nigro-striatal mechanisms. In particular, melatonin or other pineal hormones evidently facilitate some degree of sensitization of postsynaptic dopamine receptors, to judge by the absence of increase of the response to apomorphine in repeated experiments after PE. Slowing of minute fluctuations of stereotyped movements in young rats after PE is yet another argument in support of the possible existence of such an effect.

Meanwhile this last observation may indicate a pulsating character of the release of pineal hormones, which is reflected in the rhythmic structure of stereotyped behavior. In fact, for example, distinct minute fluctuations of the melatonin level have been found in human plasma [8]. Probably for this reason, removal of the pineal gland leads to defects in the temporal organization of the dopaminergic mechanisms of the brain.

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AN ACTIVE ANALOG OF INACTIVE "SLEEP PEPTIDE"

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Delta sleep-inducing peptide (DSIP) is a nonapeptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu), isolated from blood flowing from the brain of rabbits exposed to low-frequency electrical stimulation of the intralaminar thalamic nuclei [13]. Despite intensive study of "sleep peptide" from various aspects [6, 12], its principal (hypnogenic) property remains at least in doubt [1, 5, 11, 14]. Data on the effect of this peptide on temperature regulation are equally contradictory [7, 15]. However, we know that slight modifications to the structure of neuropeptides, aimed at making them more resistant to proteolysis, such as by substituting certain L-amino acid residues for their optical D-isomers, can in some cases lead to a significant increase in their biological activity [3]. The study of proteolysis of the DSIP molecule through the action of brain enzymes in vitro has shown that the primary event is removal of the N-terminal tryptophan residue [9, 10]. The D-Trp^1 analog of DSIP was accordingly synthesized and its physiological activity studied.

METHODS

This peptide was synthesized by the classical method among other members of the group of structural analogs of DSIP [4]. The dry peptide was kept in a refrigerator at between 0 and

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TABLE 1. Action of Peptide (D-Trp[1]) DSIP on Sleep and Body Temperature

Experimental conditions	Mode of injection	Dose per kilogram body weight	Number of rabbits	Number of experiments	Amount of sleep (median), % of 3-hourly trace		Change in body temperature during 3 h of experient (median)
					slow	paradoxical	
Background	—	—	32	9	34,2 (14,9—56,1)**	6,4 (0,2—13,5)*	0 (—1,0—+0,5)*
Control	Intraventricularly	20 µg	38	9	39,8 (14,5—73,2)	2,5 (0—12,6)	0,7 (—0,2—+2,4)
Peptide	The same	0,7 µg	8	5	60,7 (38,5—75,0)*	3,8 (0—6,1)	1,6 (0,8—+1,9)*
	» »	7 µg	13	8	49,8 (39,8—74,1)*	4,4 (0—16,5)	1,8 (0,4—+2,2)*
	» »	70 µg	6	5	59,4 (37,2—64,8)*	3,3 (0—8,5)	1,6 (1,0—+2,0)**
Background	—	—	11	3	47,4 (29,6—56,1)	7,3 (3,7—10,5)	0 (—0,4—+0,3)
Control	Intravenously	20 ml	12	3	44,8 (33,3—71,3)	7,4 (4,5—11,9)	0,2 (—0,5—+0,4)
Peptide	The same	3 µg	4	3	45,6 (34,6—54,1)	5,0 (1,6—8,3)**	0,4 (0—+0,6)
	» »	30—70 µg	5	3	56,7 (47,5—68,1)**	8,0 (5,5—12,9)	0,2 (0,1—+0,3)
	» »	100—300 µg	3	3	48,2 (46,5—53,3)	5,4 (3,7—6,0)	0,2 (0,1—+0,3)

Legend. *P < 0.01 compared with control, **P < 0.05 compared with control by U test. Number of waves shown in parentheses.

5°C. A weighed sample of the freshly obtained peptide was dissolved in physiological saline immediately before each injection. Experiments were carried out on adult male Chinchilla rabbits. First, under local anesthesia, electrodes were inserted into the cranial bones of the animals above the frontal, parietal, and occipital cortex and the olfactory bulb, to record the ECG, and nichrome wire electrodes also were inserted intraorbitally to record the electrooculogram (EOG) and into the neck muscles to record the EMG. In addition, a cannula made from a syringe needle (external diameter 1 mm) was introduced into one of the lateral ventricles. The correctness of insertion of the cannula and preservation of the flow of CSF through the ventricular system were verified by means of a barbiturate function test [8]. For this purpose 250 µl of an acidified 2% solution of medinal was injected into the ventricle. If the medinal reached the periaqueductal gray matter, 5 min after the injection nystagmus developed. Final verification of the position of the tip of the cannula and of the flow of CSF was carried out by determination of the spread of dye at the end of the experiments at autopsy. After recovery for 1 week, with daily adaptation to the conditions of recording, the experiments began; in the course of them the EEG, EOG, and EMG were recorded for 3 h during the daytime, simultaneously in a pair of rabbits in the course of natural behavior, in compartments of a small experimental chamber. The chamber was kept in the general laboratory room and illuminated with scattered daylight, and was not soundproofed. The polygram was recorded repeatedly in each experimental animal in the following three situations: 1) background, recording before injections; 2) control, recording began 30 min after intraventricular injection of physiological saline in a volume of 20 µl/kg at the rate of 20 µl/min; 3) experiment, recordings were made after similar injection of a solution of the peptide in the same volume and in doses of 0.7, 7, and 70 µg/kg (about 0.7, 7, and 70 nmoles/kg). Some of the animals also were given an intravenous injection of the peptide in doses of 3, 30-70, and 100-300 µg/kg, in a volume of 20 ml/kg, into the marginal vein of the ear.

The results were subjected to statistical analysis by the Wilcoxon—Mann—Whitney nonparametric test [2].

RESULTS

It will be clear from Table 1 that under normal conditions (background) the rabbits spent 40-50% of their time asleep, and paradoxical sleep accounted for 6-7% of the recording time. The procedure of intraventricular injection (control) itself caused a small but significant increase in the duration of slow sleep (+15% compared with the background, taken as 100; P < 0.05), and considerable inhibition of paradoxical sleep (—60% compared with the background; P < 0.01). The total duration of sleep was unchanged. Injection of the peptide caused a marked increase in slow sleep (+40% of the control, taken as 100; P < 0.01) on account of shortening of the periods of waking, mainly during the 2nd and 3rd hours of recording (Fig. 1), with no change in the amount of paradoxical sleep. The cyclic character of sleep and the reactivity of the animals were undisturbed. The body temperature, which was significantly increased (by 0.7°C) under the influence of intraventricular injection, increased by a further 0.9°C through the action of the peptide. No significant differences were found between the three doses used (0.7, 7, and 70 µg/kg) in their hypnogenic or pyrogenic effects. The barbiturate and morphological control showed that hypnogenic effects were noted only if the solution reached the periaqueductal gray matter and (or) the floor of the fourth ventricle.

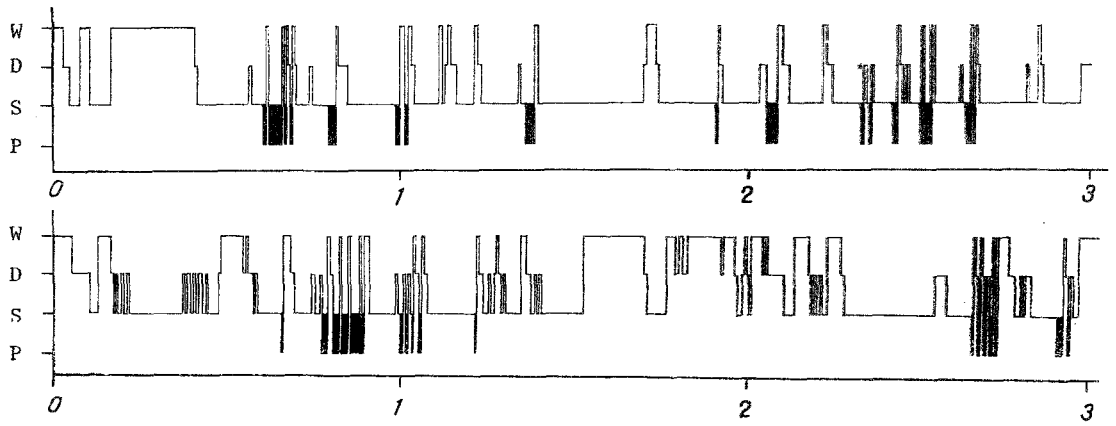

Fig. 1. Diagrams of two experiments on one animal. Above, after intraventricular injection of 7 μg/kg of peptide (D-Trp[1]) DSIP; below, after similar injection of physiological saline (75 μl). Abscissa, time (in h); ordinate, state: W) waking, D) drowsiness, S) slow sleep, P) paradoxical sleep. Slow sleep occupies 66.5% of recording time after injection of peptide and 51.3% in control, paradoxical sleep 8.2 and 6.1% respectively. Arrow indicates time of injection.

After intravenous injections of the peptide the increase in slow sleep (+25% of the control and background; $P < 0.05$) took place within a narrow range of doses (30-70 μg/kg). After injection both of lower (3 μg/kg) and of higher (100-300 μg/kg) doses, no effect on slow sleep was observed, only some inhibition of paradoxical sleep. The body temperature did not change significantly after intravenous injections of the peptide. However, the results of experiments with peripheral injection of the peptide require further verification, for they were obtained on only the minimal number of animals ($n = 3$).

Thus the aminopeptidase-resistant (D-Trp[1]) analog of DSIP, when tested on rabbits, unlike the original DSIP, exhibits marked hypnogenic and pyrogenic activity. According to some reports, "sleep factors" have their greatest action if administered "out of phase" with the circadian rhythm of sleep and waking. It can be tentatively suggested that if (D-Trp[1]) DSIP is administered at night, when rabbits are normally more active, an even stronger influence on sleep will be observed.

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