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Effects of Intracerebroventricular Injection of Delta Sleep-Inducing Peptide (DSIP) and an Analogue on Sleep and Brain Temperature in Rats at Night

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OBÁL, F., JR., A. TÖRÖK, P. ALFÖLDI, G. SÁRY, M. HAJÓS AND B. PENKE. *Effects of intracerebroventricular injection of delta sleep-inducing peptide (DSIP) and an analogue on sleep and brain temperature in rats at night.* PHARMACOL BIOCHEM BEHAV **23**(6) 953–957, 1985. —The effects of ICV injections of DSIP and ω-amino-caprilyl-DSIP (C-DSIP) on the sleep-wake activity and brain temperature (T_{br} were studied in rats. The substances (7 nmol/kg) were injected at dark onset, and the sleep-wake activity and T_{br} were recorded for 24 hr (dark and light periods, 12 hr each). Relative to the control recordings obtained after artificial CSF injection, the duration of sleep did not increase after either DSIP or C-DSIP. The only significant reaction was an increase of W 6 to 9 hr after the injection of either peptide. The course of T_{br} after DSIP and C-DSIP was also indentical to that recorded after the injection of artificial CSF. It seems that DSIP administered in a single ICV injection at dark onset does not promote sleep. The increase in W might be attributed to an indirect effect of DSIP or to a degradation product of the peptide.

DSIP Sleep Brain temperature Sleep-promoting substances

THE delta sleep-inducing peptide (DSIP) was isolated from rabbits and later synthesized as a substance with sleep-promoting character [20, 21, 29]. DSIP-like immunoreactivity has been demonstrated in various parts of the brain [7,16], and it has also been found in the plasma [15]. An increase of sleep was reported in response to the intracerebroventricular (ICV) or intravenous (IV) administration of DSIP to various animals (see [8,19] for reviews) and man [25], and thus DSIP has come into consideration as a naturally-occurring sleep-promoting substance (see [32]).

Nevertheless, the results concerning the effects of DSIP on sleep are not without contradictions. DSIP has been found to be ineffective in some experiments [17,31], and the idea that the substance is implicated in sleep regulation has also been challenged. Interestingly, only a few studies [31] have attempted to investigate the effects of DSIP on sleep during the circadian active period, and long-term records after DSIP treatment are also scarce. In the present experiments, DSIP and a DSIP analogue, ω-amino-caprilyl-DSIP (C-DSIP), were injected ICV at dark onset into rats, and the sleep-wake activity and brain temperature (T_{br}), a useful indicator of body temperature in studies on sleep [23], were recorded throughout the 12-hr dark and the subsequent 12-hr light periods. The reactions were compared with those obtained after the injection of artificial cerebrospinal fluid (CSF). Since the optimum effect of DSIP in inducing delta-sleep after ICV administration has been reported at 6–8 nmol/kg [8,26], the substances were injected in a dose of 7 nmol/kg.

METHOD

Male CFY rats were used. The body weights of the animals were between 300 and 350 g at the time of the DSIP injection.

Under pentobarbital anaesthesia (50 mg/kg), golden jewelery screws were implanted over the frontal and parietal cortices and the cerebellum for EEG recording. Silicon diodes (type 1N 4148) cemented over the parietal cortex served for T_{br} measurements. It has been demonstrated that the T_{br} recorded in this way closely follows the course of the temperature measured with an intraperitoneal transducer, though the absolute value of T_{br} is about 1°C lower than that recorded in the abdominal cavity at an ambient temperature of 21°C [23]. For implantation of the guide tube into the lateral ventricle, the description provided by Tobler and Borbély [31] was followed. The ICV injections were made via a 33-gauge stainless steel cannula.

Five days before the experiments, the placement of the cannula and the intact drainage of the ventricle were tested via the drinking response elicited by angiotensin [5]. Thus, 100 ng angiotensin in a volume of 1 μl was injected into the ventricle. Provided that it reaches the 3rd ventricle, this substance elicits drinking in about 2 min by stimulating

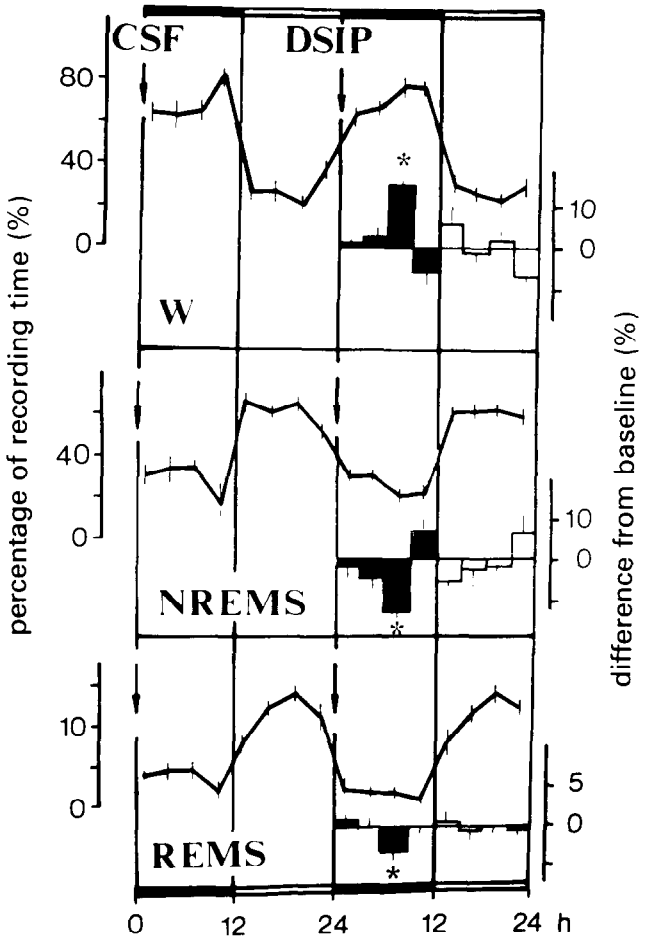

FIG. 1. Effects of ICV injection of DSIP (7 nmol/kg) on the sleep-wake activity of rats (n=7), as compared to the sleep-wake activity after the injection of artificial CSF. The mean values (±SE) for each vigilance state were computed for consecutive 3-hr periods after the injection of artificial CSF (baseline) and DSIP, and expressed as percentages of the recording time. Histograms show mean differences (±SE) from the baseline values (as percentages of recording time). Black and open columns indicate dark and light periods, respectively. Asterisks denote significant differences (at least $p<0.05$, paired t-test, 2-sided) with respect to the baseline values.

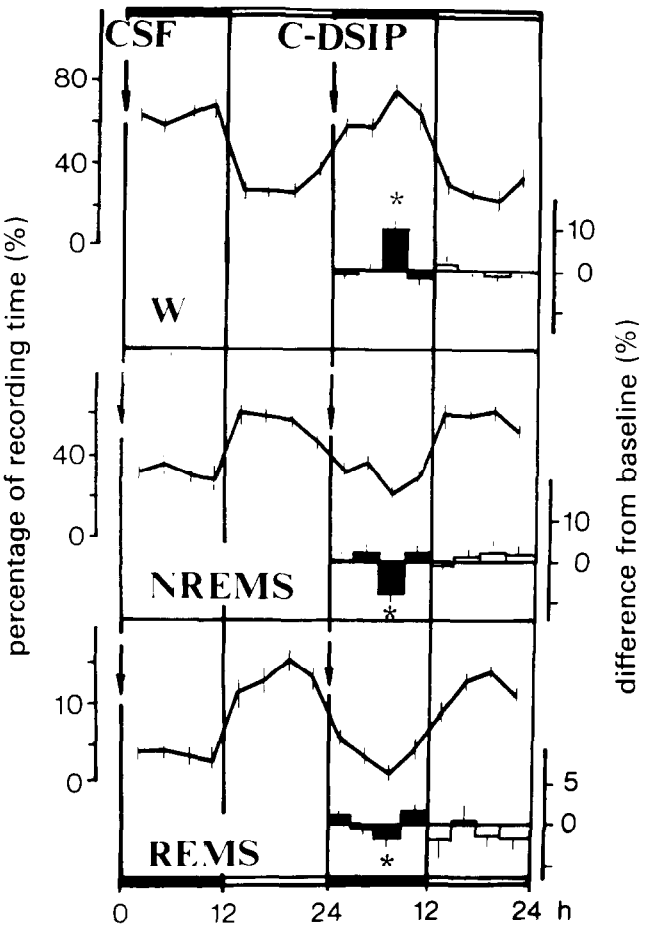

FIG. 2. Effects of ICV injection of ω-amino-caprilyl-DSIP (C-DSIP, 7 nmol/kg) on the sleep-wake activity of rats (n=11) as compared to the sleep-wake activity after the injection of artificial CSF. The mean values (±SE) for each vigilance state were computed for consecutive 3-hr periods after the injection of artificial CSF (baseline) and C-DSIP, and expressed as percentages of the recording time. Histograms show mean differences (±SE) from the baseline values (as percentages of the recording time). Black and open columns indicate dark and light periods, respectively. Asterisks denote significant differences (at least $p<0.05$, paired t-test, 2-sided) with respect to the baseline values.

preoptic structures [6]. Animals with a positive drinking test were selected for the experiments with DSIP or C-DSIP. After the sleep-wake recordings, the rats were retested for their reaction to angiotensin, and those which failed to respond were discarded from the computation of the results. Finally, trypan blue was injected into the ventricle and the cannula placements were checked in frozen sections. The present results relate only to those animals which met all these criteria. Thus, 7 and 11 rats were evaluated in the sleep-wake experiments with DSIP and C-DSIP, respectively, while the effects of DSIP on T_{hr} were studied in 6 rats.

The animals were allowed at least 10 days to recover after the operation. During this period they lived continuously connected to the recording cable in individual Plexiglas cages, with water and food available in the recording chamber. The ambient temperature was regulated at 21°C, and low-level white noise was maintained. The animals had been raised and kept on a light-dark cycle of 12 hr each, with lights on from 8:30 to 20:30 hr. Light was provided by daylight-type neon light tubes. For the sleep-wake experiments with DSIP, however, the light-dark cycle was reversed (lights on from 20:30 to 8:30 hr) 21–24 days before the recording.

In order to habituate the rats to the injection procedure, they received artificial CSF ICV for 5 days before the experiments. The artificial CSF (Na^+ 127.6 mmol/l, K^+ 2.5 mmol/l, Ca^{2+} 1.3 mmol/l, Mg^{2+} 1.0 mmol/l, Cl^- 134.5 mmol/l) was prepared according to Myers [22]. The artificial CSF injections were timed as for administration of the peptides, i.e., 10 to 15 min before dark onset. A volume of 3 to 3.5 μl was injected in about 1 to 2 min. Both DSIP (Hoffmann-LaRoche) and C-DSIP were dissolved in artificial CSF immediately before use and were injected in a dose of 7 nmol/kg.

After 5 days of CSF treatment, the sleep-wake activity was recorded for 24 hr following the CSF injection on day 6.

TABLE 1

MEAN PERCENTAGES (±SE) OF EACH VIGILANCE STATE IN THE FIRST HOUR OF THE DARK PERIOD, I.E., IN THE FIRST HOUR AFTER INJECTION OF ARTIFICIAL CSF, DSIP OR ω-AMINO-CAPRILYL-DSIP (C-DSIP)

	C-DSIP (n=11)		DSIP (n=7)	
	CSF	C-DSIP	CSF	DSIP
W	75.8 ± 5.4	84.5 ± 3.4	69.3 ± 6.0	70.6 ± 5.6
NREMS	22.2 ± 5.0	13.9 ± 3.0	26.8 ± 6.2	25.8 ± 5.0
REMS	2.0 ± 0.7	1.6 ± 0.6	3.9 ± 1.4	3.6 ± 0.7

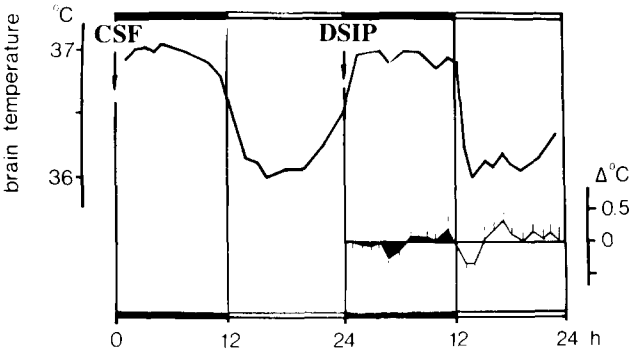

FIG. 3. Effects of ICV injection of DSIP (7 nmol/kg) on the brain temperature (T_{br}) in rats (n=6), as compared to T_{br} after the injection of artificial CSF. The curves at the top show the mean T_{br} calculated for consecutive 1-hr periods after artificial CSF (baseline) and DSIP. At the bottom, the mean differences (±SE) from the baseline values are presented. Shaded areas indicate dark periods.

DSIP or C-DSIP was then administered before the next dark period, and the recording was continued for another 24 hr.

The EEG and the motor activity of the animals (recorded as cable movements by means of piezoelectric force recorders) were used to score the vigilance states in 40-sec intervals. According to standard visual criteria, wakefulness (W), non-REM sleep (NREMS) and REM sleep (REMS) were distinguished. The records obtained after artificial CSF injection were regarded as baselines, and the effects of DSIP and C-DSIP were evaluated with respect to the corresponding baseline values by means of the paired *t*-test (2-sided). In the experiments in which the effects of DSIP on T_{br} were studied, T_{br} was recorded at 20-sec intervals throughout the baseline and DSIP days.

The amino acids 2-3-4 (Ala-Gly-Gly) of DSIP were replaced by ω-amino-caprilic acid to obtain C-DSIP. The analogue was produced by standard solid-phase synthesis via DCC coupling of the amino acids. Tert.-butyloxycarbonyl amino acids and Asp, Ser and Glu with benzyl side-protection group were used. Final cleavage and deprotection was performed by means of HF. Purification was carried out by preparative MPLC and HPLC, and the substance was subjected to amino acid analysis, thin layer chromatography and analytical HPLC. The characteristic physical constants of the C-DSIP were as follows: T.L.C. R_f^1=0.65 (n butanol:acetic acid:ethyl acetate:water=1:1:1:1), R_f^2=0.097 (n butanol:acetic acid:water=4:1:1); R_t=19 min (RP-HPLC, ODS Hypersil, 125×4 mm (5 μm) in 0.01 M ammonium acetate (pH=4.0), cont. 17% acetonitrile); amino acid analysis: $Trp_{0.82}$, $Aca_{0.71}$, $Asp_{1.12}$, $Ala_{0.98}$, $Ser_{0.98}$, $Gly_{1.08}$, $Glu_{1.18}$.

RESULTS

The baseline recordings after the ICV injection of artificial CSF showed large circadian variations for each vigilance state (Figs. 1 and 2). The highest amounts of W and NREMS were obtained in the dark and light periods, respectively, while REMS peaked in the second part of the light period.

The similarities of the baseline sleep-wake records for the rats in the normal (Fig. 2) and reversed (Fig. 1) light-dark cycles are obvious from the figures. In order to substantiate the identity of the sleep profiles, the 24-hr baseline records in the two groups of animals were compared through analysis of variance, by using the mean percentages of the vigilance states calculated for 1-hr periods. In agreement with the large circadian variations, analysis of variance showed significant time effects for each vigilance state (W: $F(23,384)=16.6$, $p<0.005$, NREMS: $F(23,384)=13.9$, $p<0.005$; REMS: $F(23,384)=15.0$, $p<0.005$). The group effects (W: $F(1,384)=0.1$; NREMS: $F(1,384)=1.8$; REMS: $F(1,384)=3.0$) and interactions (W: $F(23,384)=1.3$; NREMS: $F(23,384)=1.3$; REMS: $F(23,384)=1.1$) were not significant.

DSIP did not affect the sleep-wake activity for 6 hours after dark onset: neither the sleep-wake percentages calculated for the first postinjection hour (Table 1) nor those dtermined for the first and second 3-hr periods differed from the baseline values (Fig. 1). Six to 9 hours after the injection, however, the time spent in W increased significantly ($p<0.01$), while the amount of NREMS was reduced ($p<0.01$). REMS also decreased ($p<0.02$). Thereafter, i.e., in the remainder of the dark period and in the following light period, the percentages of the vigilance states were at the baseline level. Significant changes were not found in the amounts of the vigilance states calculated for the 12-hr diurnal periods.

The reaction to C-DSIP was similar to that described for DSIP (Fig. 2, Table 1). Thus, the sleep-wake activity was not affected for 6 hours after the injection. A significant increase in W ($p<0.05$) was found between postinjection hours 6 and 9, while both NREMS and REMS decreased ($p<0.05$). In the next 3 hr of the dark period and during the light period, the sleep-wake activity did not differ from the baseline records. The sleep-wake percentages calculated for the 12-hr diurnal periods did not display any significant changes.

After the injection of artificial CSF, T_{br} proceeded as predicted by the circadian rhythm; it was high at night and low during the light period (Fig. 3). Administration of DSIP was without any effect on T_{br}.

DISCUSSION

Although DSIP was originally isolated and synthesized with the aim of finding a sleep factor, on reviewing its effects on sleep reported from various laboratories, Graf and Kastin [8] came to the conclusion that the results were controversial. The present observations seem to make the picture even more complicated. While the failure to induce sleep by means of ICV administration of DSIP was in agreement with

the findings reported by Tobler and Borbély [31], an increase in W was hardly to be expected. As concerns the lack of any T_{br} reaction to DSIP, our observations corroborate the report that DSIP injection did not affect rectal temperature in rats at an ambient temperature of 22°C [34].

Attempts were made in our study to control the factors suggested as interfering seriously with the sleep-promoting effect of DSIP [19]. (1) It seems that DSIP provides protection against emotional stress [24,30]. The sleep-enhancing effect may therefore be related to a reduction of adverse stress reactions accompanying the treatment of the animals [31]. With this in mind, we tried to habituate the rats not only to the recording situation, but also to the injection procedure, including the timing of the DSIP administration. (2) Peptide activity has been reported to decrease in aging solutions [33]. Accordingly, the substances were always dissolved immediately prior to their use. (3) It has been demonstrated that the ICV injection of large volumes results in a non-specific reduction of the motor activity in rats [3]. Thus, DSIP was administered in a volume of 3 to 3.5 μl. (4) A bell-shaped dose-response curve, with a maximum reaction to 6–8 nmol/kg DSIP, has been found to be characteristic of the sleep-inducing effect of DSIP injected ICV [26]. The use of an optimum dose has been emphasized repeatedly, and the negative results reported by Tobler and Borbély [31] were attributed to the large dose of DSIP they administered [19]. A dose of 7 nmol/kg was therefore chosen for our experiments. (5) An effort was made in our studies to use animals in which not only the proper placement of the cannula, but also the functioning drainage of the ventricle could be verified. (6) Finally, the reversal of the light-dark cycle may influence the effects if time for adaptation of the circadian rhythm to the artificial light-dark schedule is not provided. The disturbances in the diurnal rhythm might seriously interfere with the action of DSIP, since it has been demonstrated that the substance affects circadian rhythms [9,10]. However, the rhythms of the sleep-wake activity of the rats in the normal and reversed light-dark schedules did not differ in our experiments, and the effects of DSIP and C-DSIP on the two groups were also identical. Therefore, our observations could not be explained by the change in the light-dark cycle.

In contrast to our failure to increase sleep by means of DSIP injected at dark onset, Ursin and Larsen [33] and Kafi *et al.* [14] found an enhancement of NREMS after the administration of DSIP to rats in the light period. It is conceivable, therefore, that DSIP acting in parallel with the high sleep pressure characteristic of rats during the light period may facilitate the sleep process, particularly when sleep is disturbed as a result of some environmental or experimental influence, while DSIP produces little effect when the sleep requirement is fully satisfied [12]. At night, however, the circadian rhythm predicts a minimum activity level for the endogenous sleep process and a high sleep threshold [2], which may make DSIP ineffective. Nevertheless, Inoué *et al.* [13] reported an increase of sleep in rats at night in response to the ICV infusion of DSIP. Thus, the differences between the injection and infusion techniques should also be considered. By means of infusion, an optimum concentration of DSIP can be maintained for a long period of time, and this may ensure that relatively weak tendencies to sleep, which have been reported in rats after meals [1,4] in the circadian active period, can be facilitated by DSIP. In contrast, DSIP in an acute injection at dark onset, is expected to be degraded quickly [11], before being able to facilitate any sleep tendency. A DSIP analogue, however, which is more resistant to enzymic breakdown than the original substance, could also be effective at night, even if it is administered in a single ICV injection. This might explain the increase of sleep in response to the injection of D-Trp^1-DSIP at night in an experiment otherwise exactly the same as described for the present studies; replacement of the N-terminal amino acid by D-Trp is expected to slow down the enzymic degradation of the substance, but with only a slight reduction of the activity [18].

However, DSIP was not ineffective in our experiments: a definite increase of W was found 6 to 9 hours after the treatment. The fact that C-DSIP produced the same effect as DSIP indicated that the increase of W could be considered a real reaction to the treatment. Although this reaction is the opposite of a sleep-enhancing action, an increased alertness was also observed during the day in man when DSIP was administered IV in the morning, i.e., at the onset of the circadian active period [25]. Moreover, Tobler and Borbély [31] found an increase in motor activity, appearing with a delay of several hours, after IP injection of a relatively large dose of DSIP to rats during the dark period. These observations contributed to the notion that DSIP is a factor of circadian programming rather than a sleep-promoting substance [8,28]. The present results may provide further evidence in support of this idea.

Wakefulness, however, increased only 6 hours after the injection of either DSIP or C-DSIP. Experiments with DSIP incubated in rat brain homogenates showed that the peptide was subjected to enzymic degradation at a high rate; the breakdown started with the release of the amino-terminal Trp and continued with the next N-terminal amino acids [11]. The C-terminal portion of DSIP was much more resistant. Similarly, the release of Trp in about 10 min has been suggested as the first step in the breakdown of DSIP administered ICV [19]. It therefore seems that DSIP was no longer present, or at least not in an effective concentration, when W increased. The substantial time lag between the injection and the reaction may suggest an indirect effect of DSIP [8]; however, the possibility that the increase in W was actually induced by some degradation product cannot be excluded. The available experimental findings concerning the reactions to the breakdown products of DSIP [26,27] did not exclude this possibility. Experiments testing the effects of the fragments of DSIP are now in progress.

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REFERENCES

1. Borbély, A. A. Sleep in the rat during food deprivation and subsequent restitution of food. *Brain Res* **124:** 457–471, 1977.

2. Borbély, A. A. Sleep regulation: circadian rhythm and homeostasis. In: *Sleep: Clinical and Experimental Aspects*, edited by D. Ganten and D. Pfaff. Berlin: Springer, 1982, pp. 83–103.

3. Borbély, A. A. and I. Tobler. Cerebroventricular infusion in the rat: depression of motor activity and paradoxical sleep. *Neurosci Lett* **12:** 75–80, 1979.
4. Danguir, J. and S. Nicolaidis. Intravenous infusions of nutrients and sleep in the rat: an ischymetric sleep regulation hypothesis. *Am J Physiol* **238:** E307–E312, 1980.
5. Dib. B. Effects of intracerebroventricular capsaicin on thermoregulatory behavior in the rat. *Pharmacol Biochem Behav* **16:** 23–27, 1982.
6. Epstein, A. M., J. T. Fitzsimons and B. J. Rolls. Drinking induced by injection of angiotensin into the brain of the rat. *J Physiol (Lond)* **210:** 457–474, 1970.
7. Feldman, S. C. and A. J. Kastin. Localization of neurons containing immunoreactive delta sleep-inducing peptide in the rat brain: an immunocytochemical study. *Neuroscience* **11:** 303–317, 1984.
8. Graf, M. V. and A. J. Kastin. Delta-sleep-inducing peptide (DSIP): a review. *Neurosci Biobehav Rev* **8:** 83–93, 1984.
9. Graf, M., J. B. Bauman, J. Girard, H. J. Tobler and G. A. Schoenenberger. DSIP-induced changes of the daily concentrations of brain neurotransmitters and plasma proteins in rats. *Pharmacol Biochem Behav* **17:** 511–517, 1982.
10. Graf, M., H. Christen, H. J. Tobler, P. F. Maier and G. A. Schoenenberger. Effects of repeated DSIP and DSIP-P administration on the circadian locomotor activity of rats. *Pharmacol Biochem Behav* **15:** 717–721, 1981.
11. Huang, J. T. and A. Lajtha. The degradation of a nonapetide, sleep inducing peptide, in rat brain: comparison with enkephalin breakdown. *Res Commun Chem Pathol Pharmacol* **19:** 191–199, 1978.
12. Inoué, S., K. Honda, Y. Komoda, K. Uchizono, R. Ueno and O. Hayaishi. Little sleep-promoting effect of three substances diurnally infused in unrestrained rats. *Neurosci Lett* **49:** 207–211, 1984.
13. Inoué, S., K. Honda, Y. Komoda, K. Uchizono, R. Ueno and O. Hayaishi. Differential sleep-promoting effects of five sleep substances nocturnally infused in unrestrained rats. *Proc Natl Acad Sci USA* **81:** 6240–6244, 1984.
14. Kafi, S., M. Monnier and J. M. Gaillard. The delta-sleep inducing peptide (DSIP) increases duration of sleep in rats. *Neurosci Lett* **13:** 169–179, 1979.
15. Kastin, A. J., P. F. Castellanos, W. A. Banks and D. H. Coy. Radioimmunoassay of DSIP-like material in human blood: possible protein binding. *Pharmacol Biochem Behav* **15:** 969–974, 1981.
16. Kastin, A. J., C. Niessen, A. V. Schally and D. H. Coy. Radioimmunoassay of DSIP-like material in rat brain. *Brain Res Bull* **3:** 691–695, 1978.
17. Kovalzon, V. M. and V. L. Tsibulski. Are there hypnogenic properties of synthetic "delta-sleep-inducing peptide (DSIP) "? *J High Nerv Act I P Pavlov* **30:** 1064–1066, 1980.
18. Kovalzon, V. M., V. N. Kalikhevich, F. Obál, Jr. and H. Kleinlogel. Hypnogenic effect of a peptide (D-Trp^1-DSIP) in rabbits and rats: preliminary data. In: *7th European Sleep Congress*, München, Abstracts, 1984, p. 176.
19. Monnier, M. and G. A. Schoenenberger. The peptidergic modulation of sleep with the delta sleep-inducing peptide as a prototype. In: *Functions of the Nervous System*, vol 4, edited by M. Monnier and M. Meulders. Amsterdam: Elsevier, 1983, pp. 161–219.
20. Monnier, M., L. Dudler, R. Gächter, P. F. Maier, H. J. Tobler and G. A. Schoenenberger. The delta sleep inducing peptide (DSIP). Comparative properties of the original and synthetic nonapeptide. *Experientia* **33:** 548–552, 1977.
21. Monnier, M., L. Dudler, R. Gächter and G. A. Schoenenberger. Humoral transmission of sleep. IX. Activity and concentration of the sleep peptide in cerebral and systemic blood fractions. *Pflugers Arch* **360:** 225–242, 1975.
22. Myers. R. D. Methods for perfusing different structures of the brain. In: *Methods in Psychobiology*, edited by R. D. Myers. London: Academic Press, 1972, pp. 169–211.
23. Obál, F., Jr., G. Rubicsek, P. Alföldi, G. Sáry and F. Obál. Changes in the brain and core temperatures in relation to the various arousal states in rats in the light and dark periods of the day. *Pflugers Arch* **404:** 73–79, 1985.
24. Scherschlicht, R., I. Marias, I. Schneeberger and M. Steiner. Model insomnia in animals. In: *Sleep, 1980*, edited by W. P. Koella and P. Levin. Basel: Karger, 1981, pp. 147–155.
25. Schneider-Helmert, D. and G. A. Schoenenberger. Effects of DSIP in man. *Neuropsychobiology* **9:** 197–206, 1983.
26. Schoenenberger, G. A. Characterization, properties and multivariate functions of delta-sleep-inducing peptide (DSIP). *Eur Neurol* **23:** 321–345, 1984.
27. Schoenenberger, G. A. and M. Monnier. Characterization of a delta-electroencephalogram-(sleep)-inducing peptide. *Proc Natl Acad Sci USA* **74:** 1282–1286, 1977.
28. Schoenenberger, G. A., M. Monnier, M. Graf, D. Schneider-Helmert and H. J. Tobler. Biochemical aspects of sleep regulations and the involvement of delta-sleep-inducing-peptide (=DSIP). In: *Sleep. Normal and Deranged Function*, edited by H. A. C. Kamphuiser, G. W. Bruyn and P. Visser. Mefar BV, 1981, pp. 25–47.
29. Schoenenberger, G. A., P. F. Maier, H. J. Tobler and M. Monnier. A naturally occurring delta-EEG enhancing nonapeptide in rabbits. X. Final isolation, characterization and activity test. *Pflugers Arch* **369:** 99–109, 1977.
30. Sudakov, K. V., V. T. Ivanov, E. V. Koplik, D. F. Vedjaev, I. I. Michaleva and A. S. Sargsjan. Delta sleep-inducing peptide (DSIP) as a factor facilitating animals' resistance to acute emotional stress. *Pavlov J Biol Sci* **18:** 1–5, 1983.
31. Tobler, I. and A. A. Borbély. Effect of delta sleep inducing peptide (DSIP) and arginine vasotocin (AVT) on sleep and motor activity in the rat. *Waking Sleeping* **4:** 139–153, 1980.
32. Ursin, R. Endogenous sleep factors. In: *Sleep Mechanisms*, edited by A. A. Borbély and J. L. Valatx. Berlin: Springer, 1984, pp. 118–132.
33. Ursin, R. and M. Larsen. Increased sleep following intracerebroventricular injection of the delta sleep-inducing peptide in rats. *Neurosci Lett* **40:** 145–149, 1983.
34. Yehuda, S., A. J. Kastin and D. H. Coy. Thermoregulatory and locomotor effects of DSIP: paradoxical interaction with d-amphetamine. *Pharmacol Biochem Behav* **13:** 895–900, 1980.