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INTERACTIONS BETWEEN SLEEP AND ENDOCRINE REGULATION

Ph.D. thesis

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PAPERS ATTACHED TO THE THESIS

I. Obál F Jr, Beranek L, Brandenberger G: Sleep-associated variations in plasma renin activity and blood pressure in the rat. *Neurosci. Lett.* 179:83-86, 1994.

II. Beranek L, Obál F Jr, Taishi P, Bodosi B, Laczi F, Krueger J M: Changes in rat sleep after single and repeated injections of the long-acting somatostatin analog octreotide. *Am. J. Physiol.* 273:R1484-R1491, 1997.

III. Beranek L, Hajdú I, Gardi J, Taishi P, Obál F Jr, Krueger J M: Central administration of the somatostatin analog, octreotide, induces captopril-insensitive sleep responses. *Am. J. Physiol. Accepted (June 22, 1999)*

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ABBREVATIONS

5-HT2	5-Hydroxytriptamine (serotonin)
ANOVA	Analysis of Variance
EEG	Electroencephalogram
GH	Growth Hormone
GHRH	Growth Hormone-Releasing Hormone
icv	Intracerebroventricular
IGF-1	Insulin Like Growth Factor-1
mRNA	Messenger Ribonucleic Acid
NREMS	Non-Rapid Eye Movement Sleep
OCT	Octreotid
PRA	Plasma Renin Activity
PTH	Parathyroid hormone
REMS	Rapid Eye Movement Sleep
RIA	Radioimmunoassay
sc	Subcutaneous
sst2-5	Somatostatin Receptor 2-5
SWA	Slow Wave Activity

1. INTRODUCTION

1.1. *Background*

Two states of sleep are distinguished: REMS and NREMS. The states of sleep are fundamentally different in terms of EEG activity, autonomic, somatic, and cognitive functions. Regulations of NREMS and REMS also differ. The structures involved in the generation of REMS reside in the brain stem (pons and mesencephalon). In contrast, forebrain structures (anterior hypothalamus/preoptic region, thalamus) have been implicated in the genesis of NREMS. The importance of these areas of the forebrain is that they are the origin of projection pathways that synchronize the activities of distant parts of the brain. Otherwise, the regulation of NREMS might be local [47]. The characteristic signs of sleep can only be recognized in homoiothermic species, mammals and birds, and it seems that the two states of sleep have evolved simultaneously. Sleep is a matter of controversy in the currently existing lower vertebrates. Rest-activity rhythms are, however, displayed by each species throughout the animal kingdom, and it is likely that the functions and regulations of sleep stem from functions and regulations of rest. During sleep, periods of NREMS and REMS alternate with a cycle (sleep cycle) specific to the species. In addition to the duration, NREMS has another measure: intensity (depth of NREMS). The intensity of NREMS is characterized quantitatively by the slow wave activity in the EEG. Deep NREMS with intense slow wave activity is observed during the first sleep cycles of the diurnal rest period. The duration of REMS epochs increases towards the end of sleep resulting in an increased time spent in REMS in the second portion of the rest period. These principles of sleep organization are easily recognized in each species with a strong circadian rhythm. Thus, in spite of the 180 ° shift between the rest periods in humans and rats (rats are active at night), there is a high degree of similarity in the organization and regulation of sleep between these species [10].

As an average, humans spend 8 hours per day asleep. Sleep is assumed to serve a fundamental function for permanent sleep loss is fatal [72]. Transient sleep deprivation is followed by a recovery, characterized by increases in the duration and intensity of sleep. Although practically each function of the body is altered during sleep the function of sleep is

not known. Sleep may promote processes which are important for the body in particular conditions such as infections, processing of foodstuffs or exposure to a warm environment. In fact, enhanced sleep is part of the acute phase reaction, and increases in sleep are observed after meal or in a mildly warm environment. Nevertheless, the primary functions of sleep have been associated with necessities arising in the central nervous system [39,44,47,57]

The importance of the profound alterations in autonomic mechanisms during sleep are well-known in human pathology as sleep-associated cardiovascular and respiratory diseases. Secretions of many hormones also vary with sleep. Currently, the biological significance of sleep-associated alterations in hormone secretions is not clear. On the one hand, these changes in hormone release may have importance for endocrinology. For example, sleep disorder and impairment of sleep-linked GH secretion in children are associated with short statue (psychosocial dwarfism) [95]. On the other hand, hormones may act as sleep-promoting or arousing substances [11] and might be involved in the regulation of sleep-wake activity. Our experiments addressed both issues: sleep-related variations in hormone secretion were studied in the case of the renin-angiotensin system whereas the studies with somatostatin are part of a series of experiment aiming to elucidate the role of somatotropic axis in sleep regulation.

1.2. *Sleep-associated variations in hormone secretion: the renin-angiotensin system*

The plasma concentrations of many hormones vary in synchrony with sleep-wake activity [91]. These variations are, however, not necessarily linked to sleep. Sleep exhibits circadian rhythm and the circadian pacemaker may also modulate hormone production and/or release. In this case, the plasma concentrations of a particular hormone co-vary with sleep but the two processes are independent. That variations in hormone secretions are in fact associated with sleep or they are subject to circadian regulation can be distinguished by means of sleep deprivation. For example, the diurnal rhythms of plasma cortisol [94] and PTH [58] are largely determined by circadian regulation and are only slight dependent on sleep (there is a sleep associated suppression in plasma cortisol concentration which is superposed on the circadian rhythm). Melatonin is perhaps the best-known hormone secreted during the dark phase. Melatonin secretion is normally synchronized with sleep in humans. If sleep is, however, shifted to the light period then sleep is not associated with melatonin release [1]. In

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contrast, a major GH surge occurs during deep NREMS [92]. During sleep deprivation, GH secretion is suppressed or greatly attenuated (a small circadian component has also been demonstrated for GH release) and the major GH surge shifts to the new bed time after sleep deprivation [90]. Prolactin also displays sleep (NREMS)-associated secretion [75,83].

Theoretically, there might also be differences in hormone secretions between NREMS and REMS. In fact, secretions of the anterior pituitary hormones tend to decrease during REMS. We think that this phenomenon has a circulatory explanation: there is a shift from carotid to vertebral perfusion of the brain during REMS [3] and the decreased pituitary blood flow may be associated with a decline in systemic hormone concentrations. Whatever the mechanism, the sleep state-related variations in pituitary hormones are very weak. Currently, changes in PRA are the only documented hormonal variations which thoroughly follow the ultradian rhythm of NREMS-REMS in humans [16].

Compared to wakefulness, PRA increases during NREMS irrespective of the time of the day when NREMS occurs [15]. Then, PRA decreases during REMS. The REMS-associated suppressions in PRA are very obvious; if serial PRA values are available it is possible to determine the periods of REMS without looking at the polygraph records. Changes in plasma aldosterone concentrations follow the variations in PRA with a time lag of 20 min [13,46]. The mechanism and the biological significance of these PRA changes are not known.

Sleep-associated variations in hormone secretions are generally studied in humans. GH is an exception; the relationship between GH and sleep has been documented in various species [68]. In addition, we are aware of a single report which describes a negative correlation between plasma corticosterone and NREMS in the rat [56]. That the animal data are scarce is perhaps due to technical difficulties. The blood samples have to be withdrawn without disturbing the animals. The usual laboratory animals (for which RIA kits are available) are small and the blood volume for repeated sampling is limited. The sleep cycles are also short: the duration of sleep cycle is between 60 and 90 min in humans whereas it is approximately 10 min in the rat. Nevertheless, experiments on animals are important because they may provide a model where the mechanism of the sleep-linked hormonal alterations can be

studied. We were particularly interested in PRA because sleep and wakefulness are normally associated with significant changes in posture and hemodynamics in humans unlike in rats, and these changes may greatly influence the release of renin, a hormone which has a fundamental role in the regulation of circulation. Large oscillations and a tonic rise in blood pressure are observed during REMS in humans [53]. In cats [34] and pigs [70], blood pressure decreases in REMS. In addition to measuring PRA, the blood pressure of the rats were also recorded in our experiments.

1.3. *Effects of hormones on sleep: the somatotropic axis in sleep regulation*

Pituitary GH secretion is stimulated and inhibited by two hypothalamic neurocrines, GHRH and somatostatin, respectively. GH acts in part directly and in part indirectly via IGF-1 in the tissues. IGF-1 is both a paracrine/autocrine produced locally and a hormone released from the liver in response to GH. The sleep-related GH secretion was discovered in humans in the late 60's [75,88]. The major daily GH release occurs soon after sleep onset in association with deep NREMS. Sleep and GH secretion may, however, sometimes dissociate. It was suggested that there are no direct causal relationships between GH secretion and NREMS. Instead, a common mechanism may stimulate both events simultaneously [69]. GHRH was isolated, characterized, and synthesized in the early 80's [35,74]. This discovery initiated intensive studies aiming to determine whether GHRH is involved in sleep regulation. A brief summary of the results is as follows.

Icv administration of GHRH elicits dose-related enhancements of sleep in rats and rabbits [28,59,66,68]. In general, GHRH stimulates predominantly NREMS. The effects of GHRH on NREMS are fairly rapid and short lasting (1 h). REMS is also stimulated in rabbits but this occurs after longer latency (in hour 2 postinjection). The changes in REMS are not consistent after icv GHRH in rats. GHRH increases sleep after intravenous administration in humans [36,45,84,85] and rats [62]. Decreases in sleep are observed in rats in response to inhibition of endogenous GHRH by means of a competitive antagonist [67], immunoneutralization [65], or in mutant rats with GHRH receptor deficiency [31]. Hypothalamic GHRH mRNA and GHRH contents vary with sleep-wake activity [20]. Sleep deprivation stimulates GHRH release [32] and synthesis [89,98], and GHRH is involved in the mediation of the enhancements in sleep

following sleep deprivation [65]. Hypophysectomy does not block the increases in NREMS elicited by exogenous GHRH [62] indicating that GH is not involved in the mediation of the NREMS-promoting activity. Microinjection of GHRH into the medial preoptic region is followed by large increases in NREMS whereas a GHRH antagonist administered into the same area inhibits NREMS [100]. The preoptic region is known to be involved in sleep regulation [87], and it also receives projections from extraarcuate GHRHergic neurons [77]. It is assumed, therefore, that the preoptic region is the somnogenic action site of GHRH. GHRH applied into the preoptic region fails to alter REMS [100]. Stimulation of REMS is regarded as an indirect effect of GHRH which might involve GH for hypophysectomy blocks the REMS-promoting activity of GHRH [62].

In addition to GHRH, other members of the somatotropic axis may also modulate sleep. Acute administrations of GH elicit increases in REMS in humans [55], rats [27], and cats [86]. Chronic overproduction of GH is associated with enhancements of EEG slow wave activity in humans [2], and immunoneutralization of GH is followed by decreases in sleep in the rat [61]. Icv administered IGF-1 has a weak sleep-promoting activity in the rat [63]. Finally, somatostatin is reported to stimulate REMS [26]. The sleep-promoting activity of GH/IGF-1 is attributed to some metabolic actions and may vary with the age and species.

GH, IGF-1, and somatostatin, however, are also part of the various negative feedback loops inhibiting GHRH. Thereby, they may also suppress sleep. In fact, an acute large dose of GH decreases NREMS [55,86]. The GH-induced inhibition of GHRH is exploited in transgenic dwarf mice: in these animals, tyrosine hydroxylase positive neurons express human GH, and the human GH released in the hypothalamus inhibits GHRH. NREMS is chronically decreased in these mice [96]. High icv doses of IGF-1 elicit prompt inhibition of both GH secretion and NREMS [64]. It is a subject of controversy whether GH/IGF-1 can directly inhibit GHRH or the effect is mediated via somatostatin. GH and IGF-1 stimulate somatostatin [6,8], and hypothalamic somatostatin inhibits both GH and GHRH [43,96].

The aim of our experiments was to determine the effects of exogenous somatostatin on sleep in rats. As reviewed in the papers attached, previous reports are inconsistent on the sleep

effects of somatostatin. Some authors found decreases in sleep [38] whereas Danguir's group described selective promotion of REMS by somatostatin in the rat [23]. Instead of somatostatin, we used a long acting analog, OCT. Somatostatin is eliminated in a few minutes whereas the half-life of OCT is between 1 to 2 h in the tissues [5]. Five somatostatin receptor types have been cloned. OCT is a strong agonist on the sst2 and sst5 receptors, and it binds weakly to sst3 receptors [73]. Inhibition of the somatotropic axis is attributed to the sst2 receptors [73]. OCT is also used in clinical practice for chronic treatment of GH producing tumors. It was of interest, therefore, whether OCT may influence sleep as a side effect of the treatment.

1.4. *Aims*

The experiments reported herein were carried out in rats and aimed to study:

1. Sleep-associated variation in PRA (Paper #1),
2. Changes in blood pressure during sleep (Paper #1),
3. The effects of systemic OCT on sleep and plasma GH (Paper #2),
4. The effects of icv OCT on sleep and GH (Paper #3),
5. The behavioral effects of icv OCT, and the role of these behavioral responses in the OCT-induced sleep alterations (Paper #3).

The experiments, including the methods, results and discussion of the data are reported in detail in the Papers attached. This essay is to provide a broad background and a perspective for our research.

2. METHODS

2.01. *Animals:* Male Wistar and Sprague-Dawley rats were used in the experiments with PRA determinations and OCT, respectively. The rats weighed 300-350 g.

2.02. *Surgical procedures:*

a) Anesthesia: a mixture of ketamine-xylazine (87 and 13 mg/kg, respectively) was injected intraperitoneally.
b) Electrodes and thermistor: Stainless-steel jewelry screws were implanted over the frontal and parietal cortices and over the cerebellum. A thermistor was placed over the parietal cortex to record cortical brain temperature.
c) Icv cannula: A guide cannula was implanted into the left cerebral ventricle. The location of the icv cannula was verified by examining the intracerebral distribution of Trypane blue injected at the conclusion of the experiments.
d) Cardiac catheter: For blood sampling, a Silastic catheter was implanted into the right atrium via the external jugular vein.
e) Aortic catheter: a chronic catheter (Silastic) was inserted into the descending aorta via the left arteria carotis communis. This catheter was used to measure blood pressure.

2.03. *Recording:* The rats were adapted to a 12 : 12 h light:dark cycle. They were housed in individual Plexiglass cages. The temperature in the recording rooms was regulated as indicated in the Papers attached (25-26 °C). Food and water were continuously available. The rats were connected to recording tethers. The tethers were attached to commutators. The motor activity was assessed by recording potentials generated in electromagnetic transducers attached to the tethers.

The EEG, motor activity, and brain temperature signals were digitized, collected by computers and stored on compact disks or video tapes in the experiments with OCT. The same system was also used to record sleep-wake activity and blood pressure. In the experiments with PRA measurements, sleep-wake activity together with marks indicating blood withdrawals and the behavior of the rats were recorded on a paper chart. The rats were watched via a closed-circuit television system.

2.04. *Determination of the states of vigilance:* Wakefulness, NREMS and REMS were distinguished according to usual criteria. The states of vigilance were scored for 8-s epochs in the experiments with OCT injections and blood pressure recording, and for 10-s epochs when PRA was determined.

2.05. *Quantitative analysis of the depth (intensity) of NREMS:* In the experiments with OCT, power density spectra were calculated by means of fast-Fourier transformation of the EEG for 8-s epochs. Mean power density spectra were determined for 8-s uninterrupted periods of artifact-free NREMS in each hour. The power density values in the delta range (0.25-4 Hz) were integrated and used to characterize EEG slow wave activity during NREMS.

2.06. *Processing of temperature data:* Brain temperature data recorded at 8-s intervals were averaged for 1-h periods.

2.07. *Injections:* Sc injections (0.1 ml / 100 g b.w.) were administered in the back of the neck. The volume of icv injections was invariably 2 μl.

2.08. *Blood sampling:* A Silastic tubing was connected to the intracardiac catheter and the tubing was routed through the wall of the chamber. The rats were free to move. The volume of each sample was 0.2-0.3 ml in the experiments for PRA measurements, and 0.1-0.2 ml when GH was determined. EDTA was used as anticoagulant in the experiments with PRA measurements, and therefore, the red blood cells could not be reinjected. In these experiments, physiological saline was used to replete the lost blood volume. In the case of GH measurements, the rats were heparinized. The samples were centrifuged and the red blood cells reinjected.

2.09. *Determination of PRA and GH:* PRA was determined by Dr. G. Brandenberger (Strasbourg, France) whereas GH was measured by Drs. J. Gardi and P. Taishi (Pullman, USA) by means of radioimmunoassay.

2.10. *Measurement of blood pressure:* A Silastic tubing was attached to the aortic catheter. The free end of the tubing was connected to a pressure transducer. The signals were collected by a computer together with the EEG and brain temperature. Mean blood pressure values were calculated for 8-s epochs.

2.11. *Behavioral testing:* In the experiments with icv OCT, the behavior of the rats was scored at 30-s intervals as drinking, feeding, grooming, scratching, exploration and lying for 45 min postinjection. The scores for grooming, scratching and exploration were pooled as general activity.

2.12. *Experimental protocols.* In general, OCT was injected in various doses immediately prior to the 12-h light period and sleep-wake activity during the light period was analyzed. Baseline recordings were obtained after administrations of vehicle, physiological saline, or a control solution. The treatment effects were evaluated with respect to the baseline day. This protocol was also used when the effects of OCT on GH were studied. In the experiments with PRA measurements, blood samples were withdrawn from consecutive NREMS and REMS epochs while the state of vigilance was continuously monitored. Sleep-associated variations in blood pressure were determined from 6-8-h records collected during the second portion of the light period.

2.13. *Statistics.* The major tests used included ANOVA and the non-parametric Friedman test. Statistics are listed in detail in the Papers attached.

3. RESULTS

3.1. *Sleep wake activity and PRA oscillations*

Sleep-associated variations in PRA could be studied in 4 consecutive samples, thereafter PRA rose irrespective of the state of vigilance. To determine whether the order of the samples influenced PRA, blood collection started in NREMS in one group of rats, and in REMS in another group. Hence, the sampling order was NREMS-REMS-NREMS-REMS and REMS-NREMS-REMS-NREMS in the two groups. The time interval between two consecutive blood withdrawals was approximately 6 min which corresponded to a 12-min cycle duration. The sleep cycles were determined prior to the blood sampling for each rat.

PRA increased in NREMS and decreased in REMS in both groups of rats (Paper #1, Fig. 1). The difference in PRA was statistically significant between NREMS and REMS. PRA was determined in a few samples collected during wakefulness: these PRA values were also significantly higher than PRA during REMS.

3.2. *Sleep-wake activity and blood pressure*

The major finding of our experiments is the demonstration of characteristic changes in blood pressure during REMS in the rat. These changes included large oscillations superimposed on a tonic rise. REMS was followed by a prompt and significant drop in blood pressure. The REMS-associated changes in blood pressure were so marked that REMS periods could be identified by inspecting the blood pressure curves (Paper #1, Fig. 2). The mean blood pressure was significantly higher during REMS than blood pressure in NREMS. Blood pressure rose during long periods of wakefulness whereas short arousals were associated with decreases in blood pressure. Since long wake periods were relatively scarce in our records, significant differences were not detected in the mean blood pressure between wakefulness and NREMS.

3.3. *Experiments with somatostatinergic stimulation*

Systemic (sc) administration of OCT is used in clinical practice for treatments of pituitary tumors, particularly somatotropinoma. We, therefore, felt important to study the effects of systemic OCT on sleep as a first step in our experiments. Both synthesis and release of GH

are suppressed after repeated administration of OCT [49]. This protocol was applied to study sleep in GH deficiency. To determine whether systemic or intracerebral action sites mediate the OCT-induced sleep alterations, sleep was also recorded after icv OCT. Plasma GH concentrations were measured after both systemic and icv OCT and used as a marker of the activity of the somatotropic axis. Systemic OCT occasionally, and icv OCT regularly elicited prompt drinking, a response characteristic to angiotensin. The role of intracerebroventricular angiotensin in the behavioral and sleep responses to OCT was studied by means of pretreating the animals with the angiotensin-convertase inhibitor, captopril, prior to icv OCT injections.

3.31. *Changes in sleep after systemic injection of OCT*

OCT was sc injected in doses as follows: 1 μg/kg, 10 μg/kg, and 200 μg/kg. A group of rats received a control solution previously reported in the literature [24]. NREMS was significantly suppressed after 10 and 200 μg/kg OCT in hour 1 postinjection (Paper #2, Fig. 1). This was followed by a tendency to increases in NREMS duration, and as a result, NREMS was not altered when calculated for the 12-h light period. SWA during NREMS enhanced significantly starting in hour 3 postinjection. REMS increased slightly after each dose of OCT without significant differences among the doses. Sleep returned to normal after 12 h, and it was not altered during the 12-h dark period (Paper #2, Table 1). Systemic OCT had no effects on brain temperature.

3.32. *Effects of systemic OCT on GH*

GH was determined in samples obtained at 30 min intervals after systemic injection of physiological saline and 200 μg/kg OCT. OCT suppressed plasma GH for 2 h. A recovery of GH secretion was observed between hours 2.5 and 4 postinjection (Paper #2, Fig. 2).

3.33. *Changes in sleep in response to repeated administration of OCT*

Two groups of rats were used. Injections were performed twice a day, before light and dark onset. Both groups received physiological saline for 5 days, and the record on day 5 served as baseline. Then, one group of rats was injected with 10 μg/kg OCT twice a day for 5 days whereas the other group received the control solution. Both groups were recorded on day 1 and day 5 of the injections of OCT or control solution.

Repeated administration of OCT induced permanent decreases in the duration and intensity (SWA) of NREMS on day 5 (Paper #2, Table 2, Fig. 3). OCT suppressed NREMS in hour 1 postinjection on both days 1 and 5 but the decreases in NREMS were more pronounced on day 1 than on day 5 of the treatment. REMS increased on day 1 and failed to change on day 5.

3.34. *Changes in sleep after icv OCT*

Four groups of rats were used. They received 0.001 μg, 0.01 μg, and 0.1 μg OCT, or the vehicle in a dilution corresponding to that of 0.1 μg OCT. Sleep-wake activity after OCT or the vehicle was compared to records after icv physiological saline in each group.

The changes in NREMS after icv OCT were similar as the sleep alterations found after systemic OCT (Paper #3 Fig. 1). Significant decreases in NREMS were observed in hour 1 postinjection after 0.01 and 0.1 μg OCT. In response to 0.1 μg OCT, NREMS continued to decrease for 2 hours. SWA in NREMS enhanced dose-dependently in hour 3 postinjection, and the increases in SWA were significant for the entire 12-h light period after the high dose of OCT.

In contrast to the systemic OCT, REMS did not change after icv OCT. Brain temperature rose following both icv physiological saline and OCT indicating a febrile response to the procedure of icv injection.

3.35. *Effects of icv OCT on GH*

Changes in GH secretion were studied after 0.01 and 0.1 μg OCT. Both groups of rats received icv physiological saline on the baseline day. OCT inhibited GH secretion for 60 to 90 min. GH releases, however, occurred 90 and 120 min postinjection in each rat injected with 0.01 and 0.1 μg OCT, respectively (Paper #3 Figs. 2 and 3).

3.36. *Captopril-sensitive behavioral responses to OCT*

Icv administration of OCT regularly elicited drinking. The behavioral responses to icv OCT (0.1 μg) were quantified in 12 rats. In addition, OCT was also tested in these rats after

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pretreatment with the angiotensin-convertase inhibitor, captopril (30 μg). The rats received double injections as follows: physiological saline + physiological saline, physiological saline + OCT, and captopril + OCT on three different days. Fifteen minutes elapsed between the two injections. Captopril or the vehicle did not influence behavior.

Fig. 4 in Paper #3 depicts the cumulative occurrences of 4 types of behavior at 30-s intervals during 45 min postinjection. OCT elicited drinking in each rat during 10 min postinjection. This was followed by significant increases in the occurrences of feeding. Lying decreased after OCT. Pretreatment with captopril inhibited the OCT-induced behavioral responses. Behavioral scores did not differ after double injections of physiological saline and captopril + OCT.

3.37. *OCT-induced sleep alterations after pretreatment with captopril*

Sleep-wake activity was recorded in a group of rats after double injections with physiological saline, captopril (30 μg) + physiological saline, and captopril + 0.1 μg OCT (Paper #3, Fig. 5). Captopril did not alter sleep. The effects of OCT injected after captopril were undistinguishable from the changes in sleep elicited by OCT without captopril (Paper #3, Fig 1). OCT continued to suppress NREMS in hours 1 and 2 postinjection, and SWA during NREMS enhanced in hour 3 and thereafter. REMS and brain temperature did not differ among the 3 treatments. Icv injections elicited febrile responses on each day of the experiment.

4. DISCUSSION

4.1 *Sleep-associated variations in PRA and blood pressure*

Our findings in rats confirm the sleep state-associated ultradian rhythm of PRA previously reported in humans. Although the sampling interval was as short as 6 min between NREMS and REMS, and the number of samples were limited by the blood loss, PRA in NREMS and REMS differed significantly. The state-associated variations in PRA were independent of the order of sampling. PRA is determined as the quantity of angiotensin generated in unit time. Since angiotensinogen is present in excess, PRA is dependent on the plasma concentration of renin. The concentration of renin is the function of the rates of secretion and elimination. The plasma half-life of renin has a rapid initial component (approx. 12 min) and a slow delayed component (approx. 60-120 min). The relatively short half-life of renin makes it possible that PRA is regulated in a minute-to-minute time scale via renin release. The current results, therefore, indicate that secretion of renin decreases in REMS and increases in NREMS.

Brandenberger's group measured PRA in a number of conditions with altered renin secretion in humans. The amplitude of PRA oscillations increase when the subjects are exposed to heat [14], in response to low sodium diet or furosemide, and decrease after administration of beta-1 blockers [18]; but the rhythm continues as long as PRA is detectable. The ultradian rhythm of PRA can also be demonstrated in hypertensive patients [17]. Variations in PRA can result from two processes: enhanced releases of renin during NREMS and/or suppressed renin secretion during REMS. Compared to wakefulness, PRA increases during sleep, and the period of increased PRA is shifted to the daylight hours if the subjects are awake at night and sleep during the day [15]. Selective deprivation of REMS fails to block rises of PRA during deep NREMS [12]. Finally, PRA correlates with SWA during NREMS in humans [51]. Collectively, these findings strongly suggest that NREMS is associated with enhanced renin secretion. The fact that we did not find higher PRA in NREMS than in wakefulness in the rat does not contradict the reports in humans. Our experiments were designed to measure PRA during the sleep cycles, and the few occasions when blood samples were taken during wakefulness were in fact short arousals. Stimulation of renin secretion, however, is not a

direct function of NREMS for enhancements in NREMS elicited by administration of the 5-HT2 receptor antagonist, ritanserin, are not associated with increases in PRA [19].

In contrast to NREMS, REMS is associated with decreases in renin secretion. The changes in blood pressure during REMS may provide a cue for an understanding of the mechanism of suppression of PRA.

Systemic blood pressure decreases during NREMS in humans [93]. Compared to wakefulness, blood pressure also tends to decrease in the rat [79]. Our records contained relatively few long wake epochs and the difference in blood pressure between NREMS and wakefulness could not be demonstrated (short arousals were characterized by decreases rather than increases in blood pressure). REMS is associated by large oscillations and a tonic rise in blood pressure in humans [82,93]. As reviewed in Paper #1, changes in blood pressure during REMS may vary with the species; often tonic decreases in blood pressure are reported in animals. It seems, however, that the methodology may also influence the actual findings. In cats, blood pressure decreases during REMS on the first days after implantation of the catheter and this is followed by REMS-associated increases in blood pressure under chronic conditions [78]. Our results confirm previous [48,101] and recent observations in rats [79] and indicate that this species provides a useful model for sleep-associated circulatory changes in humans. It cannot be excluded, however, that there might be differences among rat strains: blood pressure tends to decrease during REMS in Sprague-Dawley rats [71] at variance with the observations in Wistar rats.

The fall in blood pressure during NREMS is attributed to a decreased sympathetic discharge and a consequent vasodilation [4,21,81]. The tonic rise in blood pressure during REMS is the function of the posterior hypothalamus [42] whereas the rapid oscillations seem to be related to the activity of the limbic system [80]. Sympathetic activity is greatly enhanced in REMS in both humans [40,81,82] and Wistar rats [79]. Sympathetic stimulation of the arterioles results in vasoconstriction predominantly in the skeletal muscles in REMS. The renal vessels may actually dilate [52]. The importance of sympathetic activity in the REMS-associated increases in blood pressure was verified in our laboratory: guanethidine-induced sympathectomy or

suppression of sympathetic discharge as a result of acute hypertension abolishes increases of blood pressure during REMS [9].

It is proposed that the sleep-associated variations in PRA are determined by changes in blood pressure during both NREMS and REMS. Decreases in blood pressure stimulate increased renin secretion during NREMS whereas the REMS-associated rise in blood pressure suppresses renin release. Thus, both sleep states contribute to the development of the PRA rhythm. The physiological significance of the oscillations in PRA is that they reflect the relative contribution of the renin-angiotensin system to the maintenance of blood pressure. In NREMS, the sympathetic vasoconstrictor tone decreases and the importance of the humoral regulation of blood pressure enhances. In contrast, the nervous control of blood vessels dominates during REMS.

4.2. *Effects of somatostatinergic stimulation on sleep*

The experiments reported herein represent the first steps in a series of studies aiming to determine whether somatostatinergic inhibition of the somatotropic axis can modulate sleep. Two approaches were used: changes in sleep were studied after acute and repeated OCT administrations. Systemic injections of OCT for 5 days cause depletion of GH [49], i.e. GH deficiency. The permanent suppression of the duration and intensity of NREMS observed on day 5 corresponds to previously reported findings after selective inhibition of GH [61]. In addition, the responsiveness to OCT was also attenuated on day 5. This might result from a down regulation of the somatostatin receptors reported previously [49].

The sleep response to acute OCT injection was in the focus of our experiments. OCT elicited biphasic changes in sleep: an immediate NREMS suppression followed by enhancements in SWA starting 2 to 3 h postinjection. Stimulation of SWA is a sign of sleep promotion. OCT, therefore, first inhibits then increases NREMS, but the second phase occurs only if the dose is high. The relatively brief sleep suppressing effect of OCT explained why some of the previous studies using long time blocks for analysis [23,24,25,26] failed to notice this action. *It is a major finding of our studies that systemic and icv injections of OCT elicited the same type of alterations in NREMS.* It is, therefore, concluded that intracerebral actions sites

mediate the OCT-induced changes in NREMS. The sleep suppressive activity of OCT might have practical significance. Frieboes et al. [30] report that systemic administration of somatostatin impairs sleep in old human subjects. Although the cause has not been elucidated, 5 out of 7 patients complained about sleep impairment in a survey of the patients receiving chronic OCT treatment for somatotropinoma at the Endocrine Unit of the A. Szent-Györgyi Medical University [7]. The effects of systemic and icv OCT were different on REMS. REMS increased after systemic OCT and did not change significantly after icv OCT. The icv injections, however, elicited fever which suppresses REMS. In fact, a tendency to slight increases in REMS could be noted after the highest dose of icv OCT. The mechanism of somatostatinergic modulation of REMS is not clear. Dose-effect relationships could not be detected after systemic OCT which might indicate an indirect action. The brain stem was suggested to mediate the effect of somatostatin on REMS [25]. If this is correct then the changes in REMS after OCT or somatostatin are independent of the somatotropic axis.

Somatostatin inhibits hypothalamic GHRHergic neurons. Theoretically, therefore, the OCT-induced NREMS suppression might result from an inhibition of GHRH. Somatostatin is, however, a widely distributed neurotransmitter in interneurons in various structures of the central nervous system [41]. OCT may, therefore, activate mechanisms, e.g. drinking and feeding, which are unrelated to the somatotropic system and may interfere with the sleep process. Although it is not reported herein, OCT also elicits vasopressin release and a rise in blood pressure [37]. The triad consisting of drinking, vasopressin secretion, and increases in blood pressure, is a characteristic response to intracerebral administration of angiotensin II. Pretreatment with captopril inhibited all these responses (including vasopressin release and the rise in blood pressure) [37]. Saralasin, an angiotensin receptor antagonist, also inhibits OCT-induced drinking [37]. These findings indicate that somatostatinergic stimulation activates intracerebral angiotensinergic mechanisms. Captopril, however, failed to inhibit the effects of OCT on NREMS. The changes in sleep, therefore, are not mediated by angiotensin or angiotensinergic behavioral responses. OCT is also a partial agonist on μ-opiate receptors [73] and opiates can modulate sleep [33]. Further experiments are required to elucidate whether μ-receptors are involved in the sleep effects of OCT.

There is a correlation between the sleep alterations and the variations in somatotropic activity after OCT. When NREMS is suppressed, GH secretion is also inhibited. The late increases in the intensity of NREMS occur when the inhibitory effect of OCT on GH declines. Somatostatin is involved in negative feed back loops which mediate GH- and IGF-1-induced inhibition of GHRH. The sleep alterations elicited by OCT are similar to those observed after intracerebral administration of a high dose of IGF-1 [64]: IGF-1 also elicits prompt suppressions of GH secretion and NREMS but injection of IGF-1 fails to evoke the behavioral responses observed after OCT. Also, NREMS tends to increase when GH secretion recovers after IGF-1. Our hypothesis is that the late increases in NREMS intensity observed after OCT (and IGF-1) might result from rebound-like enhancements in the activity of the sleep-promoting GHRHergic system. Further experiments in our laboratory aim to address this issue.

Sleep deprivation enhances GHRH mRNA levels and decreases somatostatin mRNA expression in hypothalamic samples [98]. The sleep-dependent variations in GHRH and somatostatin suggest a reciprocal interaction between these peptides in sleep regulation similar to their interaction in endocrine regulation.

There are two pools of GHRH-containing neurons in the hypothalamus [22,77]. The majority of the hypophyseotropic GHRHergic neurons resides in the arcuate nucleus. The arcuate nucleus also contains somatostatinergic interneurons which inhibit the intraarcuate GHRHergic neurons [50]. In correlation with the pulsatile GH secretion, GHRH and somatostatin mRNA levels vary reciprocally in the arcuate nucleus [97]. The intraarcuate GHRH and somatostatin, however, do not exhibit circadian variations, and instead of an inhibition, sleep deprivation stimulates intrarcuate somatostatin [89]. It is likely that these neurons are not involved in sleep regulation.

GHRHergic neurons dispersed in the periventromedial area and in the parvicellular portion of the paraventricular nucleus project to the basal forebrain, the sleep-promoting action site of GHRH, and they may also modulate GH secretion [22,77]. GHRH mRNA expression is stimulated by sleep deprivation and vary with the diurnal rhythm in these neurons [89].

Promotion of NREMS is attributed to the activity of the extra-arcuate GHRHeric neurons. In addition to the intraarcuate somatostatinergic neurons, regulation of GH secretion also involves the hypophyseotropic somatostatinergic neurons in the periventricular nucleus. These neurons project to the median eminence and they are not responsive to sleep deprivation [89]. Therefore, currently it is not known where the somatostatinergic neurons reside which are involved in sleep regulation via possible interaction with the extra-arcuate GHRHergic neurons.

In conclusion, our experiments indicate that somatostatin, which provides an inhibitory control for the somatotropic axis, also suppresses NREMS. This observation is in agreement with the findings that negative feed backs in the somatotropic system, i.e. high GH and IGF-1 concentrations, inhibit both GH secretion and sleep. Although regulation of GH secretion and NREMS are subjected to the same feed backs, the neuronal networks of these functions are separable. In addition, current results indicate that somatostatin also controls the intracerebral angiotensinergic system and thereby it is involved in the regulation of water balance. This is a somatostatinergic function which is independent from sleep regulation.

SUMMARY

Sleep and endocrine regulation interact. There are two aspects of this interaction: *i)* sleep may modulate hormone secretions, and *ii)* hormones may influence sleep. Our work addressed both issues. *Ad i)* Sleep-associated variations in PRA were studied in rats as an example for hormone secretions modulated by sleep. *Ad ii)* The somatotropic axis is a major hormone system which influences sleep-wake activity. Hypothalamic GHRH is a well-documented sleep-promoting substance. Somatostatin mediates negative feed back in the somatotropic axis by inhibiting both GHRH and GH. The aim of our experiments was to study the effects of a long-acting somatostatin analog, OCT, on sleep in the rat. *Results: i)* PRA decreases in REMS and increases in NREMS in rats confirming previous observations in human subjects. These changes in PRA are inversely related to variations in systemic blood pressure. *ii.* Systemic and icv administrations of OCT elicit prompt suppressions in NREMS and late, rebound-like increases in the intensity of NREMS 2 to 3 h postinjection. OCT also stimulates drinking and feeding. The behavioral responses are blocked by icv captopril indicating the involvement of intracerebral angiotensin. The effects of OCT on sleep, however, are not altered by captopril. The OCT-induced suppression in NREMS correlates with the inhibition of GH-secretion, and the late increases in NREMS intensity occur when the inhibition of GH secretion vanishes. *Conclusion:* The sleep-associated variations in PRA provide an example for a hormone secretion indirectly modulated by sleep. It is assumed that the observed changes in renin secretion are elicited by changes in blood pressure due to sleep-associated variations in the sympathetic vasoconstrictor tone. In contrast, the somatotropic axis is involved in the regulation of sleep. Together with previous observations, the current findings indicate that the negative feed back mediated by somatostatin not only inhibits somatotropic activity but also suppresses sleep. Accidentally, our experiments revealed that somatostatin may be involved in the control of central angiotensinergic mechanisms regulating water and electrolyte balance. This function of somatostatin is independent from the somatotropic axis and sleep regulation.

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