Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6240–6244, October 1984
Neurobiology

Differential sleep-promoting effects of five sleep substances nocturnally infused in unrestrained rats

(rat circadian sleep rhythm/delta-sleep-inducing peptide/muramyl dipeptide/prostaglandin D_2/uridine)

SHOJIRO INOUÉ*, KAZUKI HONDA*, YASUO KOMODA*, KOJI UCHIZONO†, RYUJI UENO‡, AND OSAMU HAYAISHI‡

*Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, Kanda-Surugadai 2-3-10, Chiyoda-ku, Tokyo 101; †National Institute for Physiological Sciences, Myodaiji, Okazaki 444; and ‡Hayaishi Bioinformation Transfer Project, Research Development Corporation of Japan, c/o Osaka Medical College, Daigakumachi 2-7, Takatsuki 569, Japan

Contributed by Osamu Hayaishi, June 4, 1984

ABSTRACT **Sleep-inducing and sleep-maintaining effects of five different putative sleep substances were compared by the same nocturnal 10-hr intracerebroventricular infusion technique in otherwise saline-infused, freely moving male rats. Delta-sleep-inducing peptide (2.5 nmol), which induces electroencephalogram delta (slow)-wave patterns, was rapidly effective in increasing both slow-wave sleep and paradoxical sleep but the effects were not long-lasting. Muramyl dipeptide (2 nmol) induced excessive slow-wave sleep in the middle of the infusion period, accompanying a simultaneous elevation of brain temperature. However, paradoxical sleep was not affected. Component B of sleep-promoting substance (2 brainstem equivalents), a partially purified extract from rats deprived of sleep for 24-hr, was markedly effective in inducing and maintaining both kinds of sleep. Prostaglandin D_2 (0.36 nmol) was more effective in enhancing sleep at the later period of the infusion period. Uridine (10 pmol) caused a mild but long-lasting increase in sleep, especially in paradoxical sleep. Thus, each substance exhibited compound-specific sleep-modulating properties.**

A number of endogenous factors are known to elicit sleep-modulating properties (for recent reviews, see refs. 1–3). Among them, the following substances have been most extensively investigated: delta-sleep-inducing peptide (δSIP; induces electroencephalogram (EEG) delta (slow)-wave patterns), factor S, prostaglandin (PG) D_2, and sleep-promoting substance (SPS). δSIP originally was detected in the venous blood of sleeping rabbits (4) and finally was identified as a nonapeptide (5). Factor S was found first in the cerebrospinal fluid of sleep-deprived goats (6), then in the brain of goats, sheep, rabbits, and cattle (7), and later in the human urine (8). This factor appears to be a muramyl peptide. "Muramyl dipeptide" (*N*-acetylmuramyl-L-alanyl-D-isoglutamine) is a substance of the closest resemblance to factor S (9). PGD_2 is a natural constitutent of the brain and proved to be a potent sleep inducer (10, 11). SPS was extracted and purified from the brainstem of rats deprived of sleep for 24 hr (12). It contains at least four active components (13, 14). Uridine and SPS-B, a partially purified extract, are two SPS components (15).

The existence of so many putative sleep substances requires elucidation as to their differential role played in the regulation of sleep. At present, however, no convincing information is available. The somnogenic effects of each sleep substance are demonstrated by experiments with a variety of assay methods in various animals. Hence, a comparison of the sleep-promoting characteristics is almost impossible by an analysis of accumulated literature. Furthermore, most workers preferably adopted a short-term assay, which might not give sufficient information on the physiological role of a sleep substance. Since sleep is largely modified by the circadian mechanism (16), day-to-day dynamics in sleep parameters should be taken into account in sleep bioassay.

Honda and Inoué (17) developed a long-term bioassay technique for the quantitative evaluation of sleep-promoting effects of SPS. A test material can be steadily infused into the third ventricle of otherwise saline-infused freely moving rats. A 72-hr to 96-hr continuous polysomnogram is analyzed with reference to the modulatory effect on the circadian sleep pattern. Based on this routine technique, the present paper deals with a comparative study of sleep-promoting effects of the above substances.

MATERIALS AND METHODS

Male rats of the Sprague–Dawley strain, raised in our closed colony on a 12-hr light/12-hr dark schedule (light period, 08:00–20:00) in a constant air-conditioned environment of 25 ± 1°C and 60 ± 6% relative humidity, were used. At the age of 60–70 days, animals were anesthesized by sodium pentobarbital (50 mg/kg of body weight) and implanted with three cortical electrodes for EEG recording, two nuchal electrodes for electromyogram (EMG) recording, and a cannula in the third ventricle for continuous infusion. In some cases, a copper–constantan thermocouple was implanted in the thalamus for monitoring brain temperature. The technique of surgery was the same as described (17, 18).

The rats were individually housed in a special cage (19), which enabled continuous monitoring of locomotor activity, EEG, EMG, and brain temperature and intraventricular infusion. A cannular feedthrough slip ring fixed above the cage guaranteed free movement of the rats. Each cage was placed in a soundproof, electromagnetically shielded chamber under the same environmental conditions as above. The rats were then continuously infused with saline solution at a rate of 20 μl/hr. Simultaneously, their EEG, EMG, brain temperature, and locomotor activity were polygraphically recorded and further processed by a computer-aided device system (17, 18).

After a week's recovery from surgery, a 3-day assay was performed: a baseline day for control recordings, an experimental day, and a recovery day. Each day started at the onset of the light period at 08:00 and ended 24 hr later. This procedure was repeated up to four times in the same rats with a recovery interval of 2–6 days after their sleep rhythms had returned to the previous baseline level. Between 19:00 and 05:00 on the experimental day (i.e., from 1 hr before the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: δSIP, delta-sleep-inducing peptide; PG, prostaglandin; PS, paradoxical sleep; SPS, sleep-promoting substance; SWS, slow-wave sleep; EEG, electroencephalogram; EMG, electromyogram.

dark onset until 3 hr before the light onset), the continuous saline infusion was replaced by a steady infusion of test solutions. They consisted of 2.5 nmol of δSIP, 2.0 nmol of muramyl dipeptide, 2 brainstem equivalents of SPS-B (an active component of SPS: for the details of purification, see ref. 15), 0.36 nmol of PGD_2, and 10 pmol of uridine. These materials were dissolved in 200 μl of saline and continuously infused at a rate of 20 μl/hr. The optimal dosage for each substance was determined by the unpublished data from a pilot experiment in the cases of δSIP and muramyl dipeptide and by the data available from our previous papers in the cases of SPS-B (20), PGD_2 (11), and uridine (18). Brain temperature was recorded in rats infused with muramyl dipeptide and uridine. Sleep records in the baseline day were compared to those in the experimental day and the recovery day and were analyzed statistically by the Student *t* test.

RESULTS

Circadian Sleep-Waking Rhythms in Saline-Infused Rats. In each experimental group, the rats continuously infused with saline solution exhibited a prominent circadian rhythmicity in slow-wave sleep (SWS) and paradoxical sleep (PS), as already reported in similar studies (11, 20, 21). The total time of SWS and PS in the light period, which is shown in Table 1 and in the cumulative curves of Figs. 1–5, was 370–420 and 50–75 min, respectively, while that in the dark period was 200–260 min and 25–50 min, respectively, as shown in the same figures and table.

Effects of δSIP. A 10-hr infusion of 2.5 nmol of δSIP resulted in a rapid increase in SWS. After the initiation of the δSIP infusion, the amount of hourly SWS increased to 30–40 min, which was almost comparable to that in the light period. The difference from the baseline was highly significant at the first few hours (Fig. 1 *Upper*). However, such an SWS-inducing effect disappeared during the second half of the 10-hr infusion period. Hourly changes in PS showed no immediate increase. The increase in sleep was also evident from the cumulative curves (Fig. 1 *Lower*). The cumulative SWS increment at the end of the dark period exceeded the baseline value by 47.1 min (18.4%). The increase of PS occurred in proportion to that of SWS and amounted to 9.1 min (19.0%). However, the final cumulative amount of both SWS and PS in the dark period was not statistically different from baseline (Table 1). The increase in sleep in the early phase of the δSIP infusion was not caused by a prolongation of episodes of SWS and/or PS, but largely by their more frequent occurrence (Table 1).

Effects of Muramyl Dipeptide. A 10-hr infusion of 2 nmol of muramyl dipeptide resulted in a rather slow increase in SWS. In the middle of the infusion period, the elevated SWS values reached a level of ca. 30 min/hr. However, due to the large variations, the values were not statistically different

Table 1. Effects of a 10-hr nocturnal intraventricular infusion of five different substances on the sleep parameters (mean ± SEM)

		Baseline		Experiment		Recovery	
	Sleep parameters	Light period	Dark period	Light period	Dark period	Light period	Dark period
		δSIP at 2.5 nmol (n = 9)					
SWS	Total time, min	401.6 ± 17.3	255.3 ± 24.4	403.6 ± 13.8	302.4 ± 21.7	410.3 ± 14.8	256.2 ± 15.5
	Episode frequency	94.2 ± 8.8	91.9 ± 8.4	99.4 ± 7.9	100.7 ± 5.1	100.0 ± 7.9	85.2 ± 6.5
	Episode duration, min	4.7 ± 0.6	2.9 ± 0.4	4.3 ± 0.4	3.0 ± 0.2	4.3 ± 0.3	3.1 ± 0.3
PS	Total time, min	57.4 ± 6.5	48.0 ± 7.0	50.7 ± 5.4	57.1 ± 8.6	56.9 ± 6.1	44.1 ± 5.7
	Episode frequency	38.5 ± 3.4	27.3 ± 2.9	31.4 ± 4.0	30.2 ± 4.8	32.4 ± 2.9	25.7 ± 3.0
	Episode duration, min	1.8 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
		MDP at 2.0 nmol (n = 7)					
SWS	Total time, min	394.9 ± 16.4	221.6 ± 15.1	372.9 ± 20.4	283.7 ± 28.8	391.0 ± 12.7	218.3 ± 20.9
	Episode frequency	120.1 ± 8.4	128.3 ± 13.3	120.1 ± 9.0	129.7 ± 8.3	113.0 ± 8.1	113.0 ± 4.0
	Episode duration, min	3.2 ± 0.3	1.8 ± 0.2	3.2 ± 0.3	2.2 ± 0.2	3.6 ± 0.3	1.9 ± 0.2
PS	Total time, min	54.4 ± 9.6	47.0 ± 8.6	60.4 ± 11.1	44.6 ± 9.8	62.3 ± 6.8	50.0 ± 10.5
	Episode frequency	3.4 ± 0.3	29.4 ± 2.8	36.3 ± 5.8	32.0 ± 5.8	37.0 ± 3.2	30.3 ± 7.4
	Episode duration, min	1.7 ± 0.1	1.5 ± 0.2	1.7 ± 0.1	1.3 ± 0.1	1.7 ± 0.1	1.7 ± 0.2
		SPS-B at 2 units (n = 9)					
SWS	Total time, min	412.6 ± 9.6	246.1 ± 15.4	406.6 ± 8.6	341.0 ± 20.4*	402.9 ± 11.4	254.6 ± 14.0
	Episode frequency	91.9 ± 8.8	93.0 ± 6.8	98.0 ± 6.2	113.4 ± 9.7	87.0 ± 4.9	84.9 ± 14.3
	Episode duration, min	4.8 ± 0.5	2.8 ± 0.2	4.3 ± 0.3	3.2 ± 0.3	4.8 ± 0.4	3.0 ± 0.2
PS	Total time, min	57.4 ± 5.8	47.7 ± 8.3	53.1 ± 5.6	71.5 ± 9.5†	38.6 ± 5.9†	58.2 ± 5.4
	Episode frequency	36.2 ± 3.4	31.4 ± 4.3	34.3 ± 2.4	43.3 ± 5.5	24.9 ± 3.1†	28.4 ± 3.7
	Episode duration, min	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.2	1.9 ± 0.1
		PGD_2 at 0.36 nmol (n = 8)					
SWS	Total time, min	369.8 ± 24.0	203.0 ± 10.4	394.5 ± 24.3	267.4 ± 9.0*	363.9 ± 18.2	231.8 ± 18.3
	Episode frequency	89.1 ± 5.4	86.0 ± 5.8	86.8 ± 6.4	104.6 ± 7.0	84.6 ± 6.9	86.9 ± 5.8
	Episode duration, min	4.4 ± 0.5	2.4 ± 0.1	4.9 ± 0.7	2.6 ± 0.2	4.5 ± 0.4	2.7 ± 0.2
PS	Total time, min	65.1 ± 8.3	35.6 ± 7.4	65.1 ± 6.9	48.6 ± 10.6	46.9 ± 2.9	37.8 ± 4.5
	Episode frequency	35.3 ± 4.5	23.1 ± 3.4	35.6 ± 4.3	28.3 ± 5.3	28.7 ± 2.4	23.9 ± 2.5
	Episode duration, min	1.9 ± 0.1	1.5 ± 0.1	1.9 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	1.6 ± 0.1
		Uridine at 10 pmol (n = 8)					
SWS	Total time, min	396.5 ± 16.7	216.7 ± 8.7	403.8 ± 12.0	262.2 ± 10.3*	402.5 ± 20.2	262.9 ± 22.1
	Episode frequency	90.5 ± 7.1	70.1 ± 5.5	89.9 ± 5.0	83.1 ± 5.2	92.5 ± 5.4	78.9 ± 7.0
	Episode duration, min	4.6 ± 0.3	3.3 ± 0.4	4.6 ± 0.4	3.3 ± 0.3	4.4 ± 0.3	3.5 ± 0.4
PS	Total time, min	74.0 ± 10.1	26.3 ± 2.7	63.9 ± 6.2	44.2 ± 6.5†	62.8 ± 6.7	41.0 ± 8.4
	Episode frequency	35.6 ± 3.3	17.0 ± 2.0	34.1 ± 3.3	25.8 ± 5.2	34.4 ± 3.5	23.0 ± 3.1
	Episode duration, min	2.0 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.7 ± 0.2	1.8 ± 0.0	1.7 ± 0.1

MDP, muramyl dipeptide; n, number of rats.
*Statistically different from the baseline of the corresponding light or dark period at $P < 0.01$.
†Statistically different from the baseline of the corresponding light or dark period at $P < 0.05$.

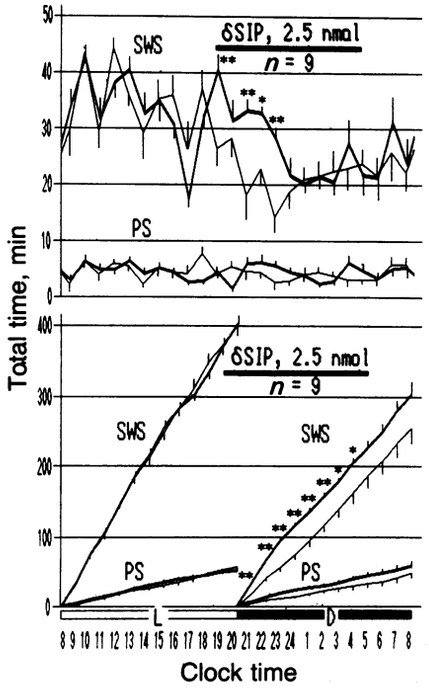

FIG. 1. Sleep-promoting effects of a 10-hr intraventricular infusion of δSIP at 2.5 nmol from 19:00 to 05:00 (indicated by a solid bar) in otherwise saline-infused rats. The upper graph shows the hourly integrated sleep amounts, while the bottom graph illustrates the cumulative values in the light (L) and dark (D) period. Thin and thick curves represent the baseline and the experimental day, respectively. Vertical lines on each hourly value indicate the range of SEM. * and **, values on the experimental day that were significantly different from that of the baseline at $P < 0.05$ and $P < 0.01$, respectively.

from the baseline level (Fig. 2 *Upper*). Throughout the effective period of muramyl dipeptide on SWS, an elevation of brain temperature by 1.0–1.5°C was observed. The cumulative curves (Fig. 2 *Lower*) show that SWS significantly increased from 5 to 12 hr after the onset of the dark period. The cumulative increment of SWS at the end of the dark period exceeded the baseline value by 62.1 min (28.0%). However, the total amount of SWS in the dark period was not statistically different from that of the baseline (Table 1). No significant change in PS was observed during the course of the muramyl dipeptide infusion. The increase in SWS was largely due to a prolongation of the episode duration but not to an increase in episode frequency (Table 1).

Effects of SPS-B. A 10-hr infusion of two brainstem equivalents of SPS-B resulted in a profound sleep-enhancing effect. The sleep records showed a continued increase in both SWS and PS. The effect appeared shortly after the initiation of the SPS-B infusion and lasted throughout the dark period. The hourly values in sleep amount were statistically different from baseline at four different points for SWS and at one point for PS (Fig. 3 *Upper*). The cumulative values of SWS started to be significantly increased 5 hr after the dark onset (Fig. 3 *Lower*). The total time of SWS in the dark period increased by 94.9 min (38.6%) as compared to baseline. This was highly significant (Table 1). The total time of PS was also significantly increased, the increment being 23.8 min (49.9%). Hence, the net increase in total sleep time in the dark period was approximately 2 hr. Similar to δSIP, the increase in sleep was caused by the more frequent occurrence of SWS and PS episodes, while their duration was less affected (Table 1). PS significantly decreased in the light period of the recovery day (Table 1), as already known in the previous fractions (21).

Effects of PGD_2. A 10-hr infusion of 0.36 nmol of PGD_2 resulted in a rapid increase in SWS and a gradual increase in PS. However, more striking and significant changes took place in the second half of the infusion period (Fig. 4). Thus, the cumulative values of SWS significantly differed from baseline from 7 hr after the dark onset until the end of the dark period. The cumulative increment of SWS and PS in the dark period was 64.4 min (31.7%) and 13.0 min (36.5%), respectively, which was larger than for any of the other substances with the exception of SPS-B (Table 1). Similar to δSIP and SPS-B, the increase was entirely due to the more

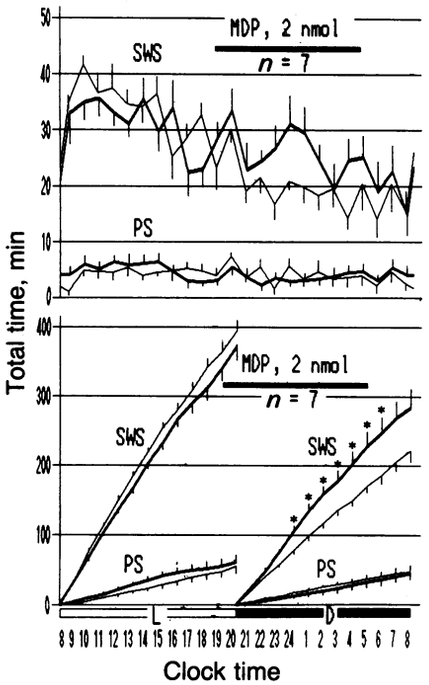

FIG. 2. Sleep-promoting effects of muramyl dipeptide (MDP). For details, see the legend of Fig. 1.

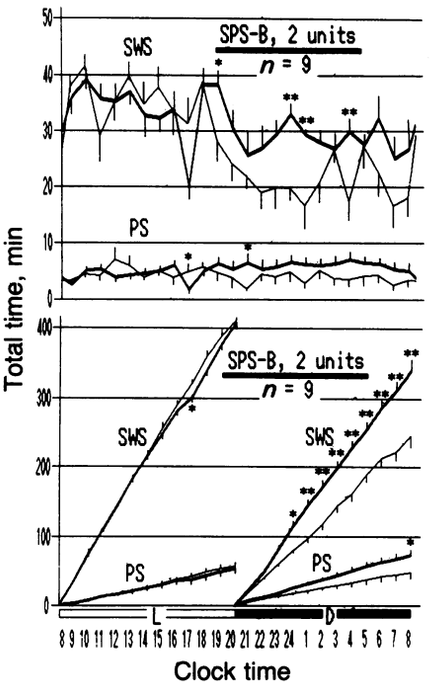

FIG. 3. Sleep-promoting effects of SPS-B. For details, see the legend of Fig. 1.

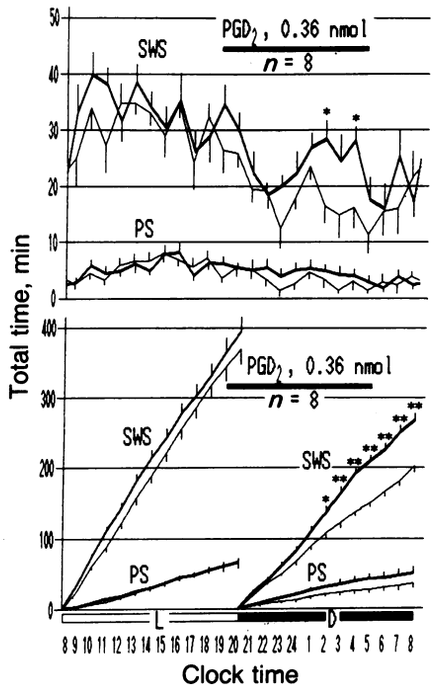

FIG. 4. Sleep-promoting effects of PGD_2. For details, see legend of Fig. 1.

frequent occurrence of SWS and PS episodes. A decrease in PS was noted in the light period of the recovery day, but the change was not statistically significant (Table 1).

Effects of Uridine. A 10-hr infusion of 10 pmol of uridine resulted in a rather mild but continued increase in both SWS and PS (Fig. 5). Consequently, the cumulative values of SWS and PS became significantly different from baseline only 7 hr and 12 hr after the dark onset, respectively. At the end of the dark period, there was a significant increment of SWS and PS by 45.5 min (21.0%) and 17.9 min (68.1%), respectively (Table 1). The large percentage-wise increase in PS was due to the relatively small baseline value. Brain temperature was not affected by the uridine administration. Similar to δSIP, SPS-B, and PGD_2, uridine increased the frequency of SWS and PS episodes, without affecting their duration (Table 1). Uridine was characterized by a long-lasting effect. At the first recovery night, almost similar increases in SWS and PS were recorded (Table 1), although the total time of neither SWS nor PS was statistically different from baseline due to the large individual variations.

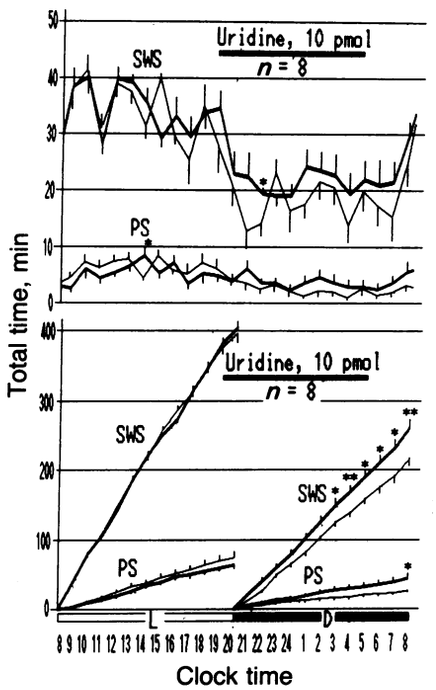

FIG. 5. Sleep-promoting effects of uridine. For details, see the legend of Fig. 1.

DISCUSSION

The laboratory rat, a member of well-known nocturnal animals, shows a regular alternation of the light-time quiescence and the dark-time activity even under a steady intraventricular infusion of saline solution (20, 21). The amount of sleep in the light period seems to be saturated and considerably stable, while the amount of wakefulness in the dark period appears to be easily affected by artificial manipulation. Hence, our bioassays were conducted by a 10-hr intraventricular infusion of sleep substances, which was initiated shortly before the onset of the dark period. The sleep-promoting effects were evaluated by the potency to modify the time-course dynamics of SWS and/or PS and the increment of the SWS and/or PS amount in the dark period.

It was found that, so far as the present bioassay technique and the dosage used were concerned, all the substances exhibited compound-specific sleep-promoting characteristics. Since the kinetic properties of these putative sleep substances are largely unknown due to the difficulty in interpreting the rate of their degradation, accumulation, and permeability, a comparative analysis of the intracerebral role in the regulation of sleep should be a matter of a future problem. Nevertheless, the following remarks might be mentioned here. δSIP was immediately effective and very potent in inducing excessive sleep, but the effect was short-lasting. Even during the course of infusion, δSIP ceased to elicit the effect. In this respect, δSIP might be regarded as a primary sleep-inducer or a trigger substance of sleep. Muramyl dipeptide was characterized by its slow SWS-promoting effect. The maximal effect was observed in the middle of the infusion period, when a marked elevation of the brain temperature took place. The sleep-enhancing effect of PGD_2 was apparent at the beginning of the infusion period but was more prominent during the later period. Thus, PGD_2 might have a property to primarily induce sleep and to secondarily activate the sleep-maintaining mechanism. SPS-B and uridine were characterized by their steady, long-lasting sleep-promoting effects. Both SWS and PS were affected. These SPS components seem to play a more or less similar basic role in triggering and maintaining sleep.

The sleep enhancement was mainly due to the frequent occurrence of sleep episodes. Since natural sleep in rats is episodic and frequently interrupted by wakefulness, especially at the dark period, a prolongation of a single SWS and/or PS episode might not be considered physiological. In this respect, the "exogenously" supplied "endogenous" substances, δSIP, PGD_2, SPS-B, and uridine, did not alter the natural sleep pattern.

Some substances are known to be related to the temperature regulation. Muramyl dipeptide caused a simultaneous increase in SWS and brain temperature. According to Krueger *et al.* (22), the pyrogenic property of muramyl dipeptide is independent from the somnogenic one, and the excessive sleep does not result from fever. As to the colonic temperature, Yehuda *et al.* (23) reported that intraperitoneally injected δSIP caused in rats hypothermia at an ambient temperature of 4°C and hyperthermia at 22°C. Ueno *et al.* (24) reported that a microinjection of PGD_2 into the preoptic area

caused hypothermia in rats. Since a close relation exists between sleep and temperature (25), sleep substances may offer an appropriate clue to investigate the mechanism involved in the interrelationship.

The differential sleep-modulatory properties of the above sleep substances suggest that sleep is regulated by a number of humoral factors that play a specific role in the dynamic process of sleep. Novel and multidisciplinary approaches to the intracerebral system may eventually clarify the mechanisms. An immunohistological mapping of δSIP (26), an autoradiographic search for a PGD_2 receptor (27), and an electrophysiological analysis of δSIP-sensitive neurons (28) are examples of such pioneer studies.

Critical reading of the manuscript by Dr. Alexander A. Borbély of University of Zurich, Zurich, is greatly appreciated. δSIP was kindly supplied by Dr. Guido A. Schoenenberger of University of Basel, Basel, and Dr. Markus Graf of the Veterans Administration Hospital and Tulane Medical Center, New Orleans. Muramyl dipeptide was a gift from Dr. James M. Krueger of the Chicago Medical School, North Chicago. This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (58480136) and funds from Chugai Pharmaceutical Co., Ltd. (Tokyo), the Suzuken Memorial Foundation (Nagoya), and the Naito Foundation (Tokyo).

1. Borbély, A. (1982) *Trends Pharmacol. Sci.* **3,** 350.
2. Drucker, Colín, R. & Valverde-R., C. (1982) in *Sleep: Clinical and Experimental Aspects*, eds. Ganten, D. & Pfaff, D. (Springer, Berlin), pp. 37–81.
3. Inoué, S., Uchizono, K. & Nagasaki, H. (1982) *Trends Neurosci.* **5,** 218–220.
4. Monnier, M., Koller, T. & Graver, S. (1963) *Exp. Neurol.* **8,** 264–277.
5. Schoenenberger, G. A., Maier, P. F., Tobler, H. J. & Monnier, M. (1977) *Pflügers Arch.* **369,** 99–109.
6. Pappenheimer, J. R., Miller, T. B. & Goodrich, C. A. (1967) *Proc. Natl. Acad. Sci. USA* **58,** 513–517.
7. Pappenheimer, J. R., Koski, G., Fencl, V., Karnovsky, M. L. & Krueger, J. (1975) *J. Neurophysiol.* **38,** 1299–1311.
8. Krueger, J. M., Bacsik, J. & García-Arrarás, J. (1980) *Am. J. Physiol.* **238,** E116–E123.
9. Krueger, J. M., Pappenheimer, J. R. & Karnovsky, M. L. (1982) *J. Biol. Chem.* **257,** 1664–1669.
10. Ueno, R., Ishikawa, Y., Nakayama, T. & Hayaishi, O. (1982) *Biochem. Biophys. Res. Commun.* **109,** 576–582.
11. Ueno, R., Honda, K., Inoué, S. & Hayaishi, O. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1735–1737.
12. Nagasaki, H., Iriki, M., Inoué, S. & Uchizono, K. (1974) *Proc. Jpn. Acad.* **50,** 241–246.
13. Uchizono, K., Ishikawa, M., Iriki, M., Inoué, S., Komoda, Y., Nagasaki, H., Higashi, A., Honda, K. & McRae-Degueurce, A. (1982) *Adv. Pharmacol. Ther. Proc. Int. Congr. Pharmacol.* **2,** 217–226.
14. Inoué, S. (1983) *Seikagaku* **55,** 445–460.
15. Komoda, Y., Ishikawa, M., Nagasaki, H., Iriki, M., Honda, K., Inoué, S., Higashi, A. & Uchizono, K. (1983) *Biomed. Res.* **4,** Suppl., 223–227.
16. Borbély, A. A. (1982) *Human Neurobiol.* **1,** 195–204.
17. Honda, K. & Inoué, S. (1978) *Rep. Inst. Med. Dent. Eng. Tokyo Med. Dent. Univ.* **12,** 81–85.
18. Honda, K., Komoda, Y., Nishida, S., Nagasaki, H., Higashi, A., Uchizono, K. & Inoué, S. (1984) *Neurosci. Res.* **1,** 243–252.
19. Honda, K., Ichikawa, H. & Inoué, S. (1973) *Rep. Inst. Med. Dent. Eng. Tokyo Med. Dent. Univ.* **8,** 149–152.
20. Honda, K. & Inoué, S. (1981) *Rep. Inst. Med. Dent. Eng. Tokyo Med. Dent. Univ.* **15,** 115–123.
21. Inoué, S., Honda, K. & Komoda, Y. (1983) in *Sleep 1982*, ed. Koella, W. P. (Karger, Basel, Switzerland), pp. 112–114.
22. Krueger, J. M., Pappenheimer, J. R. & Karnovsky, M. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6102–6106.
23. Yehuda, S., Kastin, A. J. & Coy, D. H. (1980) *Pharmacol. Biochem. Behav.* **13,** 895–900.
24. Ueno, R., Narumiya, S., Ogorochi, T., Nakayama, T., Ishikawa, Y. & Hayaishi, O. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6093–6097.
25. Heller, H. C. & Glotzbach, S. F. (1977) in *International Review of Physiology, Environmental Physiology*, ed. Robertshaw, D. (University Park Press, Baltimore), Vol. 2, pp. 147–188.
26. Constantinidis, J., Bouras, C., Guntern, R., Taban, C. H. & Tissot, R. (1983) *Neuropsychobiol.* **10,** 94–100.
27. Yamashita, A., Watanabe, Y. & Hayaishi, O. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6114–6118.
28. Normanton, J. R. & Gent, J. P. (1983) *Neuroscience* **8,** 107–114.