Pharmacology Biochemistry and Behavior, Vol. 66, No. 4, pp. 721–727, 2000

0091-3057/00/$–see front matter

ELSEVIER

PII S0091-3057(00)00242-2

Intracerebroventricular and Locus Coeruleus Microinjections of Somatostatin Antagonist Decrease REM Sleep in Rats

JUSSI TOPPILA, PIA NIITTYMÄKI, TARJA PORKKA-HEISKANEN AND DAG STENBERG

Institute of Biomedicine, Department of Physiology, P.O. Box 9, FIN-00014 University of Helsinki, Helsinki, Finland

Received 02 November 1999; Revised 17 January 2000; Accepted 31 January 2000

TOPPILA, J., P. NIITTYMÄKI, T. PORKKA-HEISKANEN AND D. STENBERG. *Intracerebroventricular and locus coeruleus microinjections of somastatin antagonist decrease REM sleep in rats.* PHARMACOL BIOCHEM BEHAV **66**(4) 721–727, 2000.—In order to study the role of endogenous somatostatin in the physiologic modulation of REM sleep (REMS), we measured the effect of intracerebroventricular (ICV) injection of somatostatin antagonist (SA) cyclo-(7-aminoheptanoyl-phe-d-trp-lys-thr(bzl)) on sleep in rats. The effect of ICV SA was also tested after 24-h REMS deprivation with the platform method. To study the role of locus coeruleus (LC) as a site of the sleep inducing action for somatostatin and galanin we microinjected SA, somatostatin, and galanin locally into LC. In all experiments, vigilance state was analyzed visually from 6 h post-injection EEG/EMG recording. Injection of 0.5 and 2 nmol of SA ICV reduced spontaneous REMS and 2 nmol dose reduced also rebound REMS after REMS deprivation when compared with controls (artificial cerebrospinal fluid vehicle). Microinjection of 0.25 nmol of SA into LC reduced REMS, whereas microinjection of somatostatin, galanin, and a combined injection of them were not effective to induce REMS. The results suggest that endogenous somatostatin may contribute to facilitation of REMS. Somatostatin receptors in the LC may be one possible mediator of this effect. © 2000 Elsevier Science Inc.

Cyclo-(7-aminoheptanoyl-phe-d-trp-lys-thr(bzl)) Galanin Neuropeptide REM sleep deprivation

PITUITARYgrowth hormone (GH) secretion is strongly associated with deep slow wave sleep (SWS) phases in several mammalian species. This may indicate that GH controlling hypothalamic neuropeptides growth hormone-releasing hormone (GHRH) and somatostatin are connected to the sleep regulatory system in the brain (45). The facilitatory effect of GHRH on SWS is well documented in humans and laboratory animals (reviewed in 24,38), but the possible effect of somatostatin on sleep is less clear. More than a decade ago it was found that long-term intracerebroventricular (ICV) infusion of somatostatin increased and depletion of endogenous somatostatin by cysteamine decreased rapid eye movement sleep (REMS) in rats (4). Subsequent studies revealed that intraperitoneal (IP) or subcutaneous (SC) injection of the long-acting somatostatin analog octreotide (SMS 201-995) caused a delayed facilitation of REMS in rats (3,6). IP injection of octreotide also counteracted the suppression of REMS caused pharmacologically by scopolamine (6) or by desipramine (8). Immunoneutralization of endogenous somatostatin by ICV infusion of somatostatin antibody decreased REMS (5) and microinjection of somatostatin antibody into the nucleus of the solitary tract antagonized carbachol-induced REMS in rats (7).

According to previous animal studies it is possible that somatostatin facilitates particularly REMS. Also in human studies, repeated IV somatostatin boluses had a tendency to increased REM density in young subjects (37), whereas in elderly subjects the same treatment decreased REMS and fragmented sleep structure (9). The site of action of somatostatin in the brain has not been identified. REMS is controlled by reciprocal activity of monoaminergic (locus coeruleus and raphe nuclei) and cholinergic (laterodorsal and pedunculopontine tegmental) nuclei located in the brainstem (13). Locus coeruleus (LC) has a dense expression of somatostatin receptors, which mediate inhibition of neuronal activity by membrane hyperpolarization (15,30). The activity of these neurons decrease during non-REM sleep (NREMS), and during REMS the firing ceases almost completely (1,13).

Requests for reprints should be addressed to Jussi Toppila, Institute of Biomedicine, Department of Physiology, P.O. Box 9, FIN-00014 University of Helsinki, Finland, Tel.: +358 9 1918565; Fax: +358 9 1918681; E-mail: Jussi.Toppila@helsinki.fi

We have previously shown that both selective REMS deprivation (REMSD) and total sleep deprivation (TSD) increased somatostatin mRNA in the rat hypothalamus measured by in situ hybridization (42,43). Increased amount of somatostatin mRNA may be a sign of enhanced production of somatostatin in the hypothalamus when sleep and particularly REMS is inhibited. If endogenous somatostatin regulates sleep, blocking the somatostatin receptors by somatostatin antagonist (SA) should reduce the amount of REMS during spontaneous sleep and during rebound sleep after REMSD.

The neuropeptide galanin is expressed in the same brain areas that are known to regulate sleep and wake, e.g., preoptic area, hypothalamus, and brainstem nuclei (20,35). Galanin increases GH secretion from the pituitary probably by influencing the GHRH-somatostatin system in the hypothalamus (26,40). Thus galanin may affect sleep by itself and/or via the GH regulatory system. In humans repeated IV boluses of galanin increased REMS during the late sleep phase and increased delta and theta activity in the EEG without affecting nocturnal plasma GH content (23). We have found earlier that 24 h REMSD increased galanin mRNA in the hypothalamus, although ICV injection of galanin did not modify daytime or nighttime sleep in the rat (44). The post-synaptic effect of galanin is usually inhibitory and mediated by three types of galanin receptors (GALR1-3) (36). In the LC, galanin is co-localized in noradrenergic cell somata (21). There are also galanin immunoreactive fibers (35) and galanin receptors that inhibit noradrenergic cells in the LC similarly as somatostatin (34). Presumably galanin and somatostatin have a synergistic inhibitory effect on the LC neurons and this might affect REMS.

In order to study the possible REMS-facilitatory effects of somatostatin and galanin, we performed a series of experiments to answer the following questions: 1) Does blockade of somatostatin receptors by ICV injected SA reduce spontaneous REMS? 2) Does ICV SA reduce rebound REMS after REMSD? 3) Is the effect of SA mediated by somatostatin receptors in LC? 4) Do somatostatin and galanin either alone or in combination increase REMS in the LC?

METHOD

Animal Preparation

Adult male Wistar rats weighing 290 to 390 g were implanted with two dental screw electrodes over the frontoparietal cortex for electroencephalography (EEG) and two neck muscle electrodes for electromyography (EMG) in combined medetomidine (Domitor® Orion Pharma, Espoo, Finland) (0.1 mg/kg SC) and pentobarbital (Mebunat® Orion Pharma) (30 mg/kg IP) anesthesia. A guide cannula for intracerebroventricular (ICV) injections (outer diameter 0.7 mm) was implanted into the lateral ventricle, or alternatively, a guide cannula for the microinjection experiments (outer diameter 0.4 mm) was aimed 2 mm above LC unilaterally with stereotaxic coordinates: 1.3 mm posterior to lambda, 1.5 mm laterally from midline, and 5.2 mm ventrally from the level of bregma and lambda (29). After the surgery the rats were allowed to recover 5 to 7 days before the recordings. After the operation and during the first post-operative day rats received 20 μg/kg buprenorphine SC (Temgesic®, Reckitt & Colman, Hull, England) for analgesia. During the recovery and the experiments the animals were kept in single animal boxes in 12/12 h light/dark cycle (150 lux at the level of the cage floor, lights on at 0800 h) with free access to standard rat chow and tap water. Before the experiments the placement of the ICV cannula was checked by injecting 0.2 μg of angiotensin II (Sigma Chemical Co., St. Louis, MO, USA, product: A9525) into the cannula. Animals that did not show a clear drinking response were not used in the experiments. After the experiments the cannula placements were verified by microscopy of 20 μm histologic cryosections made of the dissected and frozen brain. Animal experiment protocols and animal housing conditions were approved by the institutional experimental animal board and by the provincial administrative board in accordance with the laws of Finland and the European convention for the protection of vertebrate animals used for experimental and other scientific purposes.

Injection System

In the intraventricular injection experiments, a steel injection cannula (outer diameter 0.4 mm) extending 0.5 mm beyond the guide was used. The cannula was connected by polyethylene (PE) tubing with a 25 μl Hamilton syringe that was fitted into a microinjection pump (CMA100, Carnegie Medicin Ab, Stockholm, Sweden). In the microinjection experiments a cannula made of fused silica capillary (Composite Metal Services Ltd., The Chase, Hallow, Worcestershire, England) (outer diameter 0.12 mm, extending 2 mm beyond the guide) was connected with a 10μl Hamilton syringe in a microinjection pump. Before the injections the injection system was first filled with distilled water and then sufficient amount of injection solution was driven into the tubing separated from the water by an air bubble.

ICV Injections of SA and Somatostatin

In the intraventricular somatostatin antagonist experiment, rats (n = 5) received in a random order either 2 μl of artificial cerebrospinal fluid (aCSF) or 0.5 nmol of SA, cyclo-(7-aminoheptanoyl-phe-d-trp-lys-thr(bzl)) (Sigma, C4801) dissolved in 2 μl of aCSF or 2 nmol of SA in the same volume. In the somatostatin experiment, 5 rats received aCSF, 0.5 nmol, or 2 nmol of somatostatin (Sigma, S9129) according to the same protocol. Injections were given between 0930 and 1030 h after sleep onset and the injection lasted 4 min (0.5 μl/min). EEG and EMG were recorded for 6 h after the injection. Every rat received altogether three injections and there were at least two recovery days between the experiments for the same rats.

ICV SA Injection After REMSD

In the REM sleep deprivation (REMSD) experiment, the platform method was used to induce 24 h REMSD. Rats were deprived of REMS in single rat boxes containing approximately 5 cm of water on the bottom and two circular platforms (diameter 7 to 7.5 cm) above the water surface. Food and fresh drinking water were freely available during the experiment. At least 3 days before the first actual deprivation, rats were deprived for 12 to 14 h overnight in order to adapt them to the deprivation conditions and staying on the platforms. The 24 h deprivations started and ended at 0900 h. After the deprivation, rats were put into their normal cages and an injection of aCSF or 0.5 nmol of SA (n = 4) was given immediately after the onset of recovery sleep after the REMSD. The recovery sleep was normally initiated within 15 min after the deprivation. A dose of 2 nmol of SA (n = 5) was studied using different rats with the same protocol. Every rat received both treatments (aCSF and SA), and there were 3 to 6 nonex-

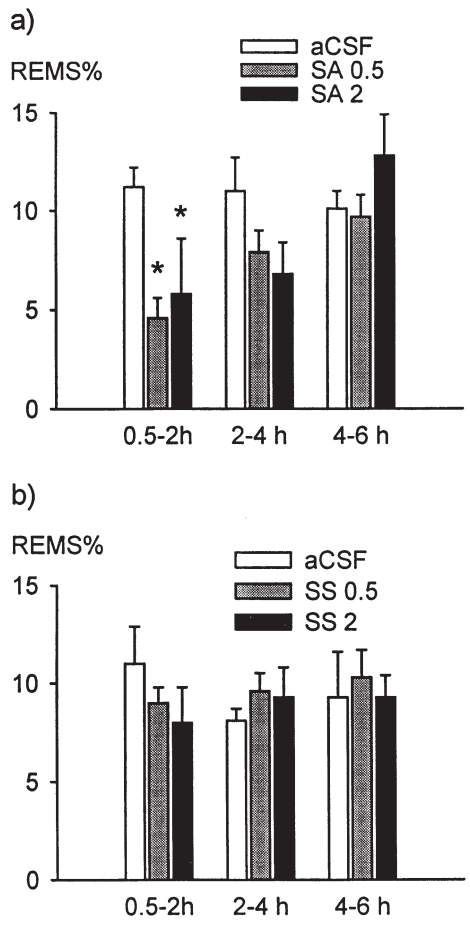

FIG. 1. The effect of ICV injected SA (a) and somatostatin (SS) (b) on mean proportions of REMS during three post-injection time periods. (a) ICV injection of aCSF (vehicle control), SA 0.5 or 2 nmol (n = 5). (b) ICV injection of aCSF, somatostatin 0.5 or 2 nmol (n = 5). Vertical bars indicate the standard error of means (SE). *RM ANOVA F (2, 8) = 4.66; $p < 0.05$, Newman-Keuls: SA 0.5 and SA 2 vs. aCSF $p < 0.05$.

periment days before the same rats were deprived and injected again.

LC Microinjections

In the first LC microinjection experiment rats (n = 8) received unilaterally 0.25 nmol of SA or somatostatin dissolved in 0.25 μl of aCSF or 0.25 μl of aCSF in a random order. There were at least two nonexperiment days between the injections. Injections were given in the morning between 0930 and 1000 h after sleep onset during 5 min (0.05 μl/min). The second microinjection experiment (n = 5) consisted of unilateral injections of 0.25 nmol of rat galanin (Bachem AG, Bubendorf, Switzerland, product: H7450) or combined injections of 0.25 nmol of galanin + 0.25 nmol of somatostatin or 0.25 μl of aCSF according to the same protocol.

Data Collection and Analysis

The EEG and EMG signals were amplified by an electroencephalograph and the amplified signal was analog-digital converted by a CED 1401 plus interface (Cambridge Electronic Design Ltd., Cambridge, England) with a sampling rate of 62 Hz. Data was displayed and stored by Spike 2 program (Cambridge Electronic Design Ltd) running in a desktop computer. During the recordings rats were observed using video camera and monitor. Collected data was categorized visually into wakefulness, non-REM sleep (NREMS), and REMS in 30-s epochs according to standard criteria (41).

TABLE 1

MEAN PROPORTION OF REM SLEEP (REMS) AND NON-REM SLEEP (NREMS) DURING 24 H REM SLEEP DEPRIVATION (0900H–0900H, LIGHTS: 0800H–2000H) WITH THE PLATFORM METHOD AT 6-H INTERVALS

	0–6 h	6–12 h	12–18 h	18–24 h
REMS%	0.1	0.3	0.1	0.0
SE	0.1	0.2	0.1	0.0
NREMS%	34.1	27.9	20.5	21.0
SE	1.8	2.2	4.0	4.1

n = 5, SE: standard error of means.

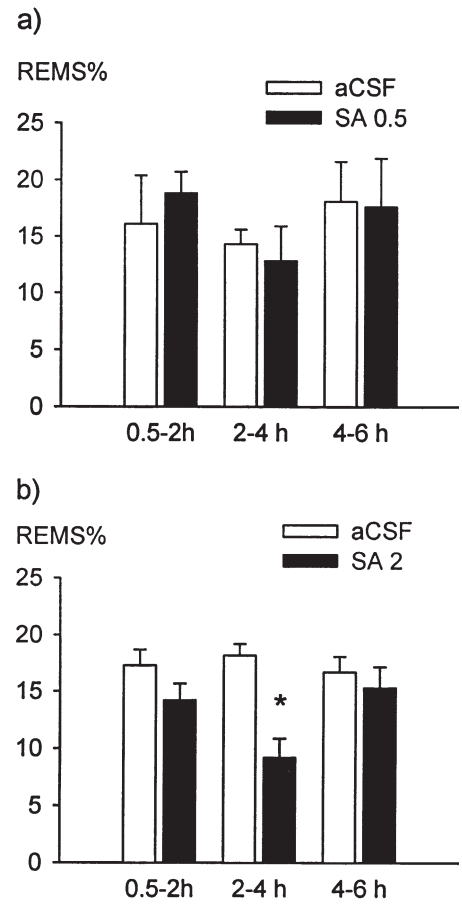

FIG. 2. The effect of ICV injected SA on mean proportions of REMS after 24 h REMSD during three post-injection time periods. (a) ICV injection of aCSF (vehicle control) or SA 0.5 nmol (n = 4). (b) ICV injection aCSF or SA 2 nmol (n = 5). Vertical bars indicate the standard error of means (SE). *Paired t-test: t (4) = 4.36, $p < 0.05$.

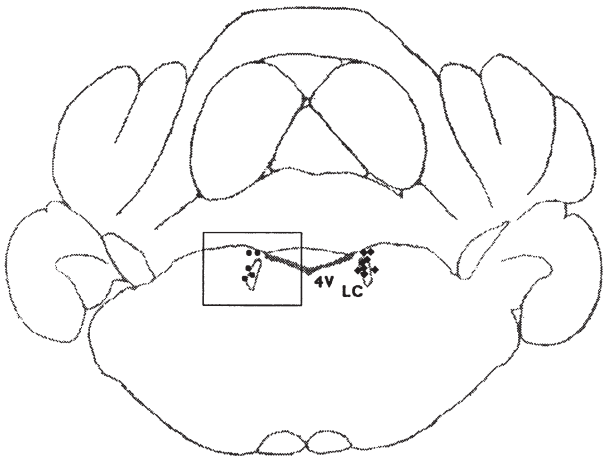

FIG. 3. Schematic illustration of the microinjection sites in the brainstem between the coronal levels of Bregma −9.3 and −9.8 (29) according to photomicroscopic verification. (right): SA/somatostatin ($n = 8$), (left): galanin/somatostatin ($n = 5$) (for clarity, plotted on the left side, actual injections were performed to the right). 4V: fourth ventricle, LC: locus coeruleus. The rectangle frame indicates the area viewed in the Fig. 4.

Transition from NREMS to REMS ("pre-REMS") was allocated equally between NREMS and REMS.

Statistics

Proportion of vigilance states per 2-h time intervals were calculated. The first 30 min after the injection was excluded because of the arousal caused by the injection procedure. Sample normality was checked by Kolmogorov-Smirnov test and equal variances of the treatment groups were checked by Levene median test with significance level $p < 0.05$. Differences between the groups were compared by analysis of variance for repeated measurements (RM ANOVA). Significant results $p < 0.05$) were tested by Student-Newman-Keuls or by Bonferroni (SA and somatostatin microinjections) multiple comparison method at significance level of $p < 0.05$. In the REMSD experiments the treatments (aCSF vs. SA) were compared by paired Student's t-test. $p < 0.05$ was accepted as a limit of statistical significance.

RESULTS

ICV SA and Somatostatin

ICV injection of 0.5 and 2 nmol of SA reduced the amount of REMS during the post injection period from 0.5 to 2 h when compared with aCSF controls (Fig. 1a). There was no difference between the two doses of SA. Later, during the periods 2 to 4 and 4 to 6 h, there was no significant difference in the amount of REMS between the treatments. SA did not affect the amount of NREMS during the 6-h post-injection recording period (data not shown). ICV injected boluses of corresponding doses of somatostatin did not affect the amount of REMS or NREMS during any post-injection time period when compared to aCSF (Fig. 1b).

ICV SA After REMSD

Platform REMSD for 24 h caused almost total inhibition of daytime and nighttime REMS during the procedure (Table 1).

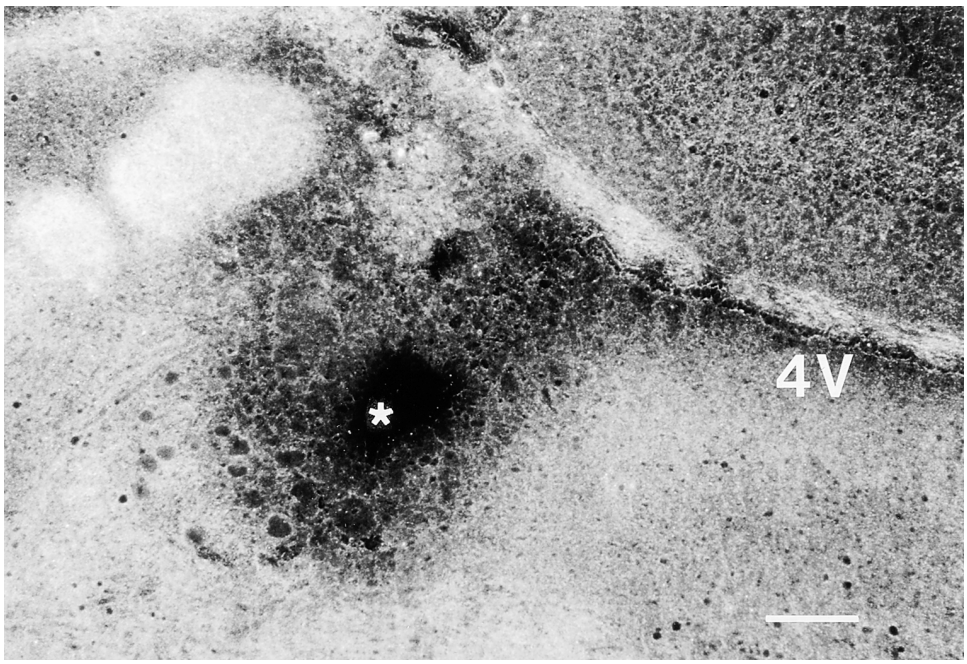

FIG. 4. Photomicrograph of the coronal section in rat brainstem at the level of Bregma −9.5 mm (unstained 20 μm cryosection in darkfield illumination). 0.25 μl of methyl blue was injected via the cannula for visualization of the unilateral injection site in the locus coeruleus. *: position of the tip of the injection cannula, 4V: fourth ventricle, scale bar: 0.2 mm.

ICV injection of 2 nmol of SA after 24 h REMSD reduced REMS during the time period 2 to 4 h after the injection when compared with aCSF (Fig. 2b). During the periods 0.5 to 2 and 4 to 6 h there was no significant difference in the amount of REMS. NREMS was not affected significantly (data not shown). Injection of 0.5 nmol of SA after REMSD did not significantly affect the amount of REMS or NREMS during the 6 h recording of recovery sleep when compared with aCSF injection (Fig. 2a).

LC Microinjections of SA, Somatostatin, and Galanin

Accepted microinjection sites were in the LC or in the immediate vicinity of the nucleus, not penetrating the fourth ventricle (Figs. 3 and 4). Microinjection of 0.25 nmol of SA into the LC reduced REMS during the post-injection period 0.5 to 2 h (Fig. 5a). NREMS was not affected significantly (data not shown). A corresponding dose of somatostatin did not affect post-injection proportions of sleep phases when compared with aCSF. Microinjection of 0.25 nmol of galanin or a combined injection of 0.25 nmol of galanin and 0.25 nmol of somatostatin did not affect REMS or NREMS during the 6 post-injection hours when compared with aCSF injection (Fig. 5b).

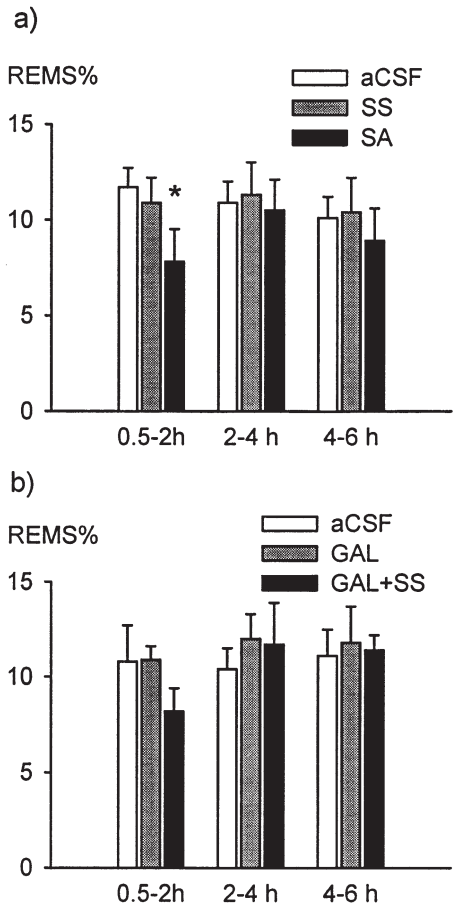

FIG. 5. The effect of LC microinjections on the mean proportion of REMS during three post-injection time periods. (a) microinjection of aCSF (vehicle control), somatostatin (SS) 0.25 nmol or SA 0.25 nmol ($n = 8$). (b) microinjection of aCSF, galanin (GAL) 0.25 nmol or combined injection of galanin 0.25 nmol and somatostatin 0.25 nmol ($n = 5$). Vertical bars indicate the standard error of means (SE). *RM ANOVA $F(2, 10) = 8.09$, $p < 0.01$, Bonferroni t-test: SA vs. aCSF $t(6) = 5.10$, $p < 0.01$.

The higher ICV dose (2 nmol) of both SA and somatostatin tended to cause in some rats motor restlessness and rotation around the rostrocaudal axis (barrel rotation) for 5 to 10 min immediately after the injection. The lower dose (0.5 nmol) and the microinjected dose (0.25 nmol) of SA, somatostatin, galanin, or a combined dose of somatostatin and galanin did not cause any observable behavioral effects in the rats.

DISCUSSION

Our results showed that SA reduces the amount of daytime REMS in rats when injected ICV, which may indicate that spontaneous REMS is facilitated by endogenous somatostatin. Both doses (0.5 and 2 nmol) gave an equal response in the magnitude and duration of REMS suppression. The 2 nmol dose tended to cause short lasting motor restlessness and barrel rotation in some rats. A similar effect of somatostatin and SA has been described in earlier studies, when these peptides were injected in high doses into CSF spaces (11,18).

The higher ICV dose of SA also decreased REMS, when REMS pressure was elevated by 24 h REMSD preceding the injection. This indicates that somatostatin may have a role in the REMS rebound after REMSD. We have previously found that after 24 h REMSD or 6 to 12 h total sleep deprivation the amount of somatostatin mRNA is elevated in the arcuate nucleus of the hypothalamus (42,43), which probably indicates an increased rate of synthesis of somatostatin peptide as a consequence of REMS inhibition. Activation of somatostatin cells may cause inhibition of GH secretion, which occurs during both REM (43) and total sleep deprivation (16) in rats. Somatostatin production in the hypothalamus could also mediate increased REMS pressure caused by lack of REMS and facilitate REMS rebound during recovery sleep. Because the effect of SA occurred after REMSD only when the higher (2 nmol) dose was used and after a delay, it is probable that REMSD activates also other REMS-facilitating transmitter systems, e.g., vasoactive intestinal peptide and prolactin (25). These factors may partially bypass the somatostatin system when it is blocked by SA, and thus reduce the effect of SA on REMS during the recovery after REMSD. The effect of SA on spontaneous and rebound REMS lasted longer than the effect of ICV injected SA on the baroreceptor reflex (17). This may be a consequence of the action of secondary neuronal systems mediating the effect of SA on REMS, whereas the effect on the baroreceptor reflex may be more direct.

A single ICV bolus of somatostatin in the morning did not increase REMS during the 6 post-injection hours recorded. In the former study of Danguir, an increased amount of REMS was found when somatostatin was infused continuously for 48 h (4). A single ICV bolus of somatostatin may be degraded too fast and/or the REMS-facilitation may require long-lasting, continuous stimulation of somatostatin receptors. In plasma, somatostatin degrades very fast, the half life is 0.4 to 3 min depending on species (28), but there is no exact information about the half-life of somatostatin in CSF and in the brain tissue. The maximal effect of ICV somatostatin and SA on the baroreceptor reflex were achieved 30 to 35 min after the injection, and a statistically significant response continued 60 min (somatostatin) and 40 min (SA) after a 2 nmol dose (17). An effect on REMS might need a longer duration of action. Furthermore, Danguir calculated REMS amount in 24-h periods that included both light and dark phase. It is possible that the increase of REMS during somatostatin infusion oc-

curs mainly during the dark phase, when the amount of natural REMS is low.

Microinjection of 0.25 nmol of SA locally into LC reduced the amount of REMS 0.5 to 2 h after the injection. This may indicate that the effect of ICV SA is partly mediated by blockade of somatostatin receptors in the LC (30). There are five cloned subtypes somatostatin receptors (SSR1-5) distributed widely in the CNS (32). Of these subtypes, at least SSR1-3 are expressed in the LC (30,33). During REMS there is a strong inhibition of LC cells that may, at least partly, be due to somatostatin released from the somatostatin immunoreactive nerve fibers present in the LC (39). These fibers originate from hypothalamus and, possibly, also from other brain areas (22). Transection of the hypothalamic periventricular somatostatin efferents decreases the amount of somatostatin in the LC (27) and according to a retrograde tracer study, the dorsal part of the paraventricular nucleus, which contains somatostatin cells, is connected to the LC (19). Nevertheless, ICV somatostatin and SA can affect LC receptors because the nucleus is located in the immediate vicinity of the fourth ventricle.

The microinjected volume (0.25 μl) was sufficient that the injected solution was able to reach the cells in the proper LC cells and the adjacent area around the nucleus (peri LC). The latter area consists of the dendrites of the LC neurons and it is probable that many of the synapses of the afferent connections to the LC cells are located here (19). Binding sites of somatostatin and galanin are concentrated to LC cells in the brainstem and local injection of these peptides decreases the activity of LC cells (reviewed in 22), which suggests that somatostatin and galanin affect LC neurons when injected into the vicinity of this nucleus.

Although somatostatin receptor antagonist (SA) had a clear effect on REMS, the agonist (somatostatin) failed to show effect when microinjected into LC. Injection of galanin, which is known to reduce the activity of LC noradrenergic neurons (34), was also ineffective as was the combination of somatostatin and galanin. Several factors may explain this: First, these peptides, which are also endogenous, may be degraded too fast in the brain tissue when administered as a single bolus. Secondly, during the first half of the light phase, when the amount of spontaneous sleep is high in the rat, endogenous somatostatin and galanin may inhibit LC cells so efficiently that injection of the same peptides fail to induce any additional inhibition to increase REMS. Microinjected somatostatin may also inhibit the release of endogenous somatostatin by activation of presynaptic autoreceptors in the somatostatin terminals. Autoregulation of somatostatin cells in the hypothalamus is possibly mediated by the SSR1 receptor subtype (12). The SA that we used might be more specific for SSR2 in the rat. SSR2 is possibly the most important receptor subtype controlling GH release in the pituitary somatotropic cells (31). Low doses up to 0.2 μg/kg SC of SA are able to antagonize the effect of endogenous and exogenous somatostatin to GH secretion in rats, while higher doses are ineffective (10), which may due to action of SA as a somatostatin agonist at higher doses (14). Novel, more potent and specific antagonists of somatostatin have recently been synthesized (2,14), but they are not yet commercially available. These compounds might resolve the receptor identity of the effect of somatostatin on REMS.

In conclusion, this study revealed that blockade of the somatostatin receptors by ICV injection of SA reduces REMS during spontaneous sleep and during recovery sleep after REMSD which supports the hypothesis that endogenous somatostatin may facilitate REMS. Local microinjection of SA into LC reduced REMS, which suggests that the effect of endogenous somatostatin on REMS may be partly mediated by the noradrenergic system in the LC.

ACKNOWLEDGEMENTS

This work was supported by the Finska Läkaresällskapet.

REFERENCES

1. Aston-Jones, G.; Bloom, F. E.: Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J. Neurosci. 1:876–886; 1981.
2. Bass, R. T.; Buckwalter, B. L.; Patel, B. P.; Pausch, M. H.; Price, L. A.; Strnad, J.; Hadcock, J. R.: Identification and characterization of novel somatostatin antagonists. Mol. Phar. 50:709–715; 1996.
3. 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 lasting somatostatin analog octreotide. Am. J. Physiol. 273:R1484–R1491; 1997.
4. Danguir, J.: Intracerebroventricular infusion of somatostatin selectively increased paradoxical sleep in rats. Brain Res. 367:26–30; 1986.
5. Danguir, J.: Internal milieu and sleep homeostasis. In: Inoué, S.; Schneider-Helmert, D., eds. Sleep peptides: Basic and clinical approaches. Berlin: Springer-Verlag; 1988:53–72.
6. Danguir, J.; DeSaint-Hilaire-Kafi, S.: Scopolamine-induced suppression of paradoxical sleep is reversed by the somatostatin analogue SMS 201-995 in rats. Pharmacol. Biochem. Behav. 30:295–297; 1988.
7. Danguir, J.; DeSaint-Hilaire-Kafi, S.: Somatostatin antiserum blocks carbachol-induced increase of paradoxical sleep in the rat. Brain Res. Bull. 20:9–12; 1988.
8. Danguir, J.; DeSaint-Hilaire-Kafi, S.: Reversal of desipramine-induced suppression of paradoxical sleep by a long-acting somatostatin analogue (octreotide) in rats. Neurosci. Lett. 98:154–158; 1989.
9. Frieboes, R. M.; Murck, H.; Schier, T.; Holsboer, F.; Steiger, A.: Somatostatin impairs sleep in elderly human subjects. Neuropsychopharmacology 16:339–345; 1997.
10. Fries, J. L.; Murphy, W. A.; Sueiras-Diaz, J.; Coy, D. H.: Somatostatin antagonist analog increases GH, insulin, and glucagon release in the rat. Peptides 3:811–814; 1982.
11. Havlicek, V.; Rezek, M.; Friesen, H.: Somatostatin and thyrotropin releasing hormone: central effect on sleep and motor system. Pharmacol. Biochem. Behav. 4:455–459; 1976.
12. Helboe, L.; Stidsen, C. E.; Moller, M.: Immunohistochemical and cytochemical localization of the somatostatin receptor subtype sst1 in the somatostatinergic parvocellular neuronal system of the rat hypothalamus. J. Neurosci. 18:4938–4945; 1998.
13. Hobson, J. A.; McCarley, R. W.; Wyzinski, P. W.: Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. Science 189:55–58; 1975.
14. Hocart, S. J.; Jain, R.; Murphy, W. A.; Taylor, J. E.; Morgan, B.; Coy, D. H.: Potent antagonists of somatostatin: synthesis and biology. J. Med. Chem. 41:1146–1154; 1998.
15. Inoue, M.; Nakajima, S., Nakamima, Y.: Somatostatin induces an inward rectification in rat locus coeruleus neurones through a pertussis toxin sensitive mechanism. J. Physiol. (Lond.) 407:177–198; 1988.
16. Kimura, F.; Tsai, C.-W.: Ultradian rhythm of growth hormone secretion and sleep in adult male rat. J. Physiol. (Lond.) 353:305–315; 1984.
17. Lin, S.-S.; Chan, J. Y. H.; Chan, S. H. H.: Facilitation of baroreceptor reflex response by endogenous somatostatin in the rat. Regul. Pept. 33:239–250; 1991.

18. Long, J. B.: Spinal subarachnoid injection of somatostatin causes neurological deficits and neuronal injury in rats. Eur. J. Pharmacol. 149:287–296; 1988.
19. Luppi, P.-H.; Aston-Jones, G.; Akaoka, H.; Chouvet, G.; Jouvet, M.: Afferent projections to the rat locus coeruleus demonstrated by retrograde and anterograde tracing with cholera-toxin B subunit and phaseolus vulgaris leucoagglutinin. Neuroscience 65:119–160; 1995.
20. Melander, T.; Hökfelt, T.; Rökaeus, Å.: Distribution of galanin-like immunoreactivity in the rat central nervous system. J. Comp. Neurol. 248:475–517; 1986.
21. Melander, T.; Hökfelt, T.; Rökaeus, Å.; Cuello, A. C.; Oertel, W. H.; Verhofstad, A.; Goldstein, M.: Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. J. Neurosci. 6:3640–3654; 1986.
22. Mounier, F.; Bluet-Pajot, M. T.; Moinard, F.; Kordon, C.; Epelbaum, J.: Activation of locus coeruleus somatostatin receptors induces an increase of growth hormone release in male rats. J. Neuroendocrinol. 8:761–764; 1996.
23. Murck, H.; Antonijevic, I. A.; Frieboes, R. M.; Maier, P.; Schier, T.; Steiger, A.: Galanin has REM-sleep deprivation-like effects on the sleep EEG in healthy young men. J. Psychiatry Res. 33:225–232; 1999.
24. Obal, F. Jr.; Floyd, R.; Kapás, L.; Bodosi, B.; Krueger, J. M.: Effects of systemic GHRH on sleep in intact and hypophysectomized rats. Am. J. Physiol. 270:E230–E237; 1996.
25. Obal, F. Jr.; Payne, L.; Kacsoh, B.; Opp, M.; Kapás, L.; Grosvenor, C. E.; Krueger, J. M.: Involvement of prolactin in the REM sleep-promoting activity of systemic vasoactive intestinal peptide (VIP). Brain Res. 645:143–149; 1994.
26. Ottlecz, A.; Snyder, G. D.; McCann, S. M.: Regulatory role of galanin in control of hypothalamic-anterior pituitary function. Proc. Natl. Acad. Sci. USA 85:9861–9865; 1988.
27. Palkovits, M.; Epelbaum, J.; Tapia-Arancibia, L.; Kordon, C.: Somatostatin in catecholamine-rich nuclei of the brainstem. Neuropeptides 3:139–144; 1982.
28. Patel, Y. C.; Wheatley, T.: In vivo and in vitro plasma disappearance and metabolism of somatostatin-28 and somatostatin-14 in the rat. Endocrinol. 112:220–225; 1983.
29. Paxinos, G.; Watson, C.: The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1986.
30. Pérez, J.; Rigo, M.; Kaupmann, K.; Bruns, C.; Yasuda, K.; Bell, G. I.; Lübbert, H.; Hoyer, D.: Localization of somatostatin (SRIF) SSTR-1, SSTR-2 and SSTR-3 receptor mRNA in rat brain by in situ hybridization. Naunyn-Schmiedeberg's Arch. Pharmacol. 349:145–160; 1994.
31. Raynor, K.; Murphy, W. A.; Coy, D. H.; Taylor, J. E.; Moreau, J.-P.; Yasuda, K.; Bell, G. I.; Reisine, T.: Cloned somatostatin receptors: identification of subtype-selective peptides and demonstration of high affinity binding of linear peptides. Mol. Phar. 43:838–844; 1993.
32. Reisine, T.: Somatostatin receptors. Am. J. Physiol. 269:G813–G820; 1995.
33. Señaris, R. M.; Humphrey, P. P. A.; Emson, P. C.: Distribution of somatostatin receptors 1, 2 and 3 mRNA in rat brain and pituitary. Eur. J. Neurosci. 6:1883–1896; 1994.
34. Sevcik, J.; Finta, E. P.; Illes, P.: Galanin receptors inhibit the spontaneous firing of locus coeruleus neurones and interact with μ-opioid receptors. Eur. J. Pharmacol. 230:223–230; 1993.
35. Skofitsch, G.; Jacobowitz, D. M.: Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. Peptides 6:509–546; 1985.
36. Smith, K. E.; Walker, M. W.; Artymyshyn, R.; Bard, J.; Borowsky, B.; Tamm, J. A.; Yao, W.-J.; Vaysse, P. J.-J.; Branchek, T. A.; Gerald, C.; Jones, K. A.: Cloned human and rat galanin GALR3 receptors. J. Biol. Chem. 273:23321–23326; 1998.
37. Steiger, A.; Guldner, J.; Hemmeter, U.; Rothe, B.; Wiedemann, K.; Holsboer, F.: Effects of growth hormone-releasing hormone and somatostatin on sleep EEG and nocturnal hormone secretion in male controls. Neuroendocrinology 56:566–573; 1992.
38. Steiger, A.; Holsboer, F.: Neuropeptides and human sleep. Sleep 20:1038–1052; 1997.
39. Sutin, E. L.; Jacobowitz, D. M.: Immunocytochemical localization of peptides and other neurochemicals in the rat laterodorsal tegmental nucleus and adjacent area. J. Comparative Neurol. 270:243–270; 1988.
40. Tanoh, T.; Shimatsu, A.; Ishikawa, Y.; Ihara, C.; Yanaihara, N.; Imura, H.: Galanin-induced growth hormone secretion in conscious rats: evidence for a possible involvement of somatostatin. J. Neuroendocrinol. 5:183–187; 1993.
41. Timo-Iaria, C.; Negrão, N.; Schmidec, W. R.; Hoshino, K.; de Menezes, C. E. L.; da Rocha, T. L.: Phases and states of sleep in the rat. Physiol. Behav. 5:1057–1062; 1970.
42. Toppila, J.; Alanko, L.; Asikainen, M.; Tobler, I.; Stenberg, D.; Porkka-Heiskanen, T.: Sleep deprivation increases somatostatin and growth hormone-releasing hormone messenger RNA in the rat hypothalamus. J. Sleep Res. 6:171–178; 1997.
43. Toppila, J.; Asikainen, M.; Alanko, L.; Turek, F. W.; Stenberg, D.; Porkka-Heiskanen, T.: The effect of REM sleep deprivation on somatostatin and growth hormone-releasing hormone gene expression in the rat hypothalamus. J. Sleep Res. 5:115–122; 1996.
44. Toppila, J.; Stenberg, D.; Alanko, L.; Asikainen, M.; Urban, J. H.; Turek, F. W.; Porkka-Heiskanen, T.: REM sleep deprivation induces galanin gene expression in the rat brain. Neurosci. Lett. 183:171–174; 1995.
45. Van Cauter, E.; Plat, L.; Copinschi, G.: Interrelations between sleep and somatropic axis. Sleep 21:553–566; 1998.