsy with typical cataplexy, born 1923–2001. The number of live births for each month in the period 1923–2001 was obtained from The Dutch Central Bureau of Statistics (Voorburg, The Netherlands). We first assessed whether the distribution of births over the months of the year changed during the 79 years of study.

To do so, the numbers of births per month in any given year were divided by the total number of births of that year, resulting in a series of 12 fractions of that year's births. The fractions were then plotted as a function of month and year, allowing any changes to be visualized. The second step of analysis concerned the comparison of birth distribution of narcoleptics with that of the population. Two tables were formed containing the absolute number of births by month and by year; one table concerned narcoleptics, the other the Dutch population. The narcolepsy table was used to add the number of births per month, resulting in 12 totals for January–December: this was the observed monthly birth distribution for the narcolepsy group. The same table was also used to calculate the number of births of narcoleptics for each of the 79 years. Both tables were used to arrive at the expected monthly birth distribution for narcoleptics, as follows. For each year, the population table was used to calculate which fraction of that year's births occurred in each of the 12 months. These 12 fractions were multiplied by the number of births of narcoleptics of that year, resulting in 12 virtual birth numbers. In this way, the number of births per year was the same in the virtual group as in the narcolepsy group, but their distribution over the months was exactly that of the general population. Adding the calculated virtual birth numbers over all 79 years for each month

resulted in 12 numbers: expected monthly birth distribution. The observed and expected monthly birth distributions were compared with the chi-square test. This technique was also used to study seasonal patterns instead of months, by pooling data from three consecutive months, starting with winter (January–March), analogous to the approach taken by other authors.[248] Finally, odds ratios were calculated per month, comparing the number of births in the particular month to the pooled number of births in the other 11 months, with the expected number of births as reference category.

RESULTS

A total of 16 699 889 live births were recorded in the 79 years of study in the Netherlands. The monthly distribution of these births did not remain stable over the years: during the 1970s the birth peak shifted from spring to autumn (Figure 8.1). The figure also shows a profound disruption for the period during World War II, which in the Netherlands lasted from May 1940–May 1945. The observed monthly birth distribution of the narcolepsy with cataplexy group did not differ significantly from the expected monthly birth distribution (v2 = 8.350, df = 11, *P* = 0.681; Figure 8.2). This also held for the secondary analyses when births were grouped per season (v2 = 1.229, df = 3, *P* = 0.745). None of the calculated odds ratios per month differed significantly from one.

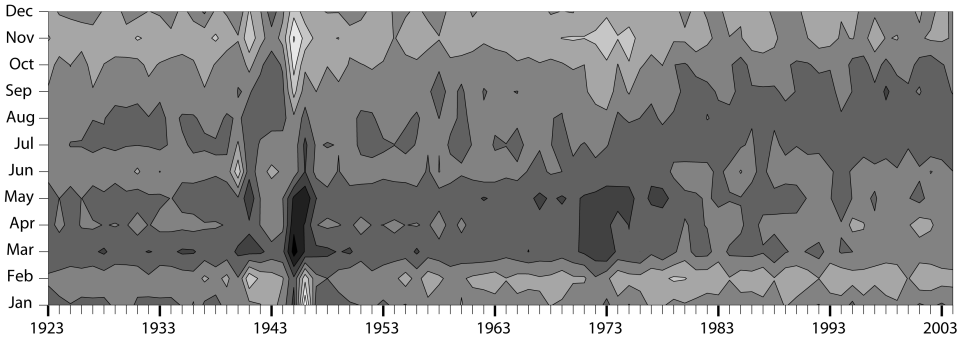

Figure 8.1 Distribution of births in the Netherlands 1923–2003. The number of births per year and month were calculated on a yearly basis to obtain the fractions of each year's births per month. The resulting table of 79 years x 12 months were used to calculate a filled contour plot, in which the range of fraction (0–1) was divided into bands of similar colour. Darker colours indicate higher fractions of births. Note the shift of birth peak from spring to autumn during the 1970s and the abrupt upheavals in birth pattern during World War II (1940–1945) (note also that the statistical analysis concerned the years 1923–2001).

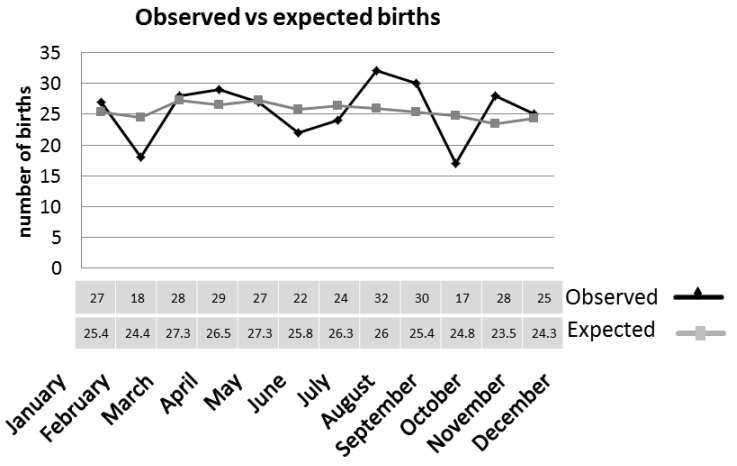

Figure 8.2 Observed versus expected births by month. The total number of births for each month over 79 years are shown for the narcolepsy group and the calculated expected number, matched for birth year. See the text for an explanation. The distributions did not differ significantly ($P = 0.94$).

DISCUSSION

This is the first study on birth month patterns in narcolepsy that takes geographical and temporal criteria fully into account. The method of analysis corrects for changes in birth patterns that occurred in the general population. Such corrections were necessary, as shown by the shift in peak from spring to autumn occurring in the 1970s, and by the marked changes occurring during the World War II. The descriptive nature of the study means that the causes of the change in the 1970s cannot be identified, even though social changes and changes in methods of contraception may have contributed.

No effect of birth month on the occurrence of narcolepsy was observed, in contrast to some earlier reports. Some authors reported a significantly different seasonality of birth month compared to that of the general population,[248-251] whereas others mentioned a predilection for births in March without stating statistical significance.[170;247] It is possible that correction factors explain the discrepancy, but this remains uncertain.

A limitation of this study is the relatively small size of the patient group. Despite this, the method used in this study has advantages over those used in previous studies. Compared to previous studies in this field, the ascertainment percentage of narcolepsy patients in our group is the highest. Furthermore, the study was performed on a very homogeneous group of narcolepsy patients. In addition, the Netherlands is a relatively small country with a high

population density and no pronounced climatic or other geographical differences between areas, providing even more uniform conditions. Finally, we suggest that the methods used in this study to correct for changing birth patterns may be used also to research birth month patterns for other diseases.

9

Delusional confusion of dreaming and reality in narcolepsy

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ABSTRACT

Study objectives: We investigated a generally unappreciated feature of the sleep disorder narcolepsy, in which patients mistake the memory of a dream for a real experience, forming sustained delusions about significant events.

Design: We interviewed narcolepsy patients and controls to establish the prevalence of this complaint and identify its predictors.

Setting: Academic medical centers in Boston and Leiden.

Participants: Patients (n = 46) with a diagnosis of narcolepsy with cataplexy, and age-matched healthy controls (n = 41).

Measurements and results: "Dream delusions" were surprisingly common in narcolepsy and were often striking in their severity. As opposed to fleeting hypnagogic and hypnopompic hallucinations of the sleep/wake transition, dream delusions were false memories induced by the experience of a vivid dream, which led to false beliefs that could persist for days or weeks.

Conclusions: The delusional confusion of dreamed events with reality is a prominent feature of narcolepsy, and suggests the possibility of source memory deficits in this disorder that have not yet been fully characterized.

INTRODUCTION

Each time we recall an event from the past, we are faced with the dual tasks of identifying the source of the memory and evaluating its veracity. In general, we can accurately determine whether a memory originated in our past experience, as opposed to in our imagination, a dream, a film, or a story. However, this process of "source monitoring" sometimes goes wrong.[252-254] Minor confusions about the source of a memory are common in the general population, as when we mistake the source of a quotation, misremember the context in which we met an acquaintance, or even believe that we actually experienced an event that we only heard about. Recently, case reports have described more severe examples of memory source confusion in patients suffering from the sleep disorder narcolepsy, in which false accusations of sexual assault occurred when patients mistook a dreamed assault for the memory of an actual event.[28;29] These reports are remarkable in that dream memories were misinterpreted as representing real, highly significant life events, leading to sustained delusions that became the basis for serious actions. Narcolepsy is a disorder of excessive sleepiness, and is not typically associated with psychotic symptoms.[255] The dramatic nature of these case reports led us to undertake the first systematic study of these "dream delusions" in narcolepsy. In a structured telephone interview, we asked narcolepsy patients and controls a series of questions about sleep, dreaming, and memory. Our goals were (1) to characterize the incidence of this phenomenon in narcolepsy patients, as compared to the general population, and (2) to describe the features of this experience.

METHODS

Participants

Narcolepsy patients and age-matched controls were recruited at two collaborating sites, Beth Israel Deaconess Medical Center in Boston, Massachusetts (n = 18), and Leiden University Medical Center in the Netherlands (n = 69). Institutional review boards at both institutions approved this research. Patients (n = 46; age 34.2 ± 10.9 [SD], 59% female) had a definite diagnosis of narcolepsy with cataplexy for a minimum of 6 months prior to the study, according to the standards of the International Classification of Sleep Disorders (ICSD-2) Diagnostic and Coding Manual.[118] Diagnoses were confirmed by interview and review of medical records, including clinic notes, overnight sleep studies, multiple sleep latency tests (MSLTs), and Human Leukocyte Antigen testing. At the time of the interviews, patients

were under treatment with a variety of medications to manage their narcolepsy, including stimulants (72% of patients; includes modafinil, amphetamine, dextroamphetamine, and methylphenidate), antidepressants (15% of patients; includes tricyclics, SSRIs, SNRIs and SARIs), and sodium oxybate (35% of patients). There were no differences in medication usage between those with and without dream delusions (chi-square tests of independence: stimulants: $P = 0.82$, antidepressants: $P = 0.64$, sodium oxybate: $P = 0.69$). Controls were recruited from the general population (n = 41; age 32.7 ± 11.6 SD, 59% female), and were screened (by self-report) to exclude the presence of any diagnosed sleep disorder. There were no differences across study sites in participant age, gender, habitual sleep schedule, dream recall, or incidence of reported confusion.

Interview procedures

Participants completed a ~30-min structured telephone interview in which they were asked a series of questions pertaining to sleep, dreaming, and memory. Following questions about their habitual sleep schedule and dream experiences, participants were asked, "*Have you ever had the experience of being unsure whether something was real, or if it was from a dream?*" 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*. To be included, a delusional episode was required to clearly persist into the waking state – 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. For purposes of analysis, participants were categorized as a "Yes" for having dream delusions if they claimed to have experiences that met this definition, and were able to provide at least one detailed example of an instance when this had occurred.

To compare general features of dreaming between narcolepsy patients and controls, participants also rated the frequency, emotionality, and intensity of their typical dream experiences on a 5-point scale.

At the conclusion of the interview, two standardized questionnaires were verbally administered – the *Boundary Questionnaire* and the *Prospective-Retrospective Memory Questionnaire*. Ernest Hartmann's *Boundary Questionnaire*, assesses the personality construct of psychological boundaries.[256;257] A "thin" boundary score (higher values) is associated with frequent and intense dreaming, as well as high interest in dreams, and the

report of unusual sleep experiences such as sleep paralysis and sleep-related hallucinations, both of which are features of narcolepsy. We administered the 18-item short form of the Boundary Questionaire.[256] The *Prospective-Retrospective Memory Questionnaire* (PRMQ) assesses subjective complaints of difficulties in remembering to carry out intentions (prospective memory), and in remembering the events of the recent past (retrospective memory).[258;259] Seventeen participants who reported dream/reality confusions meeting our criteria additionally reported to the laboratory for a face-to-face interview in which they described the qualities of these experiences in greater detail.

RESULTS

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 (χ^2 = 40.1, $P < 10^{-10}$; Figure 9.1). The severity of these delusions was striking. One man, after dreaming that a young girl had drowned in a nearby lake, asked his wife to turn on the local news in full expectation that the event would be covered. Another patient experienced sexual

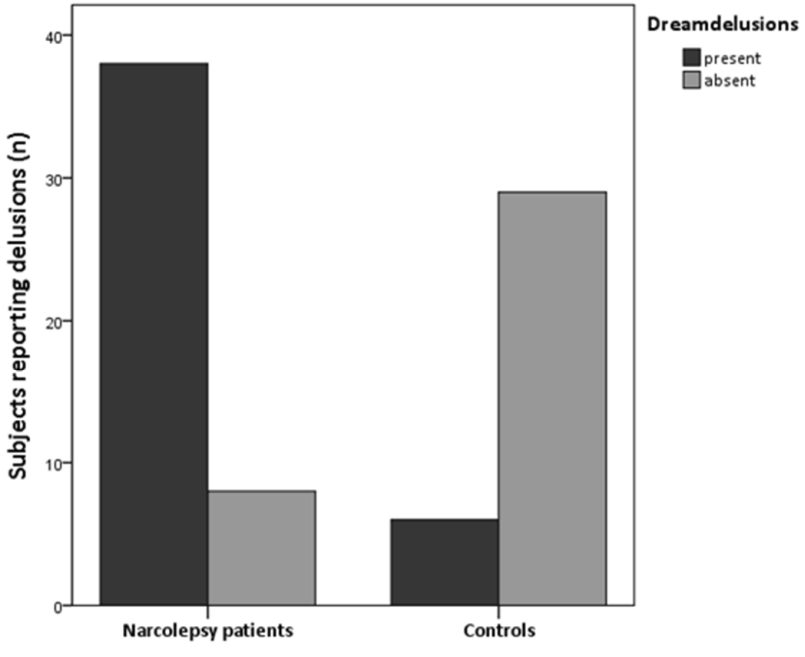

Figure 9.1 Prevalence of dream-reality confusion. Patients with narcolepsy were much more likely to report mistaking dream experiences as true memories, in comparison with age-matched healthy controls.

dreams of being unfaithful to her husband. She believed this had actually happened and felt guilty about it until she chanced to meet the 'lover' from her dreams and realized they had not seen each other in years, and had not been romantically involved. Several patients dreamed that their parents, children or pets had died, believing that this was true (one patient even made a phone call about funeral arrangements) until shocked with evidence to the contrary, when the presumed deceased suddenly reappeared. Although not all examples were this dramatic, such extreme scenarios were not uncommon.

All narcolepsy patients reporting dream delusions provided multiple examples of such occurrences. Two-thirds of patients (65%) who completed the follow-up interview 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 control participants who reported delusions, only 2 (5% of all control subjects) had experienced this more than once in their lives.

The classic hypnagogic and hypnopompic hallucinations of narcolepsy are fleeting images and feelings linked to the current environment, and patients recognize the hallucinatory nature of the experience within seconds of awakening. In contrast, the experiences reported here were much longer lasting, persisting into stable wakefulness. In follow-up interviews (see Methods), patients reported that although some delusions resolved within minutes after awakening, they often persisted for hours, days, or even weeks.

In line with prior literature,[260;261] narcolepsy patients rated their dreams as substantially more vivid ($t_{83} = 3.79$; $P = 0.0003$) and more emotional ($t_{82} = 5.25$; $P < 10^{-6}$) than the age-matched controls. They also reported recalling dreams more frequently than controls ($t_{84} = 3.16$; $P = 0.002$), and scored higher on the *BQ* than controls (indicating that patients had "thinner" boundaries; $t_{85} = 1.98$; $P = 0.05$). However, we found no evidence that dream delusions were related to an abnormal quantity or quality of dream experience in narcolepsy. Within the narcolepsy sample, neither *BQ* scores nor any other measure of dreaming differentiated between those who did and did not experience confusions (all *P*s > 0.1).

While prior research has largely failed to find objective memory dysfunction in narcolepsy,[26;262;263] subjective complaints of memory difficulty are common.[264] Here, narcolepsy patients scored higher than controls on the PRMQ for both retrospective memory problems ($t_{85} = 3.71$, $P = 0.0004$) and prospective memory problems (difficulties in remembering to carry out intentions; $t_{85} = 4.20$, $P = 0.00007$). However, memory impairment as measured by the PRMQ did not discriminate between narcolepsy patients with and without dream delusions.

DISCUSSION

Our data reveal an underappreciated memory problem in narcolepsy, in which patients are prone to delusionally believe that dreamed events actually occurred. These "dream delusions" are a special case of memory source confusion, a well-described phenomenon in which the origin of a particular memory is misattributed.[253] The conflation of dream experiences with actual events has previously been described in healthy controls.[265;266] However, in this sample of narcolepsy patients, the incidence and severity of dream delusions was striking, and far greater than that seen in controls. These observations suggest that something about the pathophysiology of narcolepsy leads to a profound confusion of memory source. Although the mechanism of dream delusions cannot be determined at this time, several possibilities present themselves. First, on the phenomenological level, our observations confirm previous reports of frequent and intense dreaming in narcolepsy. Thus, it is possible that patients mistake dream experiences for real events because the vividness of their dreams prevents the use of perceptual realism as a cue in discriminating the dreamed from the real. Our data did not provide support for this hypothesis, as dream vividness ratings did not discriminate between patients with and without delusions. However, the possibility that these delusions are caused by an abnormal intensity of dream experience in narcolepsy certainly cannot be ruled out.

Alternatively, dream delusions may be just one manifestation of a more general memory deficit in this disorder. Consistent with this possibility, we found evidence of subjective memory difficulties in narcolepsy, as assessed by the PRMQ. Again, however, this measure did not discriminate between patients with and without delusions. To our knowledge, no prior study has examined any form of memory source confusion in narcolepsy. Thus, it cannot be said at this time whether the delusions observed here are specific to dreaming, or whether narcolepsy patients might be equally prone to confuse the origin of other memories, for example mistaking imagined events or stories they have heard as personal experiences. Future studies employing standard source monitoring tasks in narcolepsy patients should be able to better determine the specificity of this complaint.

Finally, dream delusions could result from an abnormality of memory encoding specific to the sleep state. The failure to discriminate memories formed during sleep from waking life experiences could be a direct consequence of the well-described neural mechanisms of narcolepsy. Narcolepsy is caused by destruction of orexin/hypocretin neurons in the lateral hypothalamus. Normally, the orexin system helps stabilize wake/sleep states, and loss of

the orexin neurons results in “state dissociation” characterized by frequent transitions between states and the intrusion of aspects of rapid eye movement sleep into waking.[267] As monoaminergic and cholinergic neurons involved in the control of sleep states are major targets of the orexin neurons, we speculate that abnormal activity in these neurons during sleep could alter the encoding of dream content in long-term memory stores, leading to its misattribution as waking memory. Disruption of sleep neuromodulation, for example, could cause features of wakefulness to bleed into REM sleep, strengthening the typically poor memory encoding during this state.

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.

10

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.[153;154] These data suggest that SXB may alter somatotropic 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 somatotropic 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 responsivity to light in hypocretin deficient mice,[198] 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[118] 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 ([6,6-2H2]-glucose and [2H5]- 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 β-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.[97;221] When compared with controls, an increased

distal skin temperature and a decreased proximal skin temperature during wake is observed in narcolepsy patients.[23] 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.[23] 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.[97] 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.[248-251] 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.[249]

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,[38] 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.[268]

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

hypocretin deficient mice[198] could be the cause. Since light is the major suppressor of melatonin synthesis,[177] 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 $GABA_B$ receptor,[76] 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.[148] 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.[174] 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 , 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.[269] 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[269] 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 patients 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.

A

Samenvatting, conclusies en toekomstperspectieven

ALGEMENE INLEIDING EN DOELSTELLINGEN VAN DIT PROEFSCHRIFT

Een kort overzicht van de symptomen, de pathofysiologie en de behandeling van narcolepsie wordt gegeven in hoofdstuk 1. Narcolepsie wordt veroorzaakt door een tekort aan de neurotransmitter hypocretine in het brein en gekenmerkt door de volgende klassieke symptomen: overmatige slaperigheid overdag, kataplexie, hypnagoge hallucinaties, slaapverlamming en een verstoorde nachtelijke slaap. Daarnaast zijn er talrijke andere symptomen zoals overgewicht en endocriene stoornissen. Natriumoxybaat (sodium oxybate, SXB) is een medicament dat veel van de symptomen van narcolepsie kan verbeteren. Bovendien kan het de hormoonhuishouding en het metabolisme beïnvloeden zoals wordt beschreven in deel I en II van dit proefschrift.

DEEL I ENDOCRIENE ONDERZOEKEN BIJ NARCOLEPSIE

In deel I beschrijven we een groot endocrienonderzoek bij narcolepsie, vóór en na 5 dagen gebruik van SXB. Acht mannelijke hypocretine-deficiënte narcolepsiepatiënten en acht controles gematcht voor geslacht, leeftijd, body mass index (BMI), waist-to-hip ratio en percentage lichaamsvet werden in dit onderzoek geïncludeerd. Alle narcolepsiepatiënten voldeden aan de ICSD II criteria[118] en ze waren allemaal HLA DQB1 * 06:02 positief. Daarnaast waren alle patiënten ten minste 2 weken vóór aanvang van het onderzoek vrij van medicatie. Geen van de controles nam medicatie. Patiënten en controles ondergingen een 24 uur durend onderzoek waarbij bloed werd afgenomen voor de meting van prolactine (PRL) en groeihormoon (GH) (10 min interval), leptine (20 min interval), en ghreline en melatonine (1 uur interval). Dit werd gedaan tijdens de baselinemeting en op de vijfde dag van SXB-toediening. Er werd twee keer per nacht 3 gram SXB ingenomen gedurende 5 opeenvolgende nachten. Proefpersonen bleven gedurende het hele onderzoek in (half)liggende positie, met uitzondering van toiletbezoek. Drie gestandaardiseerde maaltijden werden genuttigd, en cafeïnevrije thee en water werden naar behoefte verstrekt. Het was toegestaan overdag te slapen, en het licht werd uitgedaan tussen 23:00 en 7:30 de volgende ochtend. Slaap werd continue polygrafisch gemeten gedurende beide onderzoeksperiodes.

Natriumoxybaat verhoogt prolactinesecretie bij narcolepsiepatiënten en gezonde controles

Hoofdstuk 2 behandelt de vraag of plasma prolactine (PRL) concentraties verschillen tussen hypocretine-deficiënte narcolepsiepatiënten en controles. Daarnaast hebben we gekeken naar het effect van SXB op PRL-afgifte in beide groepen. De PRL-concentratietijdreeksen werden geanalyseerd met een automatisch deconvolutieprogramma, approximate entropy (ApEn) en Cosinor-analyse. ApEn is een modelonafhankelijke statistiek die gebruikt wordt om de regulariteit van een tijdreeks te kwantificeren, hier PRL-afgifte. Zowel basale als pulsatiele PRL-secretie, pulsregulariteit en -frequentie, ApEn en 24-uurs parameters waren vergelijkbaar in patiënten en controles. SXB-toediening veroorzaakte een soortgelijke nachtelijke toename in PRL-secretie, een vervroeging van de acrophase, en een afname van de ApEn bij patiënten en controles. Het effect op de slaap van SXB bestond uit een toename in diepe slaap die vergelijkbaar was bij patiënten en controles. Bij dit gedetailleerde onderzoek werd op beide meetmomenten geen veranderde PRL-secretie vastgesteld bij hypocretine-deficiënte narcolepsiepatiënten. Daarom is het onwaarschijnlijk dat hypocretine een regulator van het lactotrofe systeem is. SXB-toediening resulteerde in een aanzienlijke toename van PRL-secretie in zowel narcolepsiepatiënten als controles. Deze bevinding is in overeenstemming met eerdere publicaties, en wordt mogelijk veroorzaakt door SXB geïnduceerde hyperpolarisatie van dopaminerge neuronen met een vermindering van dopamine-afgifte, door neuronen die de PRL-secretie reguleren.[75]

PRL-secretie is niet veranderd bij hypocretine-deficiënte narcolepsiepatiënten ten opzichte van gezonde controles.

SXB-toediening verhoogt PRL-secretie in narcolepsiepatiënten en controles.

Effect van SXB op groeihormoonsecretie bij narcolepsiepatiënten en gezonde controles

In hoofdstuk 3 wordt de groeihormoon (GH) secretie, en het effect van SXB-toediening op GH en slaap bij narcolepsiepatiënten en controles beschreven. Dit is relevant, omdat GH-verlaging gewicht kan doen toenemen.[153] De GH-concentratie tijdsreeksen zijn geanalyseerd met AutoDecon en ApEn. Basale en pulsatiele GH-secretie, pulseregulariteit en -frequentie, en ook ApEn waarden waren vergelijkbaar in patiënten en controles. Na SXB toediening werd in beide groepen een ruime verdubbeling gevonden van slow-wave sleep (SWS)

en, niet onbelangrijk, van de crosscorrelatie tussen GH-concentratie en SWS. Daarnaast veroorzaakte SXB-toediening een significante toename in het totale 24-uurs GH-secretie bij narcolepsiepatiënten, maar niet bij controles. GH heeft een sterk lipolytisch vermogen, terwijl GH-deficiëntie leidt tot vermindering van de lean body mass en toename van vetmassa.[153;154] Deze data suggereren dat SXB, naast het consoliderend effect op nachtslaap in narcolepsiepatiënten, ook de somatotrofe tonus verandert. Het is dan ook verleidelijk om te speculeren dat het vermeende gewichtreducerende effect van SXB gedeeltelijk veroorzaakt wordt door het stimulerende effect op de somatotrofe as.

GH-secretiekarakteristieken zijn niet verschillend tussen patiënten en controles.

SXB verhoogt de totale 24-h GH-secretie alleen bij narcolepsiepatiënten.

Totale plasma ghreline- en leptineconcentraties bij narcolepsiepatiënten en gematchte controles: basale concentraties en respons op natriumoxybaat

In hoofdstuk 4 wordt onderzocht of het totale plasma ghreline- en/of leptineconcentraties veranderd zijn bij hypocretine-deficiënte narcolepsiepatiënten vergeleken met controles, en of ghreline- en leptineconcentraties beïnvloed worden door SXB. Er werd geen verschil waargenomen in 24-uurs totale ghrelineconcentraties of postprandiale suppressie van ghreline tussen patiënten en controles. Evenmin werd er een invloed van 5 dagen SXB-toediening op de ghrelineconcentratie gevonden. Er was geen significant verschil tussen de 24-uurs totale leptineconcentratie en basale en pulsatiele secretie tussen beide groepen. De gemiddelde leptine pulsfrequentie was licht, maar significant verhoogd bij narcolepsiepatiënten in beide condities. De klinische relevantie van deze bevinding is onduidelijk. De resultaten maken het in ieder geval onwaarschijnlijk dat veranderingen in plasma ghreline- of leptineconcentraties een rol spelen bij de toegenomen BMI bij mensen narcolepsie. Ook is het niet aannemelijk dat plasma ghreline- of leptineconcentraties van invloed zijn op het effect van SXB-toediening op gewichtsverlies.

Leptinesecretie en ghrelineconcentraties zijn niet afwijkend bij narcolepsiepatiënten.

Veranderd circadiaan ritme van melatonineconcentraties in hypocretine-deficiënte mannen

In hoofdstuk 5 is onderzocht of melatonineconcentraties verschillen tussen narcolepsiepatiënten en controles, en of SXB-toediening melatonine-afgifte beïnvloedt. 24-uurs melatonineconcentraties verschilden niet tussen narcolepsiepatiënten en controles, noch vóór, noch tijdens SXB-toediening. Echter, het percentage 24-uurs melatonineconcentratie dat overdag werd uitgescheiden was significant hoger in narcolepsiepatiënten, zowel vóór als tijdens SXB-toediening. Een recent onderzoek liet een verminderde reactie op licht zien in hypocretine-deficiënte muizen.[198] Het is voorstelbaar dat hypocretine neurotransmissie betrokken is bij de licht-geïnduceerde suppressie van melatoninesecretie. Het effect van hypocretine op melatoninesecretie is bij mensen waarschijnlijk bescheiden, omdat men een groter verschil mag verwachten als er helemaal geen hypocretine aanwezig is. Onze bevindingen laten zien dat plasma-melatonineconcentraties overdag zijn toegenomen bij narcolepsiepatiënten (als percentage van de gemiddelde 24-uurs afgifte). SXB heeft geen effect op de gemiddelde melatonineconcentratie.

Plasma-melatonineconcentraties overdag zijn toegenomen bij narcolepsiepatiënten (als percentage van de 24-uurs afgifte).

DEEL II METABOOL ONDERZOEK BIJ NARCOLEPSIE

Glucose- en vetmetabolisme bij narcolepsie en het effect van natriumoxybaat: een hyperinsulinemische-euglycemische clampstudie

In hoofdstuk 6 werd onderzocht of narcolepsiepatiënten meer insuline-resistent zijn dan gezonde controles. Om deze vraag te beantwoorden hebben we negen hypocretine-deficiënte narcolepsie-met-kataplexie-patiënten, en negen leeftijd-, geslacht-, BMI- en vetpercentage-gematchte controles geïncludeerd. Alle narcolepsiepatiënten voldeden aan de ICSD II criteria[118] en ze waren allemaal HLA DQB1 * 06:02 positief. Geen van de controles gebruikte medicatie en als patiënten medicatie gebruikten, staakten ze deze ten minste twee weken voorafgaand aan het onderzoek. De onderzoeksdagen begonnen om 08:30 na gedurende de nacht gevast te hebben. Een hyperinsulinemische-euglycemische clamp gecombineerd met stabiele isotopen ([6,6-2 H 2] - glucose en [2H 5] - glycerol) werd uitgevoerd op baseline.

Daarnaast werd bij zeven patiënten een tweede clamp uitgevoerd na drie maanden van klinisch succesvolle SXB-behandeling. Glucose-omzetting per eenheid seruminsuline (GDR) was significant hoger bij narcolepsiepatiënten vergeleken met controles, terwijl β-cel-functie vergelijkbaar was. Er bestond een neiging tot lagere glycerolproductiesnelheid bij patiënten, wat kan duiden op een afgenomen lipolyse. SXB-behandeling induceerde een trend in de verlaging van de GDR en een afname van de endogene glucoseproductie per eenheid seruminsuline. Na SXB-behandeling was lipolyse toegenomen, en lichaamsgewicht gedaald bij narcolepsiepatiënten. Dit laat zien dat narcolepsiepatiënten meer insulinegevoelig zijn en wellicht een lagere lipolyse hebben dan gematchte controles. SXB stimuleert lipolyse bij narcolepsiepatiënten, wat mogelijk deels verantwoordelijk is voor het gewichtsverlies tijdens de behandeling. Hoewel SXB de neiging heeft om de systemische insulinegevoeligheid te verminderen, doet het de hepatische insulinegevoeligheid toenemen. Dit suggereert weefselspecifieke effecten van SXB.

Narcolepsiepatiënten zijn insulinegevoeliger dan gematchte controles.

SXB reduceert gewicht bij narcolepsiepatiënten.

De effecten van natriumoxybaat op kerntemperatuur- en huidtemperatuur-regulatie bij narcolepsie

In hoofdstuk 7 worden metabole resultaten beschreven van het onderzoek wat beschreven is in deel I van dit proefschrift. Het begin van slaap wordt doorgaans voorafgegaan door een toename in (distale) huidtemperatuur en een afname in kerntemperatuur.[97;221] Bij narcolepsiepatiënten wordt er tijdens waak een toegenomen distale huidtemperatuur en een afgenomen kern temperatuur gevonden ten opzichte van controles.[23;25] In dit onderzoek hebben we gekeken naar het effect van korte termijn SXB-toediening op huid- en kerntemperatuur. Huidtemperatuur werd gemeten met gebruik van I-buttons, en kerntemperatuur door de Jonahcapsule, bij acht hypocretine-deficiënte mannelijke narcolepsiepatiënten en acht gematchte gezonde controles. Tijdens de baselinemeting hadden patiënten overdag een significant lagere kern temperatuur en proximale huidtemperatuur dan controles. SXB verhoogde bij patiënten de proximale huidtemperatuur tot normale waarden, maar er werd geen verschil in kerntemperatuur gevonden na SXB-toediening. De verlaagde proximale huidtemperatuur overdag bij narcolepsiepatiënten ten opzichte van controles is eerder beschreven.[23] Echter in tegenstelling tot onze bevindingen,

vond men bij het eerdere onderzoek ook een hogere distale huidtemperatuur. De combinatie van toegenomen distale huidtemperatuur en verlaagde proximale huidtemperatuur leidde in eerder onderzoek tot een hogere distale-proximale gradiënt (DPG). Een toegenomen DPG is onder gecontroleerde omstandigheden een goede voorspeller voor sleep onset bij gezonde controles.[219] Bovendien werd bij narcolepsie een korte inslaaplatentie gevonden bij een toename van proximale en distale temperaturen en in mindere mate ook bij een toename van de DPG, hoewel geen van deze onderzoeken gekeken heeft naar spontaan indutten bij narcolepsiepatiënten. Baseline-analyse van temperatuurdata rondom spontane dutjes in (half)liggende positie uit het huidige onderzoek, leverde geen goed voorspellende waarden op bij narcolepsiepatiënten. Verrassend genoeg was een toename in distale huidtemperatuur en DPG wel voorspellend voor naderende slaap overdag tijdens SXB-toediening. We concluderen dat SXB-toediening het slaap-waakpatroon evenals de huidtemperatuurprofielen bij narcolepsiepatiënten normaliseert. Daarnaast herstelt SXB de relatie tussen huidtemperatuur en sleep onset bij narcolepsiepatiënten.

Narcolepsiepatiënten hebben overdag een lagere kerntemperatuur en proximale huidtemperatuur dan gematchte controles.

Met SXB-toediening wordt DPG overdag voorspellend voor naderende slaap.

SXB normaliseert proximale huidtemperatuur.

DEEL III ANDERE ASPECTEN VAN NARCOLEPSIE

Geboortemaand is geen risicofactor voor narcolepsie met kataplexie in Nederland

Hoofdstuk 9 beschrijft seizoensveranderingen in geboortepatronen van de hele Nederlandse bevolking over een periode van 79 jaar, vergeleken met het geboortepatroon van Nederlandse narcolepsiepatiënten. Onderzoek naar geboortepatronen wordt verricht om een auto-immuun oorzaak voor ziekte aannemelijker te maken. Voor een auto-immuunaandoening geldt vaak een door het seizoen bepaalde verhoogde of verlaagde kans op het ontwikkelen van de aandoening. Geboortemaand- en jaar werden genoteerd van 307 patiënten met niet-familiaire narcolepsie met kataplexie, die in Nederland geboren zijn tussen 1923 en 2001. Het aantal levendgeborenen per maand en per jaar van de gehele Nederlandse populatie voor dezelfde periode werd gebruikt om een virtuele dataset van verwachte geboorten per

maand te creëren en dit te vergelijken met de geboortemaand van de narcolepsie-met-kataplexie-patiënten in hetzelfde jaar. Waargenomen en verwachte aantallen per maand werden vergeleken met behulp van de Chi-kwadraattest. Eerdere publicaties[247-250] vonden een geboortepiek in het voorjaar, wij vonden een piek van narcolepsiegeboorten in augustus. Deze piek was echter niet anders dan de verwachte geboortegetallen in de bevolking. Daarnaast werden er geen andere verschillen gevonden tussen de verwachte en de geobserveerde aantallen geboortes. Concluderend werd er geen effect van geboortemaand gevonden op het voorkomen van narcolepsie bij een onderzoek naar 307 patiënten als er gecorrigeerd werd voor geboortepatronen van de gehele bevolking. Dit in tegenstelling tot eerdere studies waarbij een aanzienlijk verschil in seizoensgebonden karakter van geboortemaand werd gevonden bij narcolepsiepatiënten vergeleken met die van de algemene populatie. Ons onderzoek is de eerste die geboortes per jaar bekeken heeft. Andere studies voegden geboortes per tien jaar samen en negeerden daarmee veranderingen in geboortepatronen door bijvoorbeeld oorlog of de introductie van hormonale anticonceptie. Daarnaast is ons onderzoek verricht in een klein land zonder grote klimaatveranderingen per regio in tegenstelling tot de grootste studie naar geboortemaand bij narcolepsie die een populatie samengevoegd heeft van de Verenigde Staten, Canada en Frankrijk.[249]

Geboortemaand bij narcolepsie kan de auto-immuunhypothese niet ondersteunen.

Verwarring tussen dromen en realiteit bij narcolepsie

In hoofdstuk 8 beschrijven we een onderzoek bij 46 narcolepsie-met-kataplexie-patiënten en leeftijd-gematchte controles. Deelnemers voltooiden een ongeveer 30-mininuten durend gestructureerd telefonisch interview waarin zij een aantal vragen met betrekking tot slapen, dromen en geheugen beantwoordden. Delusionele episodes werden gedefinieerd als toestanden waarin de deelnemer volledig wakker was, maar onzeker of een herinnering gedroomd was of echt, of overtuigd was dat een herinnering echt was, en later ontdekte dat het toch gedroomd moest zijn. Kortstondige gevoelens van verwarring bij de overgang van slaap naar waak werden uitgesloten omdat korte verwarring een bekend gevolg is van hypnagoge en hypnopompe hallucinaties die karakteristiek zijn voor narcolepsie. Delusionele verwarring tussen droom en realiteit kwam erg veel voor bij narcolepsie. Al met al rapporteerde 83% van de narcolepsiepatiënten dat zij dromen verwarden met realiteit, vergeleken met 15% van de controles. Alle narcolepsiepatiënten die droomdelusies rapporteerden konden

meerdere voorbeelden van zulke gebeurtenissen noemen. Tweederde van de patiënten (65%) rapporteerde minstens één keer per week een droomdelusie te ervaren, en op twee na (95%) hadden ze allemaal minstens één keer per maand een dergelijke ervaring. Dit in tegenstelling tot zes controles die droomdelusies hadden, waarvan er slechts twee (5% van alle controles) dit meer dan eens hebben meegemaakt in hun leven. Kortom, delusionele verwarring tussen droom en realiteit komt veelvuldig voor bij narcolepsiepatiënten, maar zelden bij controles, en verdient daarom meer aandacht in de dagelijkse praktijk.

Verwarring tussen droom en realiteit is een veelvoorkomend symptoom van narcolepsie.

TOEKOMSTPERSPECTIEVIEVEN

Het onderzoek beschreven in dit proefschrift levert nieuwe aanknopingspunten voor toekomstig onderzoek en heeft daarnaast implicaties voor de klinische praktijk. Ten eerste moeten de onderzoeken, net als bij bijna alle onderzoeken, worden gerepliceerd, bij voorkeur in grotere groepen. Mannen en vrouwen met narcolepsie zijn vergelijkbaar aangedaan, maar ons endocriene onderzoek werd alleen bij mannen uitgevoerd. Toekomstige onderzoeken zouden zich ook moeten richten op vrouwen en kinderen. Wanneer deze onderzoeken worden uitgevoerd in premenopauzale vrouwen, moet rekening worden gehouden met de menstruele cyclus, en dienen alle vrouwen te worden gemeten op dezelfde dag van hun cyclus. Bij kinderen zal het niet haalbaar zijn om hetzelfde onderzoek te herhalen, wat voornamelijk te wijten is aan de hoeveelheid bloed die afgenomen moet worden voor de analyses. Maar een minder uitgebreid protocol, met minder bloedmonsters en een langere follow-up is van groot belang. Pubertas praecox komt veel voor bij narcolepsie in de kinderjaren,[38] en het is voorstelbaar dat deze kinderen ook andere endocriene afwijkingen hebben. Bovendien verhoogt SXB, dat off-label voorgeschreven wordt aan kinderen, bij volwassenen met narcolepsie groeihormoon- en prolactine-afgifte. Hoewel de toename van deze hormonen binnen normale grenzen bleef bij volwassen mannen, is het mogelijk dat bij kinderen deze grens overstegen wordt. Bovendien kunnen de gevolgen van een dergelijke toename in hormoonconcentraties heel anders zijn bij het zich ontwikkelende kind. Daarom, en om veiligheidsredenen, is een trial met SXB met inbegrip van hormonale controle bij kinderen nodig.[268]

Het percentage van melatonine dat overdag uitgescheiden wordt was aanzienlijk hoger bij narcolepsiepatiënten dan bij controles. We hadden de hypothese dat verlaagde responsiviteit

op licht, zoals gevonden werd bij hypocretine-deficiënte muizen,[198] een oorzaak zou kunnen zijn. Omdat licht de grootste onderdrukker van melatoninesynthese is,[177] is het denkbaar dat afwijkingen in hypocretinefunctie lichtgeïnduceerde onderdrukking van melatoninesecretie kunnen verstoren. Om deze hypothese te testen moet het protocol worden herhaald in het donker. Als een verstoorde lichtgeïnduceerde onderdrukking de oorzaak is van het veranderde afgiftepatroon bij narcolepsiepatiënten, zou men geen verschillen tussen patiënten en controles vinden bij een protocol uitgevoerd in het donker.

Afgezien van het effect van SXB op de $GABA_B$-receptor is het werkingsmechanisme vooralsnog niet opgehelderd.[76] Uit onze data is gebleken dat SXB de relatie tussen SWS- en GH-secretie versterkt, zodat het effect ervan, op zijn minst gedeeltelijk, zou kunnen worden veroorzaakt door een verhoging van de GHRH activiteit. SXB verhoogt ook de regulariteit van de GH-secretie. Dit kan betekenen dat SXB gelijktijdig endogene somatostatinerelease bevordert, omdat is aangetoond dat negatieve feedback secretoire regulariteit verhoogt.[148] Toekomstig onderzoek zou deze hypothese moeten bevestigen.

We hebben totale ghrelineconcentraties en niet het biologisch actieve hexatropine (met een octanoylgroep) gemeten. Hoewel er een hoge correlatie bestaat tussen het totale ghreline en hexatropine,[174] blijft de mogelijkheid bestaan dat er een verandering bestaat in de actieve vorm. Een toekomstig onderzoek zou moeten uitwijzen of er ook een hoge correlatie bestaat tussen beide vormen van ghreline bij narcolepsiepatiënten. Het is onwaarschijnlijk dat leptine en ghreline betrokken zijn bij de mechanismen die ten grondslag liggen aan een verhoogde BMI bij mensen met narcolepsie. Ook lijken leptine en ghreline niet betrokken te zijn bij de effecten van SXB op GH-secretie en gewichtsverlies. Echter, ons onderzoek is onder strikte laboratoriumomstandigheden uitgevoerd met gestandaardiseerde maaltijden op vooraf bepaalde tijden. Het is mogelijk dat er in het dagelijks leven, met andere leefstijlen en etenstijden, toch veranderingen gevonden worden. Om dit vast te stellen zou er een studie gedaan kunnen worden onder minder gecontroleerde condities met zelfgekozen voedings- en bedtijden.

Met de hyperinsulinemische-euglycemische clampstudie werd aangetoond dat narcolepsiepatiënten insulinegevoeliger zijn dan gematchte controles. Daarom is het minder aannemelijk dat narcolepsiepatiënten meer vatbaar zijn voor het krijgen van diabetes type 2. Het is van belang om de oorzaak hiervan te achterhalen omdat het implicaties kan hebben voor de behandeling van diabetes of zelfs preventie hiervan. Er was een trend in de richting van een lagere lipolyse bij narcolepsiepatiënten, wat voor een deel zou kunnen verklaren waarom ze

overgewicht krijgen. Toekomstig onderzoek in grotere groepen met genoeg power is nodig om op te helderen of er een daadwerkelijk verschil in lipolyse bestaat. Tot nu toe blijft het onduidelijk waarom narcolepsiepatiënten in gewicht toenemen, doorgaans kort na het begin van de ziekte. Onderzoek in een respiratiekamer, waarbij energiegebruik berekend wordt op basis van de uitgeademde lucht tijdens het verblijf in de kamer, in combinatie met dubbel gelabeld water, waarmee energiegebruik over langere perioden bepaald kan worden middels isotopen in de urine, en meting van de fysieke activiteit zou kunnen helpen de vraag te beantwoorden of een veranderd energiegebruik en/of lichamelijke activiteit een oorzaak is voor het overgewicht bij narcolepsie.

De temperatuurverschillen die gevonden werden tussen narcolepsiepatiënten en controles, en de normalisatie van huidtemperatuur onder SXB, werden verkregen onder strikt gecontroleerde laboratoriumomstandigheden waarbij de deelnemers gedurende 24 h in (half)liggende positie bleven. Een houding waarvan bekend is dat het de huidtemperatuur beïnvloedt en een houding waarin mensen doorgaans niet 24 uur in blijven. Het zou interessant zijn om huid- en kerntemperatuur te meten in een ambulante toestand, waarbij de deelnemers zoveel mogelijk proberen hun normale dagelijkse bezigheden vol te houden. Daarnaast bleken onder SXB een toegenomen distale huidtemperatuur en DPG voorspellend voor een opkomende slaapperiode overdag. Wanneer slaapperiodes voorspeld kunnen worden door temperatuurwisselingen in het dagelijkse leven, komen wellicht praktische toepassingen in zicht. Het zou mogelijk kunnen zijn om ongewenste slaap te voorkomen door temperatuursensors te koppelen aan een alarmsysteem tijdens autorijden of monotone taken.

Het is nog altijd onbekend waarom sommige mensen goed functionerende hypocretinecellen verliezen en daarmee narcolepsie krijgen. Hoewel er steeds meer aanwijzingen komen voor een auto-immuunoorzaak, is het bewijs nog steeds niet geleverd. Om een auto-immuunoorzaak te bewijzen moet voldaan zijn aan de gemodificeerde Witebskycriteria,[269] inclusief direct bewijs uit overdracht van pathogeen antilichaam of pathogene T-cellen; indirect bewijs gebaseerd op overbrenging van de auto-immuunaandoening in proefdieren, en indirect bewijs vanuit klinische aanwijzingen. Onderzoeken naar geboortemaanden zijn een heel indirecte manier om een auto-immuunoorzaak voor een aandoening aannemelijker te maken. Wij konden dit niet bevestigen met ons onderzoek naar geboortemaand. Onderzoekers komen steeds dichter bij het bewijs voor een auto-immuunoorzaak van narcolepsie, maar er is nog steeds veel werk te verzetten voordat aan de gemodificeerde Witebskycriteria voldaan wordt en auto-immuniteit is bewezen.

Tot slot hebben we met ons onderzoek naar delusionele verwarring tussen droom en realiteit een ondergewaardeerd, maar veel voorkomend probleem bij narcolepsie onthuld. Het kan nuttig zijn om deze verwarringen te benoemen bij de anamnese omdat het op deze manier herkend kan worden als symptoom van de aandoening. Gezien de hoge prevalentie van dit probleem bij narcolepsie is het van belang om uit te zoeken of dit ook veel voorkomt bij andere hypersomnieën en daarbij zijn specificiteit te bepalen. Hoewel het onderliggende mechanisme van de droomdelusies niet duidelijk is, is het duidelijk dat mensen met narcolepsie vaak moeilijkheden hebben met het onderscheiden van het gedroomde met de realiteit. Hiermee samenhangend hebben patiënten het idee dat ze in het algemeen meer moeite hebben met zowel retrospectief als prospectief geheugen. Deze observaties onderstrepen de mogelijkheid dat er iets mis kan zijn met het brongeheugen bij narcolepsiepatiënten wat vooralsnog nog niet opgehelderd is. F-MRI-onderzoeken kunnen behulpzaam zijn om onderliggende mechanismen op te helderen door tijdens geheugentaken verschillen in activiteit aan te tonen in de hippocampus, prefrontale of in andere hersengebieden. Slaap zou tijdens f-MRI-onderzoeken geregistreerd moeten worden en geavanceerde neuropsychologische tests zijn nodig om de specifieke geheugenproblemen die bij narcolepsie beschreven worden, maar nooit aangetoond zijn met gangbare tests, te detecteren. Daarnaast zou het interessant zijn om narcolepsiepatiënten met droomdelusies te vergelijken met narcolepsiepatiënten zonder droomdelusies om een betere verklaring voor het probleem te vinden.

Er wordt veel onderzoek naar narcolepsie verricht en ik heb daar mijn steentje aan bijgedragen, en hoop dat in de toekomst te blijven doen totdat het niet meer nodig is.

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Curriculum Vitae

Claire Donjacour was born in Veghel April 20, 1970. In 1987 she finished secondary school at the Mgr. Zwijsen College in Veghel and started her career becoming an operating room nurse at the Academic Hospital Leiden (AZL later LUMC). She finished this training in 1991 and worked in the AZL until September 1993. Then she got employed at the OR of the Rijnland hospital Leiderdorp for six months and switched to the Westeinde hospital in The Hague. In 1995 she obtained her colloquium doctum to study psychology. Consecutively she started studying psychology at the Leiden University next to her full time job as operating room nurse at the Westeinde Hospital. In 1998 she switched to the University of Amsterdam to specialise in clinical- and neuropsychology and obtained her Master of Arts degree in 2000. She then continued working as an operating room nurse at the Westeinde hospital and got a research position supervised by prof. dr. G.A. Kerkhof, at the sleep centre of the same hospital. In 2001 she started medical school at the University of Leiden. She received her propedeuse in 2002 and her medical degree in August 2005. One month later she became an intern at the Diaconessenhuis Leiden. In this year she went to Ghana to work as a volunteer for Care to Move. During medical school she kept on working as an operating room nurse for 3 days a week. During her clinical rotations she worked one day per week in the OR and one evening as an MRI technician at the radiology department of the LUMC. In addition she worked as a volunteer for the African Albino Foundation, and was a member of the Local and National organisation of final year students (LOCA). Narcolepsy research started during her rotations and became an official PhD project in 2007. Part of her research is done in Maastricht (supervised by prof. dr. K.R. Westerterp) and at the Beth Israel Deaconess Medical Centre of Harvard university in Boston. Neurology training started October 2006 and ended in June 2014 at the LUMC (prof. dr. R.A.C. Roos). One year of this training took place at the Westeinde hospital (prof. dr. M.J.B. Taphoorn). During the final year of her neurology training she worked for 3 months at the Rijnland Hostpital in Leiderdorp (dr. A.A. vd Plas) and at SEIN Heemstede (dr. R.D. Thijs). Since August 2014 she works as a neurologist in sleep and epilepsy at SEIN Zwolle and Groningen.

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List of publications

Donjacour CE, Aziz NA, Overeem S, Kalsbeek A, Pijl H, Lammers GJ. Glucose and Fat Metabolism in Narcolepsy and the Effect Of Sodium Oxybate: A Hyperinsulinemic-Euglycemic Clamp Study. Sleep 2014; 37:795-801.

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