Exploring the relationships of gamma-hydroxybutyrate and sleep on metabolism, physiology, and behavior in humans

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CHAPTER 1

Thesis Introduction and Aims

1.1 Thesis Introduction

This thesis is divided into two discrete parts. The first part focuses on the impact of the drug sodium oxybate in various populations, especially in humans with narcolepsy, both with and without cataplexy. The second part of the thesis focuses on better understanding the relationship of sleep, eating, and metabolism in healthy humans.

1.2 Abstract for Part I - Neurobiological and Clinical Effects of Sodium Oxybate

Neurobiology of Gamma-hydroxybutyrate and Sodium Oxybate

Gamma-hydroxybutyrate (GHB) is an endogenous short chain fatty acid and a, mostly oral, pharmacological compound that has been utilized in a variety of ways. Endogenously, GHB is synthesized locally within the CNS, mostly from its parent compound gamma-Aminobutyric acid (GABA). Evidence suggests a role for GHB as a neuromodulator/neurotransmitter. Under endogenous conditions and concentrations, and depending on the cell group affected, GHB may increase or decrease neuronal activity by inhibiting the release of neurotransmitters that are co-localized with GHB. Sodium oxybate (SXB) is the sodium salt of GHB and is used for the exogenous oral administration of GHB. Supraphysiological concentrations of GHB from exogenous administration are likely to produce qualitatively different neuronal actions than those produced by endogenous GHB concentrations. After exogenous administration, most of the observed behavioral effects appear to be mediated via the activity of GHB at $GABA_B$ receptors, as long as the concentration is sufficient to elicit binding, which does not happen at endogenous concentrations. Endogenous and exogenous GHB is rapidly and completely converted into CO_2 and H_2O through the tricarboxylic acid cycle (Krebs cycle).

Clinical Effects of Sodium Oxybate

Sodium oxybate has been observed to modulate sleep in nonclinical study participants, and sleep and wakefulness in clinical populations, including groups with insomnia, fibromyalgia, narcolepsy. Furthermore, multiple measures of daytime sleepiness and cataplexy demonstrated consistent short- and long-term improvement in response to nighttime SXB therapy. The most common reported adverse events include dose-related headache, nausea, dizziness and somnolence. In this thesis, with my collaborators, I characterized the sleep effects of SXB in individuals with Parkinson's disease (PD) and further characterized the sleep effects of SXB in individuals with narcolepsy.

In PD, many patients experience excessive daytime sleepiness and numerous nocturnal sleep abnormalities. To determine the safety and efficacy of SXB in this population, we enrolled 38 individuals in a multicenter, open-label, PSG study. The administration of nightly SXB correlated with significant improvements in

subjective sleepiness, subjective sleep quality, reduced fatigue severity, and a mean increase in time spent in slow wave sleep (SWS).

In narcolepsy, previous research indicates that nightly SXB administration reduces nocturnal sleep disruption. In this thesis, I present the findings from two clinical trials that provided an opportunity to further characterize these sleep-related effects in this population. The first study enrolled 278 individuals and evaluated treatment with SXB as monotherapy or in combination with modafinil and performed polysomnography (PSG) and the Maintenance of Wakefulness Test (MWT) at baseline and after 4 and 8 weeks. The second study enrolled 228 individuals across the United States, Canada, and Europe and randomized them to receive 6 and 9 g/night and measured nocturnal sleep after 8 weeks of treatment. In both studies, the individuals on SXB demonstrated several significant dose-related changes to their sleep compared their baseline measures. These include significant increases in time spent in total sleep time, SWS, and delta power. At the 6 and 9g/night doses, we observed a significant median decrease in nocturnal awakenings, and a significantly decreased in stage 1 sleep and the frequency of nocturnal awakenings in first and second study, respectively. In addition to its previously established efficacy for the treatment of cataplexy and EDS in narcolepsy, we further demonstrated that nightly SXB administration significantly impacts measures of SWS, wake after sleep onset, awakenings, total sleep time, and stage 1 sleep in a dose-related manner.

Differences in Abuse Liability between 'Illicit GHB' and Sodium Oxybate

There are distinct differences in the accessibility, purity, dosing, and misuse associated with illicit GHB compared to pharmaceutical SXB. Gamma-hydroxybutyrate sodium and SXB are the chemical and drug names, respectively, for the pharmaceutical product Xyrem® (sodium oxybate) oral solution. However, the acronym 'GHB' is also used to refer to illicit formulations that are used for non-medical purposes. In a review paper, we highlight important differences between illicit GHB and sodium oxybate with regard to their relative abuse liability, which includes the likelihood and consequences of abuse. Data are summarized from the scientific literature; from national surveillance systems in the U.S., Europe, and Australia (for illicit GHB); and from clinical trials and post-marketing surveillance with sodium oxybate (Xyrem). In the U.S., the prevalence of illicit GHB use, abuse, intoxication, and overdose has declined from 2000, the year that GHB was scheduled, to the present and is lower than that of most other licit and illicit drugs. Abuse and misuse of the pharmaceutical product, SXB, has been rare over the 5 years since its introduction to the market, which is likely due in part to the risk management program associated with this product. Differences in the accessibility, purity, dosing, and misuse of illicit GHB and sodium oxybate suggest that risks associated with illicit GHB are greater than those associated with the pharmaceutical product SXB.

Metabolic Effects of Sodium Oxybate

The effects of SXB on various endocrinological parameters has been explored, including its impact on melatonin, ghrelin, growth hormone, insulin, cortisol and glucagon, endocannabinoids, and more. Because ghrelin and leptin interact with hypocretin neurons to influence energy homeostasis, we evaluated whether human hypocretin deficiency, or the narcolepsy therapeutic SXB, alter the levels of these hormones. To do this, we assessed 8 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. Blood samples of total ghrelin and leptin were collected over 24 hours at 60 and 20-min intervals, respectively, during two study occasions: baseline, and during the last night of 5 consecutive nights of SXB administration (2 x 3.0g/night). At baseline, mean 24-h total ghrelin and leptin levels were not different between hypocretin deficient narcolepsy patients and controls. Furthermore, sodium oxybate did not significantly affect the plasma concentration of either one of these hormones. Therefore, 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.

1.3 Abstract for Part II - Sleep, Eating, and Energy Regulation

Sleep and Eating

Substantial epidemiological evidence shows a significant association between reduced sleep and increased body weight, and a multitude of energy-regulation mechanisms have been explored to better understand this association. Cross-sectional and prospective data show that short-duration sleepers have modified eating behaviors, including altered within-day eating timing, increased snacking behavior, and increased calories from beverages. Controlled, prospective research demonstrates an increase in caloric intake on the day following one to several nights of partial sleep restriction in normal weight adults. While it is common for self-reported hunger to increase after sleep deprivation, some studies show no difference in hunger between the sleep-rested and sleep-deprived conditions, despite difference in food selection behavior. The increase in caloric intake may in part be explained by reduced satiety.

Some increase in caloric intake after sleep loss would be expected to accommodate for increased energy expenditure from additional wake time. Indeed, several studies show that homeostatic factors contribute to increased caloric intake after sleep loss; however, this increased caloric intake seems to exceed the level expected to accommodate for energy expenditure associated with the additional time. It has been argued that altered hedonic-valuation factors increase portion size and alter food selection after sleep loss.

A well-described and consistent response to sleep loss is impaired alertness, which can be observed in multiple ways, including decreases in both subjective and objective alertness measurements. Moreover, it has been hypothesized that sleep deprivation-induced cognitive impairments, such as reduced alertness and attention, result from instability in the wake state and that such an increase in moment-to-moment variability of attention impairs a wide variety of cognitive tasks, including goal-directed activities. Eating is one such goal-directed activity with food decisions being made 200 times or more each day. Eating is influenced by a wide variety of factors. Internal factors include metabolic state, health beliefs and objectives, emotional state, and the behavioral and metabolic consequences of dietary habits. External influences include food presentation and environmental conditions. External factors can shift awareness away from internal drivers of food intake, potentially causing diminished accordance with health goals and internal signals of satiety. Thus, the act of self-monitoring food intake volume and food type, as well as satiety requires awareness, which is influenced by alertness, which is influenced by sleep.

We hypothesized that lowered alertness would lead to less surveillance in both total caloric intake and decisions about the types of food one eats. In the current study, we examined whether experimentally induced changes in subjective or objective alertness were associated with changes in total calorie consumption, and calorie consumption based on several categorizations, including the healthfulness, the tempting nature, and the caloric density of the offered foods.

Metabolic Effects of a 4-Day Outdoor Trip Under Simulated Paleolithic Conditions

The observation that the emergence of common Western diseases (WD) – from obesity to coronary heart disease to cancers – takes place with much greater prevalence as societies migrate from natural-living cultures to those that increasingly assume the characteristics of wealthier, modernized societies, has been well documented. This is highlighted clearly by, for example, the drastically increased prevalence of obesity and diabetes in recently urbanized vs rural-living indigenous peoples. For instance, in Nauru, since the 1920s, royalties for the natural resource phosphate has allowed these people to become one of the world's richest *per capita*. This wealth, however, has also afforded a rapid change in lifestyle. In this population, the first case of type 2 diabetes was noted only in 1925. Now, however, the Nauruans are the world's most obese people (92.8%), have the highest blood pressure in the Western Pacific region, and two-thirds of their population over age 55 suffer from type 2 diabetes. For approximately 84,000 generations, humans lived under hunter and gatherer conditions. But recently, humans have endured dramatic change from their native lifestyle with the occurrence of the agricultural, industrial, and digital revolutions. Despite the massive technological innovation that has taken place during these revolutions, they have all occurred within a relatively recent time frame. These innovations have enabled humans to live in a manner that is discordant with expectancies of our genes, which were largely established during our pre-agricultural past.

The metabolic dysregulation that appears to accompany the rural-modern lifestyle transition is supported by an abundance of evidence indicting elements of the modern lifestyle as causative in WD. These include, but are not limited to: overnutrition, low dietary fiber intake, sugar-rich diet, physical inactivity, vitamin D deficiency, psychosocial stress, sleep deprivation and circadian rhythms disturbances, and more. Therefore, the shift from a natural to a modern lifestyle likely promotes a gene-environment mismatch, which causes metabolic dysregulation, which causes disease. In contrast to single-intervention studies, our study aimed to have participants emulate a modern-day, Paleolithic-like lifestyle pattern during a short nature trip – which included multiple alterations from the default lifestyle pattern of modern living – to assess signs of favorable metabolic changes. We hypothesize that adopting a more Paleolithic-like lifestyle pattern will yield favorable and observable effects on metabolism, even in the short term.

1.4 Aims

Part I - Neurobiological and Clinical Effects of Sodium Oxybate

GHB is an active metabolite of the inhibitory neurotransmitter GABA. SXB is the sodium salt of GHB and is used for the exogenous oral administration of GHB. GHB has a suggested role as a neuromodulator/neurotransmitter. After exogenous administration, most of the observed behavioral effects of SXB appear to be mediated via the activity of GHB at $GABA_B$ receptors. GHB has been shown to have modulatory activity on multiple neurotransmitter systems, as well as hormonal and metabolic effects.

In Chapter 2, I review the biology and pharmacology of GHB and SXB, as well as their modulatory effects on neurotransmitters, hormones and metabolism. I then review the evidence supporting a modulatory effect of GHB and SXB on sleep and wakefulness, both in healthy and in clinical populations. I examine the data showing that GHB can dose-dependently decrease sleep onset latency, promote delta activity and enhance sleep maintenance in healthy populations. Next, I provide an overview of the effects of GHB in enhancing mood and prosocial behavior in healthy populations and discuss its putative antidepressant effects.

In the following section of Chapter 2, I review the effects of GHB and SXB in clinical populations. I present data indicating that GHB may improve sleep in patients with insomnia. I also review the evidence indicating a beneficial effect of GHB/SXB in decreasing sleep disruption in patients with fibromyalgia and, importantly, in also decreasing pain, fatigue and overall multidimensional function. Next, I review the evidence of the effects of GHB/SXB in modulating sleep in patients with neurodegenerative diseases, specifically Parkinson's and Alzheimer's disease. I also discuss the association between sleep impairment and Alzheimer's disease pathogenesis and explore the potential therapeutic benefit of the neurobiological effects of GHB on those pathogenic processes. Finally, I analyze the effects of GHB/SXB in narcolepsy and discuss how SXB has consistently shown short- and

long-term improvement on various properties of sleep, including increases in SWS duration and delta power, and a reduced number of night-time awakenings.

In Chapter 3, I examine the safety and efficacy of SXB in subjects with Parkinson's disease (PD) and sleep disorders. In a multicenter, open-label, polysomnographic study[1], we hypothesized that using SXB as a specific treatment for nocturnal sleep abnormalities could also decrease the excessive daytime sleepiness often observed in the PD population. In the 27 subjects with PD who completed the study, receiving a mean dose of 7.8 g SXB per night for 6 weeks, it was observed that SXB was well tolerated, increased SWS, improved subjective nighttime and daytime sleep problems, and decreased daytime fatigue. These results suggested that nocturnal administration of SXB can have beneficial effects on excessive daytime sleepiness and fatigue in PD and that the potential therapeutic effects of SXB for PD-associated sleep dysfunctions are worth pursuing in controlled trials using objective measures of daytime sleepiness.

In Chapter 4, I build upon previous studies indicating that nightly SXB administration reduces nocturnal sleep disruption in narcolepsy. Aiming at further characterizing the sleep-related effects of SXB in the context of narcolepsy, we conducted a double-blind, placebo-controlled study in patients with narcolepsy treated with SXB as monotherapy or in combination with modafinil[2]. The intent-to-treat population consisted of 222 patients randomized to receive treatment with placebo (n=55), SXB (n=50), modafinil (n=63), or SXB + modafinil (n=54). Treatment efficacy was assessed using overnight polysomnography (PSG), Maintenance of Wakefulness Test, Epworth Sleepiness Scale scores and daily diary recordings. After 8 weeks, significant changes in sleep architecture were observed among patients receiving SXB as monotherapy or in combination with modafinil, whereas no significant changes in PSG parameters were found after treatment with placebo or modafinil alone. Changes induced by SXB or SXB + modafinil included increased stage 3 and 4 sleep and delta power, and decreased nocturnal awakenings, showing that SXB can significantly reduce measures of sleep disruption and significantly increase SWS in patients with narcolepsy.

In Chapter 5, I review the differences in the accessibility, purity, dosing, and misuse between illicit GHB and pharmaceutical SXB (Xyrem®)[3]. Although gamma-hydroxybutyrate sodium is the chemical name for sodium oxybate, the acronym GHB also refers to the illicit formulations used for non-medical purposes. In this review, I emphasize critical differences between illicit GHB and pharmaceutical SXB, namely in what concerns their relative abuse liability and its consequences. Based on data from the scientific literature, from national surveillance systems in the United States, Europe, and Australia for illicit GHB, and from clinical trials and post-marketing surveillance for pharmaceutical SXB, I discuss how the prevalence of illicit GHB use, abuse, intoxication, and overdose has declined in the United States since it became an illegal drug in the year 2000, how and why the abuse and misuse of pharmaceutical SXB has been rare since its introduction to the market, and how and why the risks associated with illicit GHB are greater than those associated with pharmaceutical SXB.

In Chapter 6, I further analyze the effects of nightly SXB administration on nocturnal sleep in narcolepsy patients. I describe the first large randomized, double-blind, placebo-controlled, parallel group trial examining the impact of SXB on sleep architecture and narcolepsy symptoms, conducted with 228 adult patients with narcolepsy/cataplexy in the United States, Canada, and Europe[4]. In patients receiving either 4.5, 6, or 9 g SXB or placebo nightly for 8 weeks, changes in sleep architecture were measured using PSG and changes in narcolepsy symptoms and adverse events were recorded in daily diaries. After 8 weeks of nightly SXB administration, a significant dose-dependent impact on the measures of SWS was observed, including increases in the duration of stage 3 and 4 sleep, and increased delta power in all dose groups; the doses of 6 and 9 g/night also significantly decreased stage 1 sleep and the frequency of nocturnal awakenings. The effects of SXB on nocturnal sleep concurred with significant decreases in the severity and frequency of narcolepsy symptoms. The results I describe in this chapter indicate that SXB may improve the sleep fragmentation that is commonly associated with narcolepsy.

In Chapter 7, I examine the link between narcolepsy, hypocretin neurons, the hormones ghrelin and leptin, and SXB. Given that narcolepsy is caused by a selective loss of hypocretin neurons and is associated with obesity, and that ghrelin and leptin interact with hypocretin neurons to influence energy homeostasis, we aimed at evaluating whether human hypocretin deficiency or SXB can alter the levels of these hormones[5]. The rationale for this study was that ghrelin and leptin hormonal disturbances induced by hypocretin deficiency could provide an explanation for the weight and ingestive behavior phenotype of the narcoleptic population, and for the influence of SXB on body weight. To do so, we studied the blood levels of ghrelin and leptin in hypocretin deficient narcoleptic patients at baseline and after five consecutive nights of SXB administration (2 × 3.0 g/night). We found no differences in ghrelin or leptin levels nor any effects of SXB on the plasma levels of either hormone. These results therefore indicated that the increased body-mass index of narcolepsy patients is unlikely to be driven by hypocretin deficiency-mediated changes in total ghrelin or leptin levels, and that the influence of SXB on body weight is unlikely to involve changes in in the secretion of the hormones. I discuss how these findings contribute to a better understanding of the hormonal phenotype of the narcoleptic population.

Part II: Sleep, Eating, and Metabolism

Obesity is a major public health issue in today's world, and it appears likely that multiple factors of modern life contribute to its cause. Concurrently, various factors of modern life promote reduced and disturbed nighttime sleep. For example, in 2012, 70 million Americans reported sleeping less than six hours, which according to the National Sleep Foundation of the United States, is below recommended levels for human health and wellbeing.

There is strong epidemiologic evidence connecting reduced sleep duration and/or disturbed sleep with an increased risk for obesity. Subsequent to, and in parallel

with these associative observations, various investigations have revealed that altered sleep may disturb energy regulation and brain-processing circuits related to appetitive behavior, thus contributing to alterations in metabolism, body weight, and body composition.

In Chapter 8, I review the latest findings relevant to sleep and energy-regulating processes. First, I examine how obesity rates have risen in both adults and children worldwide, and how the prevalence of obesity may be related to both sleep duration and sleep quality. Next, I discuss epidemiological studies that seek to answer whether sleep times really have decreased over the last 60 years. In addition, I discuss how epidemiological evidence of sleep length and quality relates to weight and energy regulation, and how sleep disturbance affects metabolic, endocrine, immune, and circadian processes. Finally, I examine how brain-processing circuits related to alertness, memory, inhibition control, mood, and reward behavior are altered by sleep loss. This literature review provides the background for the human studies I contributed to in this field, which are presented in detail in chapter 9 and chapter 10.

In chapter 9, I explain how manipulation of a single night sleep influences participants' food preferences[6]. In that study, we hypothesized that lowered alertness by artificially restricting a person's natural sleep period would alter total calorie consumption or modify preference for the source of calories from a range of healthy and unhealthy foods. We show that ecologically-relevant impairments in both subjective and objective alertness is associated with altered eating behaviors and food choice. We also show that some of these effects differentiate between whether the alertness impairment was subjective (i.e., sleepiness) or objective (e.g., reaction time) in nature. Overall, impairments in alertness associated with increased caloric intake, preference for less healthy foods (as rated by the participants' subjective rating of the food), and consumption of more calorically-dense food options. These findings suggest that reduced alertness after modest sleep loss may alter a person's food choices towards less healthy, more calorically-dense foods.

In chapter 10, I examine how a short-term return to Paleolithic eating, living, and sleeping conditions via a several-day nature trip may have beneficial effects on human physiology[7]. The recent shift from natural to modern living environments may cause an unintended mismatch between genes shaped for a natural environmental niche and typical modern lifestyles. For example, blue light from hand-held electronic devices prolongs sleep onset and shifts circadian timing, reduced sleep promotes the consumption of calorically dense processed foods, and modern diet can disrupt sleep quality. Using a within-participant design, we examined whether a four-day and four-night intervention aimed to mimic Paleolithic-like living conditions could alter metabolic and physiological parameters in two groups of 14 volunteers. Participants lived outdoors without tents, hiked extensively during the day, and had reduced and delayed caloric intake (two meals per day after noon). Body weight, body mass index, body fat, visceral fat, and waist-hip ratio all significantly decreased. In addition, metabolic parameter

values, such as fasting glucose, basal insulin, and fatty liver index, decreased, which suggests that a brief nature trip may be an effective intervention to improve modern metabolic health.