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GENERALIA

Editorial foreword. Experimental work in the field of sleep research was energetically furthered by the efforts of the eminent physiologist and Nobel Laureate Walter Rudolf Hess, in whose memory the Zürich lecture series 'The Principle of Order in Physiology' was held on March 14th, 1981. The following report documents the first controlled clinical experiments testing the delta-sleep-inducing-peptide on chronic sufferers of insomnia. In order that these findings may reach as wide a readership as possible, we are placing this brief but important report under the heading 'Generalia'.

The influence of synthetic DSIP (delta-sleep-inducing-peptide) on disturbed human sleep*

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Summary. The effects of acute intravenous administration of synthetic DSIP, 25 nmoles/kg b.wt, on disturbed human sleep were tested in 6 middle-aged chronic insomniacs. The results were: longer sleep duration and a higher quality of sleep with fewer interruptions; slightly more REM-sleep, but no day-time sedation or other side effects though the sleep enhancing capacity was seen for up to 6 h of night sleep. Sleep-promoting effects occurred only in the second hour after injection, in the first hour a slight arousing effect was indicated. The study corroborates the findings of previous investigations in healthy subjects and shows that DSIP has a normalizing influence on human sleep regulation.

Introductory remarks

DSIP (delta-sleep-inducing-peptide), a nonapeptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu: mol.wt 849), has been isolated from extracorporeal dialysate of cerebral sagittal venous blood from rabbits during electrical stimulation of the thalamus (Monnier and Hösli, 1965). The final isolation, amino acid analysis, sequence and synthesis, and the delta EEG (sleep)-inducing properties of the synthetic compound were first reported in 1976-1978 (Schoenenberger et al., 1977, 1978). The sleep-promoting and related general physiological effects of the compound after intraventricular (brain), intravenous and intraperitoneal application in different mammalian species have since been confirmed (Kafi et al., 1979; Monnier et al., 1977; Nagasaki et al., 1980; Polc et al., 1978). The endogenous distribution of DSIP-like material, its proteolyte degradation by brain extracts/slices as well as its passage through the blood-brain barrier have been demonstrated (Huang and Lajtha, 1978; Kastin et al., 1978, 1979). We published a summary of different effects of DSIP in various systems in 1979. A bell-shaped dose-response curve and acute or immediate as well as delayed or late effects have been reported (Schoenenberger and Monnier, 1979). A critical comment on the state of the experimental findings and problems has recently appeared (Kastin et al., 1980). The first report on experiments in man was published in 1980 from our sleep laboratory (Gnirss et al., 1980; Schneider-Helmert et al., 1981).

In a first human study we administered 25 nmoles/kg b.wt DSIP at 09.00 h by very slow intravenous infusions to 6 healthy middle-aged volunteers of both sexes in a double-blind, placebo-controlled laboratory study with continuous polygraphic recording on 12 channels simultaneously, behavioural observation by video monitoring, repetitive performance tests as well as subjective evaluations, and polysomnographic control of night sleep following the experimental days. Laboratory tests were made prior to and following the complete 60-h test period. Standardized visual and automatic analyses of these complex measurements yielded the following results:

a) No incompatibility, no forced sedation and no cardio-respiratory reaction or any adverse effects were noted;

* In honor of W.R. Hess, to mark the centenary of his birth.

b) immediately following DSIP infusion the subjects reported feelings of relaxation or mild pressure to sleep;
c) within 130 min following DSIP infusion, the sleep time increased by 58.8% (median);
d) after that time, neither subjective evaluation, performance in a stress test nor power spectral analysis of the EEG indicated sedation following awakening and reactivation of the subjects;
e) the night sleep recordings, after DSIP application in the morning, i.e. 13–22 h after its infusion, still indicated longer sleep time with both increased REM-sleep and NREM-sleep along with reduced waking and drowsiness.

It was concluded that DSIP actually displayed sleep-sustaining properties for at least some hours after application, and this was supposed not to be by means of forced sedation, as is known for a variety of psychoactive substances, but rather because it plays a part in situations normally conducive to sleep. In an extension of this study, 3 subjects volunteered for a repetition with varied infusion rates of the same DSIP dose. We concluded from these additional experiments that the infusion rate, i.e. the initial phase concentration, is an important factor for the effectiveness of DSIP in man, and that a parabolic relation between infusion rate and sleep-promoting effects is probable, with an optimum in the range of 2- to 6-min injection time. Blois et al. (1980) then confirmed such a delayed sleep-promoting effect of DSIP in normal sleepers but found a slight arousing effect immediately after the injection, which was given as a bolus, i.e. high initial phase concentration.

Improvements of *normal* sleep are difficult to demonstrate when substances or mechanisms are concerned which are supposed to be physiological, in contrast to hypnotics. Therefore, insomnia models have been developed and used. Under such experimental conditions DSIP counteracted the arousing and sleep-preventing effects of low morphine doses (0.1 mg/kg, cats), stress after exposure to dogs barking (rabbits), and i.p. injection of d-amphetamine (15 mg/kg, rats) (Scherschlicht et al., 1979; Scherschlicht, 1980; Yehuda et al., 1980). In accordance with these animal experiments the purpose of this work was to investigate the effects of acute DSIP applications on *pathological human sleep patterns,* i.e. those of defined chronic insomniac patients, taking carefully into account the experience gained from the preceding human studies.

Methodology

As an *experimental sample,* middle-aged, severe chronic insomniacs, otherwise healthy, were chosen, i.e. subjects (Ss) with a type of sleep disturbance that is relatively constant and would only respond to a major influence on sleep regulation. They were selected after physical and psychological examination and gave informed consent to participation in this study. They were 4 female Ss, aged 36, 44, 51 and 54 years, and 2 male Ss, 38 and 48 years old, all complaining primarily of unstable sleep with too many awakenings and difficulties in staying asleep; this was objectively confirmed by the sleep recording data of the placebo (p) nights.

Since acute pharmacological trials are extremely susceptible to environmental factors, and since the duration of DSIP effects in man is not yet exactly known and could even exceed 24 h to a minor degree (Blois et al., 1980; Schneider-Helmert et al., 1981), an *experimental design* was chosen that minimized or compensated for the influence of intervening variables, time trends and sequential effects upon the results. 2 Ss at a time underwent simultaneous polygraphic recordings in the sleep laboratory for 5 consecutive nights. The bed-times were individually kept fixed for all 5 nights according to the Ss' habitual schedule. The first 2 nights served for adaptation to the experimental condition with placebo (p) injections and complete recordings. The treatments on the 3 test nights were randomly attributed within each pair of Ss either to the sequence p-DSIP-p or DSIP-p-DSIP. It follows from this procedure that; 1. there is a low risk of influences on the test period from nonspecific positive (placebo) or negative (injection) treatment factors because of extended prior adaptation; 2. the effects of singular environmental variables, e.g. meteorological factors, were neutralized because they affected at the same time one S under p and one under DSIP treatment; 3. an underlying trend during the test period for a given S was mathematically eliminated by comparing the data of the middle test night (with DSIP or p treatment, respectively) with the basic slope as defined by the values of the preceding and the following night (both with p or DSIP, respectively). All treatments, recordings and visual analyses were carried out under *double-blind conditions.*

The treatment was applied immediately before bedtime by slow intravenous injections over 4 min of 6 ml saline solution containing (or not, for p condition) DSIP 25 nmoles/kg b.wt; i.e. a dose and injection rate that had been found to be in the effective range for sleep enhancement in normal Ss (Gnirss et al., 1980; Schneider-Helmert et al., 1981).

Measurements and statistics: Extensive laboratory tests were made in each S before and after the study for control of toxicity. Sleep was polygraphically recorded and analyzed according to standardized criteria (Rechtschaffen and Kales, 1968). The following rating scales and questionnaires were given daily for collecting self-estimations: a morning questionnaire concerning sleep and present status and an evening diary concerning day-time events and present

status, both including questions on possible side effects as incidentally seen with psychoactive substances; the Stanford sleepiness scale (Hoddes et al., 1973) as a continuous measure of activity during daytime, and the EWL (Janke and Debus, 1978), which is an adjective list for the dimensions activity, concentration, mood and anxiety, which was completed before and after each sleep recording. DSIP effects were evaluated with the t-test on the data of test night No 2 and the means of test nights Nos 1 and 3 for every S, taking into account the individual treatment sequence.

Results

The laboratory controls were all normal and the questionnaires did not reveal any side effects.

While the latency to sleep onset was unchanged and intrasleep or final waking times were not significantly affected by DSIP as compared to p treatment, there were tendencies ($p < 0.1$) toward a lower number of awakenings and a higher total sleep efficiency (ratio time asleep/recording time) (cf. table 1). Clearly significant effects of DSIP were found on variables indicating quantitative and qualitative improvement of sleep; the time and efficiency of actual sleep (stage 2 + slow-wave-sleep + REM-sleep) increased, whereas the relative amount (%) of sleep stage 1 (light sleep or drowsiness) and the number of arousals (interruptions of sleep by intervening stage 1 or wakings) per h sleep decreased. Concerning the sleep architecture, there was a tendency toward more REM-sleep, as an increase with $p < 0.1$ was noted in REM-sleep percent, REM-/NREM-sleep ratio, and in the number of sleep cycles per night. Effects on slow-wave-sleep (stages 3 + 4) could not be tested, because its representation within the different nights varied too much as a characteristic effect of the specific age range of our sample (cf. normative data in Williams et al., 1974).

There was no DSIP effect upon the day-time mental concentration, mood or anxiety. A comparison of EWL evening pretreatment/presleep ratings with morning/postsleep ratings could indicate effects of night sleep with DSIP treatment on various mood and activity scales. Table 2 shows the deviation of overnight changes on DSIP vs placebo-treated nights. Only increase of introversion and greater reduction of irritation were marginally significant. The Stanford sleepiness scale ratings from morning to evening following the test nights showed no significant difference in day-time activity or sleepiness respectively, though the mean sleepiness scale indicated even higher alertness in the morning after DSIP than after p nights (fig. 1).

For an analysis of the response time to DSIP administration, 60-min steps of the sleep recording data were analyzed individually for the crucial variables waking (W), stage 1 (S1) and total sleep time excluding S1 (TST2), all measured in min. The results are shown in figure 2. After a small arousing tendency within the

Table 1. Effects of DSIP treatment on main polygraphic sleep measures

Variable	Mean deviation (SD)	Significance (p)
Within total night sleep recording epoch:		
Number of awakenings	−6.0 (8.2)	<0.1
Waking time within sleep period (min)	−10.0 (38.0)	n.s.
Final waking time (min)	−15.0 (40.5)	n.s.
Total sleep efficiency (%)	+4.9 (8.1)	<0.1
Sleep efficiency excl. S1 (%)	+8.1 (5.8)	<0.01
Within sleep period (from sleep onset to final awakening):		
Number of arousals per h sleep	−3.6 (3.9)	<0.05
% sleep stage 1 (S1)	−4.4 (3.7)	<0.025
Total true sleep time (min)	+32.25 (22.9)	<0.01
% REM-sleep	+2.3 (3.7)	<0.1
Ratio REM-/NREM-sleep	+3.6 (5.8)	<0.1
Number REM-/NREM-sleep cycles	+0.3 (0.4)	<0.1

Table 2. Overnight changes on EWL scores: Mean deviations on DSIP vs placebo nights (% of total scale points) and significance

	Mean deviation	Significance (p)
Readiness for performance	+ 2.7%	n.s.
Introversion	+19.1%	<0.1
General well-being	+ 5.9%	n.s.
General deactivation	+11.5%	n.s.
Emotional irritation	− 7.4%	<0.1
Anxiety	+ 3.8%	n.s.

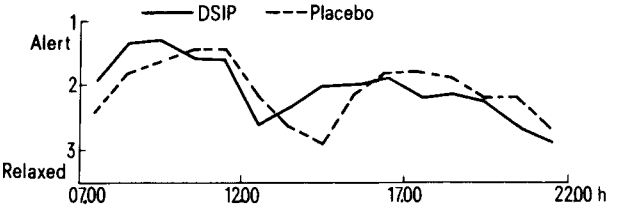

Figure 1. Mean sleepiness ratings on days after test nights with DSIP or placebo treatment.

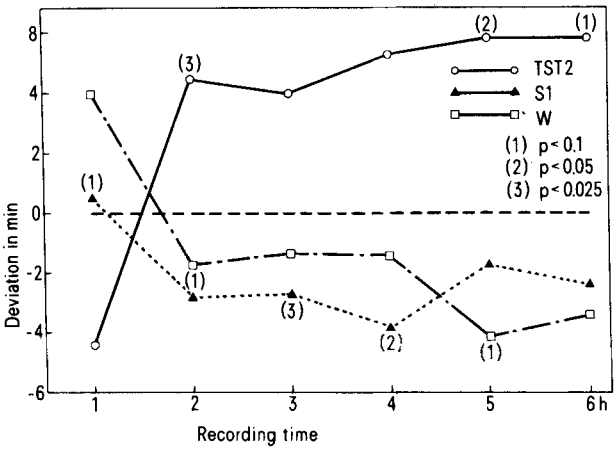

Figure 2. Analysis of 60-min steps of polygraphic night sleep recordings for wake time (W), stage 1 = drowsiness (S1) and true sleep (TST2). The points indicate mean deviations (in min) of values after DSIP treatment from placebo nights (= zero).

first 60-min epoch, significant sleep improvements were present from the 2nd through the 5th recording h after DSIP administration.

Discussion

The results of this first experiment with insomniacs corroborate the somnogenic properties of DSIP as suggested by previous studies in normal sleepers (Blois et al., 1980; Gnirss et al., 1980; Schneider-Helmert et al., 1981). The nonapeptide was of course named 'delta-sleep-inducing' because of its effect in the original experiments in rabbits (Monnier et al., 1972; Schoenenberger and Monnier, 1977), but there is now convincing evidence that DSIP has rather complex actions on sleep regulation (Graf et al., 1981a, b). In the chronic severe insomniacs of this study, acute application of DSIP induced an increase of actual sleep, which became more stable and resistant against interrupting tendencies, along with a reduction of drowsiness. Additionally, a tendency for enhancement of REM-sleep is noteworthy, because reduced REM-sleep is a common, though minor, symptom of insomnia, and analogous effects of DSIP were found in an insomnia model with cats given morphine (Scherschlicht et al., 1979). DSIP thus seems to improve disturbed sleep and to bring about physiological patterns. Yet no sedation was noted either in a short-term range of up to 4 h after injection as tested in the previous day-time experiment (Gnirss et al., 1980; Schneider-Helmert et al., 1981) or in the long-term range overnight, in this experiment. On the contrary, the morning alertness was slightly higher with DSIP treatment on the preceding evening than with placebo, according to subjective judgements. There was no indication of any side-effect or incompatibility. The tendency of the overnight mood changes towards higher introversion and lower irritation after DSIP treatment as compared to placebo is difficult to interpret. It could be a direct psychotropic effect, but we would rather attribute it to improved sleep after DSIP.

Considering the intravenous application and rapid degradation of DSIP (Huang and Lajtha, 1978; Marks et al., 1977), it is surprising that responses to DSIP injection became manifest only in the 2nd h but persisted throughout the night, which is in accordance with the findings of Blois et al. (1980). If, however, the effects of DSIP are even extended to the sleep of the 2nd night after administration, such a delayed action would definitely not have yielded false positive results in this study; on the contrary, the experimental design avoided sequential effects of the treatment and would in this eventuality rather have given false negative results for immediate DSIP action. On the other hand, the administration of DSIP immediately before bed-time, together with the delay of its sleep enhancing action, may be responsible for the lack of shortening of sleep onset time, which is in contradiction to animal studies (Polc et al., 1978; Scherschlicht et al., 1979).

Conclusions

Sleep is not a separate function, but is closely related to day-time psycho-physiological functions, and vice versa. This becomes especially evident in sleep disturbances, which therefore, as well as treatment effects, have to be considered as part of the sleep-wake rhythm (Schneider-Helmert, 1981). New findings in chronobiological research on DSIP in rats, and the specific binding of DSIP as well as the increase of its concentration in the pinealis, hypophysis and hypothalamus after tryptophan injection (Graf et al., 1980, 1981a, b) indicate an action on a high level sleep-wake organization rather than a direct effect only on limited mechanisms that are involved either in sustaining cortical activity or inducing sleep. Though we do not yet exactly know the mode of action of DSIP in man, the findings of our human studies lend support to the speculative view (Graf et al., 1980) that DSIP is a neuromodulator which could feed 'programs' into the complex, psychosomatic process of sleep regulation. The experiments with healthy and insomniac human adults thus far characterize DSIP action as a 'soft' enhancement of sleep with, at the same time, a complete lack of deactivation or other hang-over effects after sleep. This lack of massive sedation in its various aspects points to an intrinsically different and new mode of action of DSIP as compared to pharmaceutical preparations that induce and maintain sleep by, and in relation to, their general depressive action on vigilance. Thus, they are non-specific with regard to sleep promotion. DSIP on the other hand seems to improve a disturbed sleep regulation directly and with no immediate influence on cortical vigilance. This view is supported in this study with an insomniac population especially by the fact that acute DSIP application regularly showed sleep improvements, as proved by the significance levels of the critical variables, but did not provoke dramatic changes or even unphysiological sleep stage distributions. Therefore the correct attribute for DSIP effects on disturbed human sleep would be 'physiological'.

Since DSIP may also have additional effects on the central nervous system (Kastin et al., 1980), further studies are needed in insomnia and in other pathological states, associated or not with sleep disturbances, for exploring the possible actions of DSIP in man.

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The animal musks and a comment of their biogenesis

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Summary. The macrocyclic compounds occurring in animal glandular secretions are reviewed. Early hypotheses for their biogenesis from fatty acids via ω and β-oxidations are found to be inadequate. Radio-active acetate was incorporated into macrocyclic ketones of muskrat (*Ondatra* sp.) preputial glandular secretion, but radio-labelled stearate, oleate, and α, ω-octadecandioic acid were not incorporated.

Introduction

Elbert Hubbard's[2] adage that a perfume is any smell that is used to drown a worse one might have been coined with the animal secretions musk and civet in mind. Significantly, these scents were, and are, favourites with men for their own use. If nowadays more women should be found wearing them, then it is perhaps mainly for their effects on other women!

That civet has long been prized in perfumery is attested to in the plays of Shakespeare: '... he rubs himself with civet ... the sweet youth's in love' (Much ado about nothing, III, 2, 45), 'The courtier's hands are perfumed with civet' (As you like it, III, 2, 60), 'Give me an ounce of civet, good apothecary, to sweeten my imagination' (King Lear, IV, 6, 133). But Shakespeare had no illusions about the nature of civet: '... the very uncleanly flux of a cat' (As you like it, III, 2, 65).

Musk has been described as the most potent of all perfumes. Allegedly, the walls of the Empress