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Effects of Repeated DSIP and DSIP-P Administration on the Circadian Locomotor Activity of Rats

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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.* PHARMAC. BIOCHEM. BEHAV. **15**(5) 717–721, 1981.—Daily intravenous evening injections of 30 nmol/kg DSIP (Delta Sleep-Inducing Peptide) in rats adapted to a constant 24 hr light:dark cycle produced changes in the circadian locomotor behavior. After 3 days the normally high locomotor activity during the dark phase was reduced while during the light (sleeping) phase the animals became relatively more active. Similar, but more rapid and more marked changes were observed (with the same schedule of injections) after 0.1 nmol/kg DSIP-P (the analogue of DSIP phosphorylated at the serine in position 7). In fact the peptide and its analogue induced a relative reversal or shift of the circadian locomotor activity phases opposite to the persisting light:dark conditions (=Zeitgeber). This suggests that DSIP exerts rather complex "programming" effects on the circadian activities and has more than just a sleep-inducing activity.

Circadian rhythm Locomotor Activity Rats Delta Sleep-Inducing Peptide Phosphorylation

DSIP (Delta Sleep-Inducing Peptide), a nonapeptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu: MW=849), has been isolated from extracorporeal dialysate of cerebral sagittal venous blood from rabbits during electric pulse stimulation of the thalamus [11]. The final isolation, amino acid analysis, sequence, synthesis and the delta EEG (sleep)-inducing properties of the synthetic compound was reported in 1976/77 [17,18]. The sleep-promoting and related general physiological effects of the compound after intracerebroventricular, intravenous and intraperitoneal administration in various mammalian species were since confirmed [5, 12, 13, 14]. A bell-shaped dose-response curve and acute as well as delayed effects were also reported after intravenous injection in normal human volunteers. These were more pronounced in insomniac patients, as revelaed by polygraphic and TV recordings as well as extensive testing of psychophysiological functions [2, 15, 16]. The endogenous distributuion of DSIP-like material, its proteolytic degradation by brain extracts/slices as well as its passage through the blood-brain barrier was demonstrated [4, 6, 7, 10]. A critical comment on the state of the experimental findings and problems [8] and a summary of different effects of DSIP in various systems [19] have been reported.

Although it has been well established that in general two basic residues (Arg or Lys) close to the serine residue constitute part of the substrate's specificity of cAMP-dependent protein kinase [9,20], no systematic search for the phosphorylation of CNS peptides containing serine but lacking the paired basic residues in its vicinity has been carried out. Taking into account the important role of phosphorylation and dephosphorylation events in peptide processing and function on a membrane and signal transmitting level [1], we synthesized the serine-phosphorylated analogue of DSIP= DSIP-P. The present study was primarily carried out in order to investigate the effect of repeated daily DSIP and DSIP-P injections on the circadian rhythm of locomotor activities in rats.

METHOD

Chemicals and Technical Equipment

DSIP was obtained on commercial order from Bachem Fine Biochemicals, Switzerland, and tested for activity, intact amino terminal tryptophan and correct sequence, excluding the presence of the β-aspartate stereoisomeric form [18]. DSIP-P was synthesized by esterification of DSIP with $POCl_3$ (US Patent No. 4.165,312). 20 mg DSIP were dissolved in 3 ml $POCl_3$ by means of 0.5 ml formic acid, stirred and kept at 0°C for 200 hr. Excess $POCl_3$ and formic acid were removed by evaporation and the material was then

lyophilized. The crude product was purified by preparative thin-layer chromatography (acetone-water 7:3 v/v) and separation on Sephadex G-15 columns. The identification and estimation of the final product using analytical TLC and degradation of the ester by alkaline phosphatase (calf intestine, Serva, GFR) revealed a pure phosphorylated analogue of DSIP (25% yield).

Rats from our own inbred strain (KBS/K60) weighing between 200–300 g were used. Locomotor activities of the animals were registered by Animex activity meters type 0 (Farad Electronics, Sweden) which were adjusted to record displacements of the whole animal. Pulse registration was performed by a microprocessor system based on Zilog/Z80. The programs for the pulse accumulation, the tapeout routines and the endfile generation were established at the University Computer Center (Biozentrum, Basle) on a PDP 11/45 (Digital Equipment Corp., USA) by means of a cross-assembler and stored in an EPROM 2708. The accumulated pulses during 5 min intervals over the whole experimental time were first written on a cassette tape which was stored on a Mag Tape of the PDP 11/45 computer.

Experimental Procedure

Eleven groups of seven female animals were adapted to constant room conditions (21°C, 75% r.h.) and a constant light (6.00–18.00) cycle for 21 days (Fig. 1). Six out of seven rats in each group were then randomly placed in single cages for an adaptation period of 3 days. On the 4th day these animals were injected IV with 0.4 ml NaCl in the evening (17.30–18.00) and registration of locomotor activity for all animals was started synchronously at 18.00. Twenty-three hr thereafter, 3 randomly chosen animals out of one group of 6 rats were injected IV with either 30 nmol DSIP or 0.1 nmol DSIP-P/kg b.w. in 0.4 ml saline. The 3 remaining animals received saline and served as controls. This scheme of injection was then continued for a total of 4 registration days. During the experiment the animals received an equal daily portion of NAFAG (Switzerland) cubes MO.890 rat chow just after injection and water ad lib.

Data Evaluation

The activity level determined during the 23 hr following the initial injection of saline served as basic control (=A). On the following four days the 3 rats out of 6 injected with saline served as internal controls (=B) and the 3 animals receiving DSIP or DSIP-P respectively were termed test animals (=C) (Fig. 1). For each animal the accumulated pulses of intervals of 200 min of a test day were compared with the sum of the corresponding intervals of the control day. The means of the quotients of the internal control animals (=B) and the test animals (=C) were additionally compared between themselves. The result was expressed in percent activity of the test animals compared to the values of the basic control day on the one hand and additionally referred to the internal control animals on the other. Possible environmental influences were detected by the comparison with the "internal" control rats. Fortran programs were written to perform the calculations and to draw the graphs as follows:

Control day: $A_a - A_f$; Test day: $B_a - B_c$; $C_d - C_f$ (I)

Calculation:

$$\frac{B_a}{A_a} = q_{B_a}, \frac{B_b}{A_b} = q_{B_b}, \ldots \qquad \frac{C_d}{A_d} = q_{C_d}, \ldots \qquad \text{(II)}$$

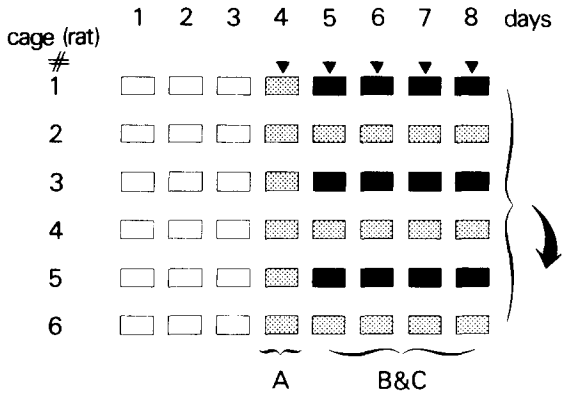

FIG. 1. Experimental design for testing motor activity in rats. Day 1–3 (open symbols)=adaptation days. Day 4=basic control with 0.4 ml NaCl IV; 5–8 experimental days; (▼): IV injections of either saline (shaded symbols)=internal controls or 30 nmol kg^{-1} DSIP/0.1 nmol kg^{-1} DSIP-P (dark symbols). From day 1–8 the animals were single housed, after 21 days of adaptation to a 12 hr light:dark (light=6.00–18.00) cycle as a group.

$$\frac{q_{B_a} + q_{B_b} + \ldots}{n_B} = \bar{q}_B \text{ (mean)}; \quad \frac{q_{C_d} + q_{C_e} + \ldots}{n_C} = \bar{q}_C \qquad \text{(III)}$$

$$\frac{\bar{q}_C}{\bar{q}_B} = Q \qquad \text{(IV)}$$

Q = % Activity of the test animals as compared to the internal controls, based on the comparison with the control days.

A = Accumulated pulses during a given interval for one rat on the control day.

B = Accumulated pulses during the same interval as for A for an internal control rat on a test day.

C = Accumulated pulses during the same interval as for A for a test rat on a test day.

a, b, c, d, e, f, = Numbering of the animals.

n_B, n_C = Number of internal control animals or test animals.

q = Quotient of a given interval during the test day as compared to the same interval of the basic control day for the same rat.

$\bar{q}$ = Mean of all q's.

RESULTS

An example of a first graphic evaluation of the locomotor activity of one experimental group of 6 rats during a first test day is shown in Fig. 2.

In order to compensate for possible differences in sensitivities of the activity meters the activity counts of the 3 test and the 3 control animals were normalized by dividing the sum for each group through the corresponding value of the control day. The quotient thus obtained for the control animals was arbitrarily set at 100%. For continuous visualization of the motor activities over time, the summed up activity counts of the 5 min intervals for the control and the test rats were then referred to this endpoint (=100%) of the internal controls. In the given example 30 nmol kg^{-1} DSIP

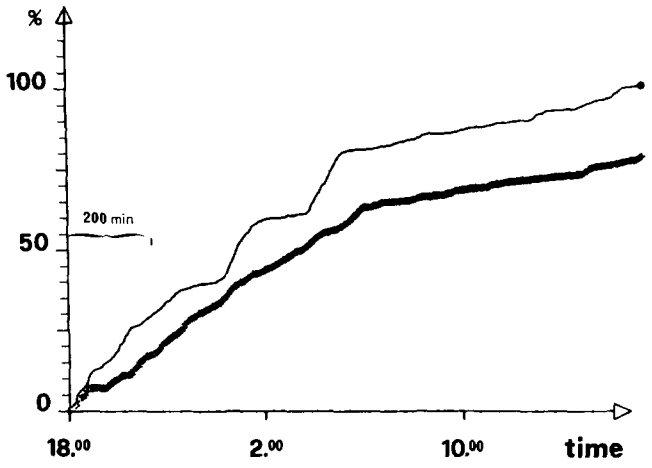

FIG. 2. Cumulative recording of the locomotor activity counts of 3 internal controls (thin line) and 3 test animals (hatched line). The impulses of the 5 min periods are expressed as % (ordinate) of the endpoint of the upper (control) curve=100%. Abscissa=time course from 18.00–17.30: 23.3 hr; 7 intervals of 200 min were used for further calculations.

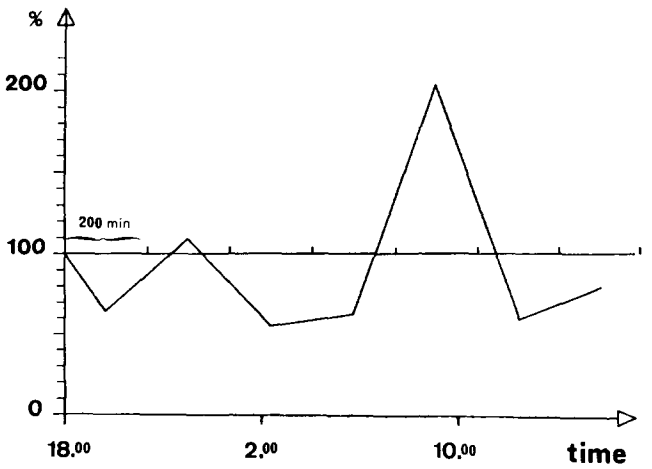

FIG. 3. Mean percentual deviations (ordinate) of the locomotor activity of the test rats of 200 min intervals over 23.3 hr (abscissa) from Fig. 2 as compared to the means of the control rats=100%. The values were additionally standardized by comparing to the corresponding intervals of the control day 4 (Fig. 1) before calculation.

IV reduced the overall motor activities of the test rats as compared to the controls. In a second step the activity counts for 200 min intervals (as indicated in Fig. 2) for the test rats and the control rats were again standardized by comparison to the counts of the identical time intervals of the control day of the corresponding animals. The means of the values of these quotients obtained for the control animals during the test day were then set at 100% and the percentual deviation of the means of the quotients of the test rats for each time interval is depicted in Fig. 3. This example shows that the effect of DSIP with respect to inducing decreased

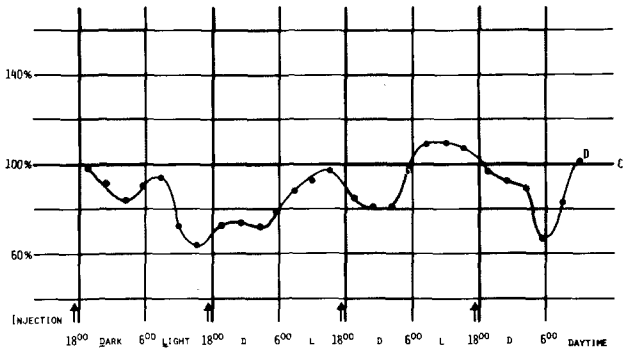

FIG. 4. Percentual deviations of the means of 200 min intervals of locomotor activities (ordinate) for all 17 rats injected IV with 30 nmole kg^{-1} DSIP (=D) compared to 17 control animals=100% (=C). Abscissa=4 experimental days (dark period=shaded: 18.00–6.00; light period=white: 6.00–18.00). ↑=injection (17.30–18.00).

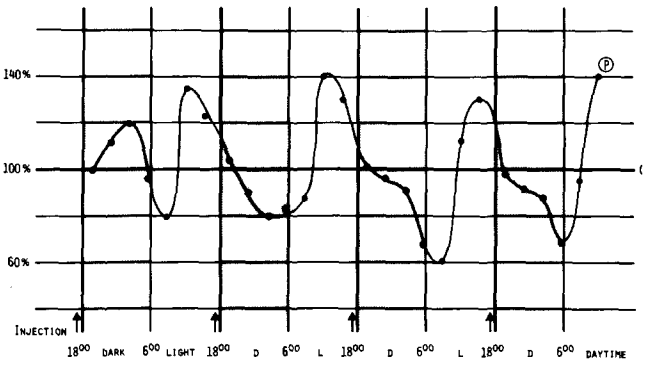

FIG. 5. Identical graphic representation as shown in Fig. 4 calculated for the test animals injected IV with 0.1 nmol kg^{-1} DSIP-P (n=15) as compared to NaCl controls (n=15).

motor activity during the night and a reversed behavior during daytime can clearly be demonstrated by this evaluation procedure and graphic display.

Therefore, an identical evaluation as shown in Fig. 3 was carried out for all 6 experimental groups treated with 30 nmol DSIP kg^{-1} IV and the 4 test days (n=34; 17 internal controls and 17 test animals; 1 control and 1 test rat were discarded). The results are summarized in Fig. 4 by the continuous curve of the deviations for the test animals vs the controls =100%. DSIP before the dark period caused a decrease in locomotor activity during the night as compared to the controls while the relative activity increased during the first half of the next light period as compared to the circadian motility of the control rats. However, during the whole second 23.3 hr the motility was decreased although still showing a slight and relatively reversed diurnal undulation. During the third 23.3 hr, i.e., after the third DSIP injection, the changes in the circadian locomotor behavior appeared more distinctly.

During the last 20 hr for the test animals a locomotor activity pattern different from that for the controls seems to be established. In contrast to day 2 and 3, in the first half of

the dark period only a slight decrease of the activities occurred which then became more pronounced in the second part of the night. From 6.00 to 14.00 this deflection normalized. This locomotor behavior appears to be in contrast to the maintained external light:dark cycle ("Zeitgeber").

According to an identical experimental protocol 0.1 nmol DSIP-P was injected to 5 groups of rats (n=30; 15 controls and 15 test animals). The results of the locomotor activities of these groups processed the same way as described are displayed in Fig. 5. After an increase in the late darkness and a decrease in the early light phase an elevation of the relative activity of the peptide-injected animals was seen. The following night DSIP-P administration produced a reduction during dark which persisted during the early daytime and a subsequent considerable increase of the locomotor activities of the test animals compared to the controls. After the first light:dark period and the decrease of the activities during the second night this increase could be interpreted as a rebound phenomenon. However, in the third 23.3 hr the phosphorylated peptide seemed to stabilize the locomotor activities of this group during the dark period followed by a decrease toward the morning. The pattern was then reversed to hypermotility after noon. This suggests that DSIP-P induced in addition to changes of the locomotor activities a shift of the circadian rhythm even when assuming the increase per se to be a rebound phenomenon. During the last 20 hr this new pattern was maintained until 14.00.

DISCUSSION

Several reports [2, 5, 13, 16] have shown that after intravenous injection DSIP exerts long lasting effects upon the EEG patterns in different animals and humans in such way that species-specific natural sleep-EEG recordings were induced. Apparently DSIP is not species-specific with respect to its effectiveness per se. The peptide, however, induces sleep behavior- and EEG-patterns of each species in a different and distinct way [5, 14, 15]. Moreover, a bell-shaped dose-response curve [19] as well as activities upon the preparation of the isolated rat head previously reported and delayed effects on motor activity in rats after repeated injections led us to the hypothesis that DSIP in addition to facilitating sleep may exert a chronopharmacological action as natural "programming" substance. This hypothesis was sustained by the following findings in normal human volunteers: no marked psychopharmacological effects such as influences on mood, stress situation or performance were found. However, the volunteers felt a subjective sleep pressure and also sleep EEG phenomena were increased immediately after the injection; but significant increases of SWS (stages 2–4) and REM-EEG patterns were more pronounced 9 hr later, i.e., the night following the morning injections [15]. This behavior as well as a shift in the diurnal mental performance evaluated by psychological tests with insomniacs (when an improvement in sleep quality rather than quantity was observed) sustained these tentative conclusions [16].

Although DSIP and DSIP-P display similar effects after repeated administration, some differences at least for the first two days of injection were observed. It appears that DSIP acts primarily by reducing the locomotor activity during the dark period and slowly inducing a shifted rhythm of the activities whereas DSIP-P immediately influences the circadian activity pattern of the rats, attaining its persisting effect on the third day. With regard to the much lower dose administered of the phosphorylated peptide (0.1 nmol kg^{-1} vs 30 nmol kg^{-1} for DSIP) and its more pronounced effect it may be speculated that DSIP-P represents the naturally active form of DSIP or that both forms exhibit two different activities by itself. In this respect possible effects of DSIP in vivo could be modulated by phosphorylation or dephosphorylation processes and the rapid disappearance of DSIP in the brain after intravenous injection and detection by RIA [7] not explained by the degradation rate [4,10] could be due to such changes of the molecular structure of DSIP. It is even conceivable that the phosphorylated DSIP analogue might in part represent the DSIP-like material found in the brain [6]. Furthermore, the suggested role of DSIP and DSIP-P respectively in regulating sleep activity within the circadian rhythm is feasible in terms of a modulating role of phosphorous-transferring events which are closely linked to energy-consuming and energy-producing processes during the rest-activity cycle.

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