Indian J Physiol Pharmacol 2000; 44 (4) : 428–434

THE EFFECT OF DELTA SLEEP-INDUCING PEPTIDE ON THE EEG AND POWER SPECTRA IN RAT

OLIVERA P. STANOJLOVIĆ*, DRAGANA P. ŽIVANOVIĆ AND VESELINKA T. ŠUŠIĆ

Department of Physiology,
School of Medicine,
University of Belgrade,
11000 Belgrade, Yugoslavia

(Received on February 3, 2000)

Abstract : The effects of delta sleep-inducing peptide (DSIP) on the EEG and power spectra of adult male Wistar rats (b.w. 180–220 g) were studied by power spectra analyses of EEG wave forms recorded continuously for 12 h after DSIP administration. The animals were given DSIP i.p. (1 mg/kg). Saline-injected rats served as the corresponding control. Recorded bursts of high amplitude EEG in the 1–9 Hz range (δ and θ) were found to be more frequent in DSIP-treated animals, while power spectra and (δ) wave activity were enhanced in comparison with the control and a statistically significant increase was registered in all experimental points after DSIP (2 h $P<0.05$; 4 h $P<0.05$; 5 h $P<0.05$; 6 h $P<0.05$; 7 h $P<0.01$; 11 h $P<0.05$). In addition, DSIP significantly elevated both the EEG output in the (δ) range and sleep activity. These results suggest that DSIP should be considered as a potential agent for the treatment of sleep disturbances in human medicine.

Key words : DSIP EEG power spectra rat

INTRODUCTION

Although studied by numerous authors, sleep still remains an enigma and its benefits at physiological, biochemical and cellular level are still far from being completely understood. As early as in 1930, there was an active debate on whether sleep represents a passive process resulting from the lack of sensory stimuli and discussion of von Economo (1) greatly contributed to this debate. Monnier et al. (2) reported the presence of a sleep-inducing factor in the venous blood of rabbits in which sleep has been induced by electrical stimulation of the thalamus. When recipient rabbits were administered the blood dialysate of the sleep-induced animals, they fell asleep and the induced sleep was characterized by a large amount of slow wave sleep (SWS) with predominant EEG activity in the (δ) band (1-4Hz) Besides; this infusion induced EEG and behavioural changes in recipient animals. The sleep-inducing factor was

*Corresponding Author

identified as a peptide consisting of 9 amino acid residues (Mr 848.98). It was shown to act at biochemical level by modifying thermoregulatory responses, inducing changes in several neurotransmitters and neuromodulators (3) and reducing stress (4, 5). In addition, it was reported to act as a potent analgesic (6) and it plays a role in programming circadian rhythm (7, 8) and during the last several years became interesting for its antiepileptic action (9–11).

The aim of the present study was to investigate the modulations of EEG activity and mean power spectra in adult Wistar rats after i.p. administration of DSIP in the dose of 1 mg/kg.

METHODS

Adult, 2-month-old male Wistar rats (170–200 g) reared in Military Medical Academy Breeding Laboratories, Belgrade, were used. They were given 25 g of food (Purina rat chow) per day and had a free access to water. The animals were maintained at ambient temperature (approximately 22°C and 12/12 h light/dark cycle with light switched on at 9 a.m.). They were housed individually in transparent plastic cages (55 × 35 × 15 cm).

For the EEG recordings three gold-plated screws were used. The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), positioned in a sterotaxic apparatus and recording electrodes were implanted over frontal, parietal and occipital cortices.

A classical EEG apparatus (a product of Alvar) with a modified output degree enabling to transfer output signals to the input circuit of 8-channel, 12-byte AN card PCL-711B (Advantech Co. Ltd.) installed into a computer was used. For digital acquisition and elaboration of the EEG signals, the corresponding software was developed enabling permanent and continuous registration of the EEG signals on a hard disk of a computer. Selected EEG power spectra were analyzed by MATLAB mathematical program. Frequency range was defined by a time constant (0.3s, lower and upper limit frequencies of 0.5 and 30 Hz). As a result of such an analysis, relative numerical values of individual EEG components were obtained. In the present study, epochs of 5-10 s were used for one hour time. The spectral power was plotted and the integrated energy signals were expressed as (μV^2/Hz or pW/Hz). Statistical significance of the differences in power spectra of the δ waves was determined by the Man Whitney U-test.

The animals were divided into two groups: 1. Control, saline-injected (n = 6) and 2. DSIP-injected (1 mg/kg, n = 6). The treatments were performed by i.p. route employing injection volume of 0.1 ml. DSIP was a generous gift of Dr. I. I. Mikhaleva of the N. I. Pirogov Medical Institute, Odessa, Russia. DSIP solution was prepared in sterile physiological saline immediately before the administration.

RESULTS

About 2 h after the application, DSIP produced EEG changes in the form of high-voltage waves with a predominant frequency in the 1-4 Hz range (δ = 81.53%) as shown in Fig. 1. Recorded bursts of high amplitude EEG in the 7-9 Hz range θ were found to be more frequent after DSIP administration as illustrated in Fig. 2. An increase of the sequential power density (pW/Hz) particularly in the δ-and θ-frequency bands was registered.

The EEG recordings from the left frontoparietal cortical leads in control saline-treated animals and after (1-12 h) DSIP injection and sequential power spectra of the corresponding EEG activity are presented in Fig. 3. Predominance of a high voltage slow wave activation (δ<4 Hz) of the EEG tracing and peak increases in EEG δ power spectra can be seen (Fig. 3).

The EEG δ power was increased following i.p. administration of 1 mg/kg DSIP in comparison with the corresponding control. Statistically significant DSIP-related differences in EEG power density of δ wave SWS episodes were observed (Man Whitney U-test) 2 h ($P<0.05$), 4 h ($P<0.05$), 5 h ($P<0.05$), 6 h (P0.05), 7 h ($P<0.01$) and 11 h ($P<0.05$) point upon DSIP administration (Fig. 4).

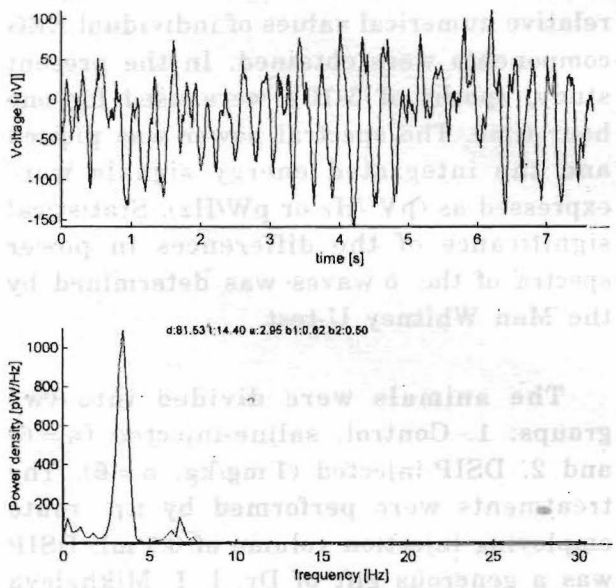

Fig. 1: Poweer spectra analysis of the EEG activity of adult Wistar rat after DSIP administration. A majority of the total spectral power (81.53%) can be seen in the 1-4 Hz range 2 h upon DSIP adminstration (1 mg/kg, i.p.).
Time calibration 1 sec, amplitude calibration 100μV.

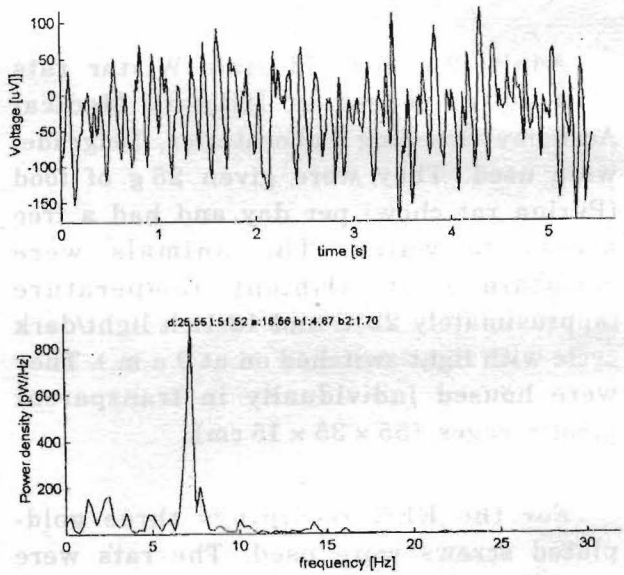

Fig. 2: EEG record and power density of DSIP-treated rats. DSIP increased bursts of high-speed tracing revealing the synchronous amplitude δ wave (7-9 Hz) activity
Time calibration 1 sec, amplitude calibration 100μV.

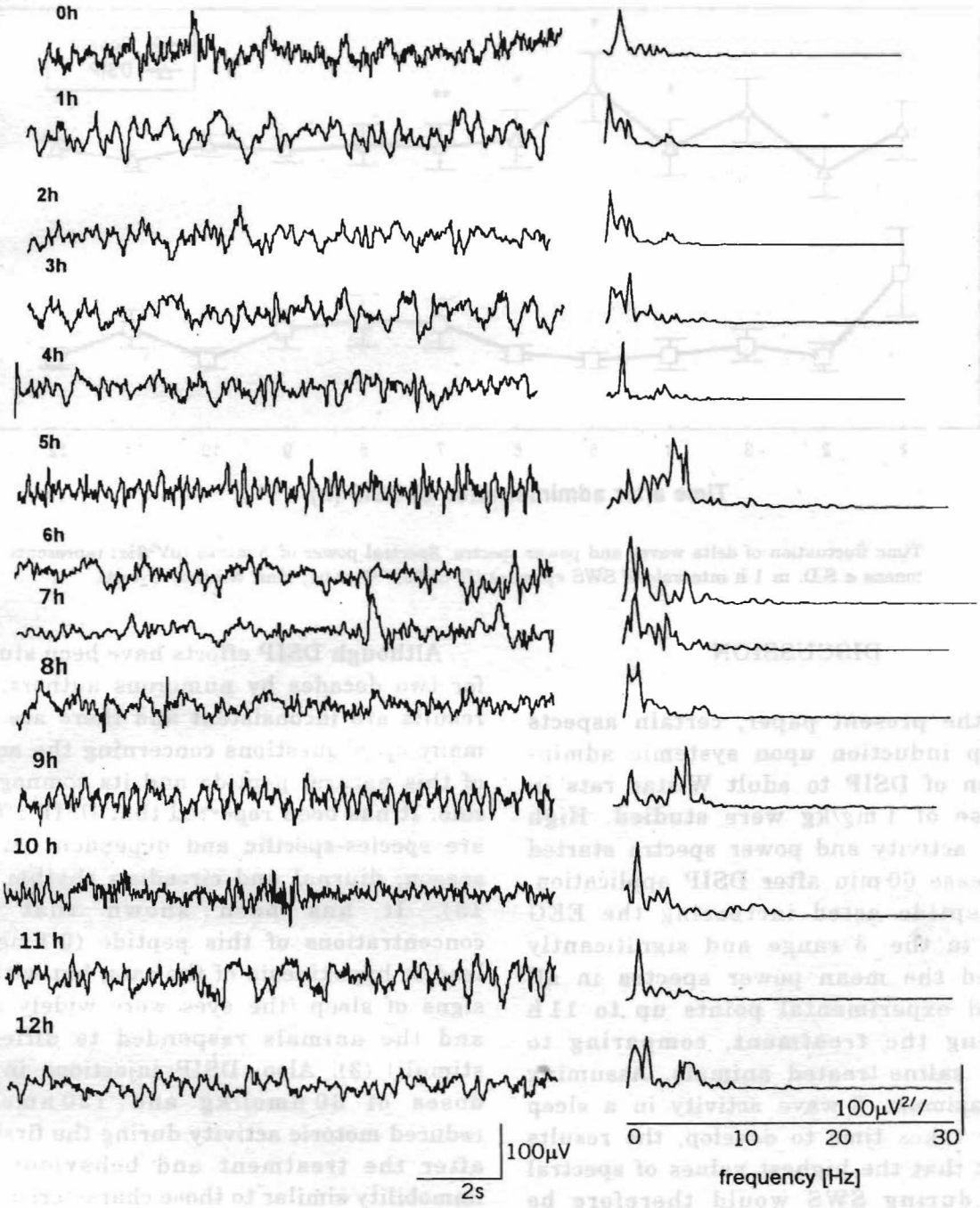

Fig. 3: Time course of change in EEG delta power spectra following DSIP administration.
Left - a continous EEG recording from the left frontoparietal cortical leads before (zero time, saline control) and after (1-12h) DSIP injection. Right - sequential power spectra of the corresponding EEG activity seen on the left ($\mu V^2/Hz$). Power spectra of thirteen consecutive EEG epochs (duration approx. 10 sec) express a tendency of being greater in a low frequency band (δ). Time calibration 2 sec, amplitude calibration 100 μV.

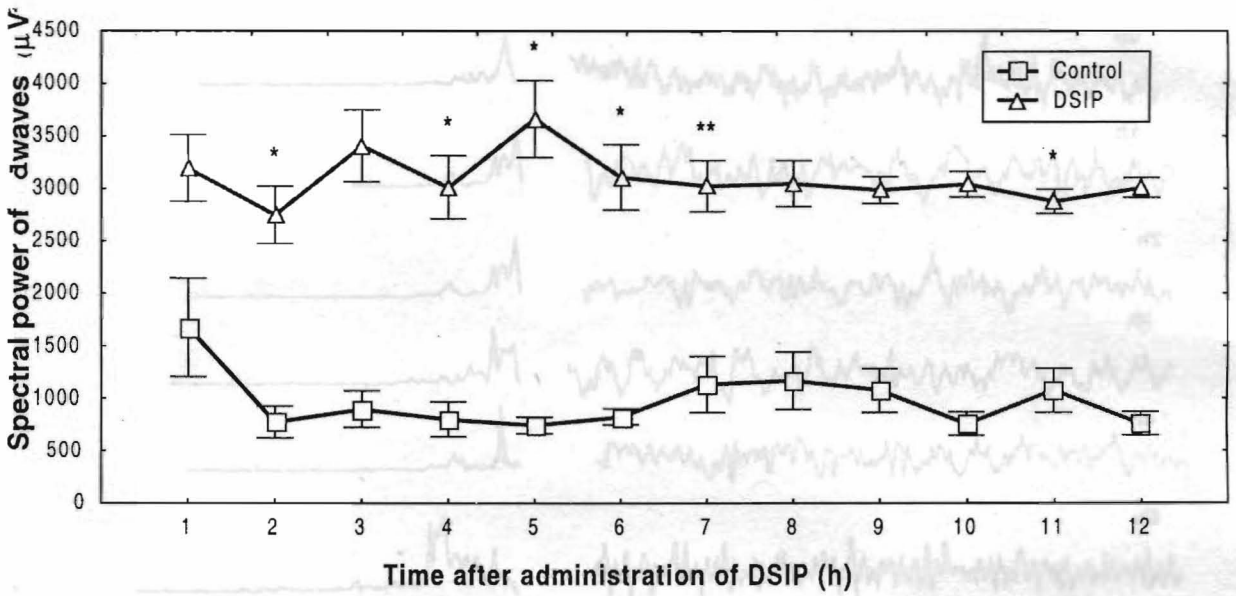

Fig. 4: Time fluctuation of delta waves and power spectra. Spectral power of δ waves ($\mu V^2/Hz$) represents means ± S.D. in 1 h intervals of SWS episodes (*P<0.05, **P<0.01, Man Wintney U-test).

DISCUSSION

In the present paper, certain aspects of sleep induction upon systemic administration of DSIP to adult Wistar rats in the dose of 1 mg/kg were studied. High voltage activity and power spectra started to increase 60 min after DSIP application. This peptide acted increasing the EEG output in the δ range and significantly elevated the mean power spectra in all checked experimental points up to 11 h following the treatment, comparing to control saline-treated animals. Assuming that maximum δ wave activity in a sleep episode takes time to develop, the results suggest that the highest values of spectral power during SWS would therefore be 1-12 h after DSIP administration. DSIP also elevated the duration and the number of episodes of SWS activity and therefore a total sleep time.

Although DSIP effects have been studies for two decades by numerous authors, the results are inconsistent and there are still many open questions concerning the action of this natural peptide and its somnogenic role. It has been reported that DSIP effects are species-specific and dependent on the season, diurnal and circadian rhythm (12, 13). It has been shown that low concentrations of this peptide (0.1 mg/kg) lead to hypokinesia of the rats, but without signs of sleep (the eyes were widely open and the animals responded to different stimuli) (3). Also, DSIP injections in the doses of 30 nmol/kg and 120 nmol/kg reduced motoric activity during the first 2 h after the treatment and behaviour and immobility similar to those characteristic for sleep were observed (14), but the clear signs of sleep were absent (15). Besides, a significant increase of δ electric activity was recorded in the rat brain upon i.p.

10. Shandra AA, Godlevskii LS, Mikhaleva II Lobenko AA, Paneko AV, Mazarati AM. DSIP and its role in the epileptic activity modulation. *Physiol J* (Russian) 1993; 79: 16–30.

11. Mendzheritskii AM, Uskova NI, Lysenko AV, Revinskii IV. The neuromediator mechanism of the additive action of the DSIP in experimental audiogenic epilepsy caused by hypokinesia. *Exp Clin Pharmacol* (Russian) 1996; 59: 8–10.

12. Graf MV, Zadine JE, Schoenenberger GA. Amphetamine-induced locomotor behavior of mice is influenced by DSIP. *Peptides* 1982; 3: 729–731.

13. Graf MV, Kastin AJ. Delta-sleep-inducing peptide (DSIP). *Neurosci Biobehav Rev* 1984; 8: 83–93.

14. Graf MV, Kastin AJ. Delta-sleep-inducing peptide (DSIP): An Update. *Peptides* 1986; 8: 1165–1187.

15. Schoenenberger GA, Monnier M, Graf M, Schneider-Helmert D, Tobler JH. Biochemical aspect of sleep regulation and the involvement of DSIP. In: Komphuisen HAC, Bruyn GW and Visser P (eds): Sleep. Normal and Deranged Function. Nefar BV. 1981: 25–47.

16. Miller LH, Turnbull A, Kastin AJ, Coy DH. Sleep-wave activity of a delta sleep inducing peptide analog correlates with its penetrance of the blood-brain barrier. *Sleep* 1986; 9: 80–84.

17. Patrick GA, Baxter CE, Harris LS. Delta sleep inducing peptide: Effects on locomotor activity and barbiturate-induced sleeping time. *Fed Proc* 1981; 40: 275.

18. Augustijns PF, Ng KY, Williams TM, Borchardt RT. Peptidyl dipeptidase A-catalyzed metabolism of delta-sleep inducing peptide in bovine brain microvessel endothelial cells: A cell culture model of the blood brain barrier. *Biochem Biophys Res Comm* 1995; 210: 987–994.

19. Yon L, Feuilloley M, Charnay Y, Vaudry H. Immunohistochemical localization of delta sleep-inducing peptide-like immunoreactivity in the central nervous system and pituitary of the frog Rana ridibunda. *Neuroscience* 1992; 47221–47240.

20. Vallarino M, Feuilloley M, You L, Charnay Y, Vaudry H. Immunohistochemical localization of delta sleep-inducing peptide (DSIP) in the brain and pituitary of the cartilaginous fish Scyliorhinus canicula. *Peptides* 1992; 13: 645–652.

21. Pu LP, Dubois PM. Fetal development of delta-sleep-inducing-peptide-like immunoreactivity in hypothalamus of Guinea pig with special regard to the prenatal colocalization with gonadotropin-releasing-hormone-like immunoreactivity. *Neuroendocrinology* 1992; 55: 66–73.