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Delta sleep-inducing peptide and its tetrapeptide analogue alleviate severity of metaphit seizures

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Abstract

The effects of delta sleep-inducing peptide (DSIP) and its tetrapeptide analogue, DSIP(1-4), on metaphit-induced audiogenic seizures were studied. Five groups of adult male Wistar rats were intraperitoneally treated with (1) saline, (2) metaphit, (3) DSIP, (4) metaphit + DSIP and (5) metaphit + DSIP(1-4). To examine blocking effects of DSIP and its analogue on fully developed metaphit seizures, the last two groups were injected after the eight audiogenic testing. The rats were stimulated using electric bell (on the top of the cage, generating 100 ± 3 dB and frequency 5–8 kHz, for 60 s) 1 h after metaphit and afterwards at hourly intervals during the experiment. For EEG recordings and power spectra, three gold-plated screws were implanted into the skull. In metaphit-treated animals, EEGs appeared as polyspikes and spike-wave complexes while the power spectra were increasing for 30-h period. The incidence and severity of metaphit-induced audiogenic seizures reached peak value 7–12 h after the injection. Both DSIP and DSIP(1-4) significantly increased power spectra of delta waves and decreased incidence of seizures, mean seizure grade and tonic component of metaphit-induced convulsions. Taken together, these results suggest that DSIP and its analogue DSIP(1-4) should be considered as potential antiepileptics.

Keywords: Antiepileptics; DSIP; DSIP(1-4); Metaphit; Audiogenic seizures; Power spectra

1. Introduction

Delta sleep-inducing peptide (DSIP) is a well-known natural somnogenic nonapeptide with many other physiological functions. In 1977, Monnier et al. isolated DSIP and afterwards many papers suggested that it stimulates slow wave sleep (SWS). DSIP is present in various organs, tissues and body fluids where it is believed to have various physiological roles nonspecific for sleep. Functional improvements were achieved by synthesizing numerous analogues of this peptide so far. DSIP analogues representing hepta- and octapeptides (also known as long), as well as tetrapeptide (termed short, used in our experiments), were synthesized with the aim of evaluating the peptide properties in sleep induction (Schoenenberger, 1984; Schoenenberger and Monnier, 1977; Schoenenberger et al., 1978).

To increase lipophilicity and resistance to proteolysis, (i.e., to stabilize the resulting peptide), amino acid sequence of the native peptide was changed in positions 1, 2 and 6, providing 11 new analogues. DSIP and its analogues were used for evaluating peptide specificity in restricting stress (Khvatova et al., 1995) and antiepileptic activity (Shandra et al., 1996). Their antiepileptic properties were assessed in seizures induced by corazol, picrotoxin, bicuculline and pharmacological kindling; DSIP analogues were shown to be more potent antiepileptics (Prudchenko et al., 1993; Shandra et al., 1993).

Metaphit (1-(1(3-isothiocyanatophenyl)-cyclohexyl(piperidine))) represents a phencyclidine (PCP) analogue differing in an isothiocyanate group in *m*-position of the aromatic ring. It was synthesized in 1985 by Rafferty et al. with the aim of designing a covalent marker for PCP receptors. This compound irreversibly binds to the PCP recognition site of the NMDA receptor complex and also to the dopamine uptake complex (Sershen et al., 1988). It

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decreases serotonergic functions (Nabeshima et al., 1989), antagonizes cocaine-induced locomotor stimulation by acylating cocaine-binding sites on dopaminergic nerve terminals (Berger et al., 1986) and acts as sigma receptor agonist in this brain area (Wang et al., 1987). Metaphit has been shown to induce audiogenic seizures after systemic and intracerebroventricular administration and to be truly epileptic in small rodents (Debler et al., 1989; Šušić et al., 1991; Živanović et al., 1998, 1999).

Audiogenic seizures expressed typical and characteristic signs of epilepsy (running, clonus and tonus), while grades from 1 to 4 (Anlezark et al., 1976) or 1 to 9 (Jobe et al., 1973) have been assigned to describe the extent and intensity of such behavioral changes.

In the present study, our efforts were focused on answering the question whether simultaneous action of metaphit and audiogenic stimulation (AGS) could be modified by DSIP or its synthetic analogue.

2. Material and methods

Adult male Wistar rats (2 months old, 170–200 g) raised in Military Medical Academy Breeding Laboratories, Belgrade, were used. They were housed individually in transparent plastic cages (55 × 35 × 15 cm) with 25 g of Purina rat chows per day and had free access to water. The animals were maintained at ambient temperature (approximately 22 °C and humidity of 50%) and 12/12-h light/dark cycle with light switched on at 9:00 a.m. None of the untreated animals screened for audiogenic susceptibility expressed seizure activity. AGS was applied for 60 s using an electric bell (on the top of the cage) generating 100 ± 3 dB and frequency of 5–8 kHz. The first stimulation was applied 1 h after metaphit administration and repeated thereafter at hourly intervals during the experiment. Audiogenic convulsive behavior was assessed by incidence of motor seizures and seizure severity grade determined as previously reported (Šušić and Marković 1993; Stanojlović et al., 2000a) using a descriptive rating scale from 0 to 3 (0 = no response; 1 = wild running only; 2 = wild running followed by clonic seizures; 3 = wild running progressing to generalized clonic convulsions followed by tonic extension of fore- and hindlimbs and tail).

For EEG recording, the rats were anesthetized with sodium pentobarbital (40 mg/kg ip), positioned in a stereotaxic apparatus and three gold-plated recording electrodes were implanted over frontal, parietal and occipital cortices. Experiments were performed in accordance with the Helsinki Declaration and animals were left to recover 7 days after the surgery. EEG apparatus (Alvar, France) with a modified output degree enabling to transfer output signals to the input circuit of an 8-channel, 12-byte AD card PCL-711B (Advantech) installed into a computer and the corresponding software were used. Length of epochs for EEG analysis depended on charac-

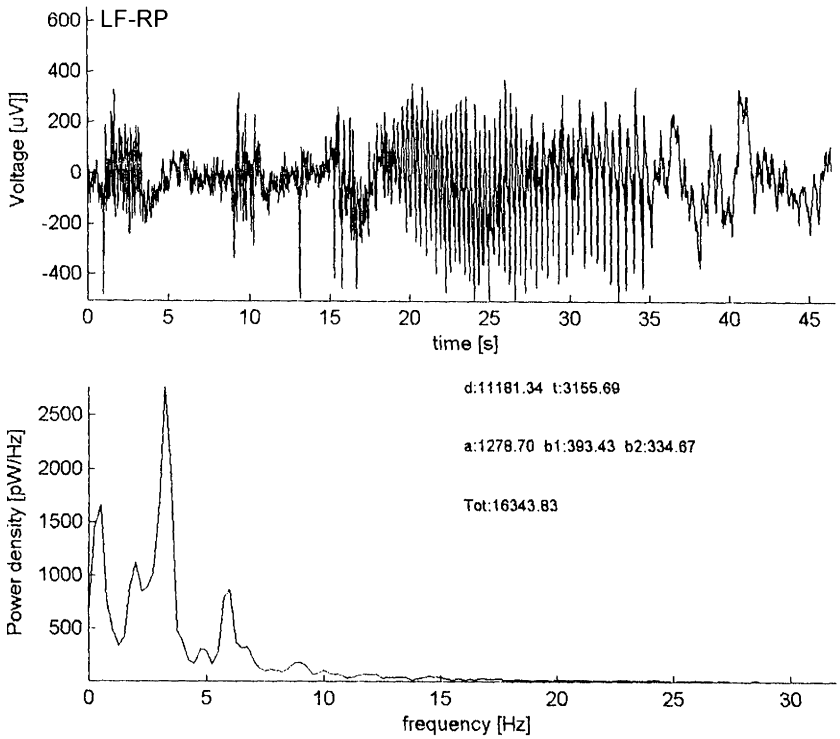

Fig. 1. EEG and power spectra manifestation of running (0–18 s) and clonic activity (18–35 s) induced by AGS (100 ± 3 dB, 60 s) in metaphit-treated rats (10 mg/kg ip). Note desynchronization and high-amplitude, low-frequency synchronized spiking activity in EEG. Total power spectra started to increase with fast tendency in the course of metaphit epilepsy. LF-RP: left fronto-right parietal cortex.

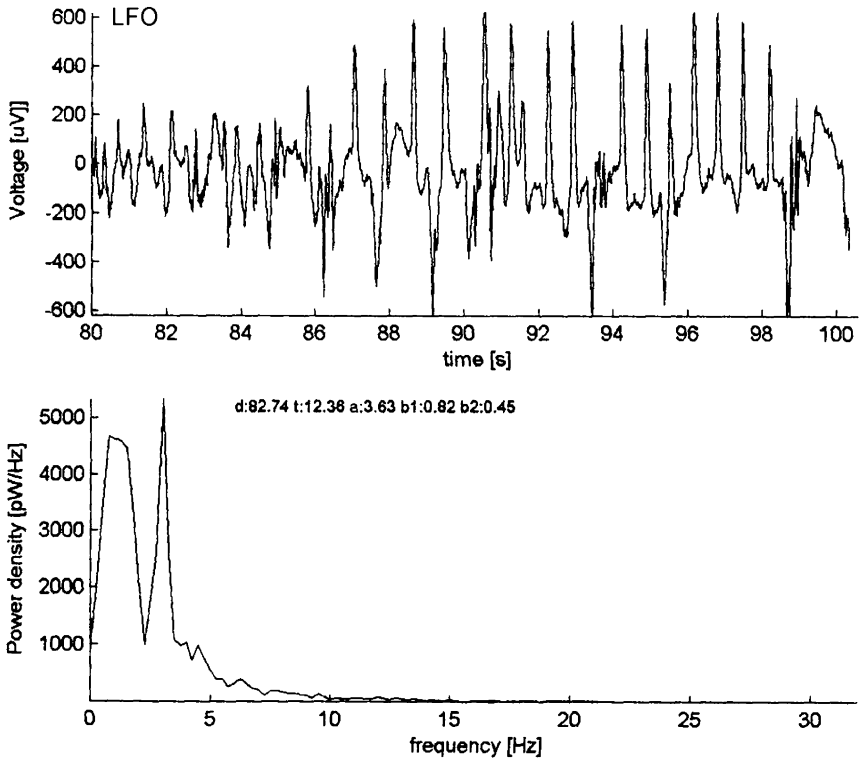

Fig. 2. Representative EEG records of complete motor seizure response (Grade 3). LFO: left frontooccipital cortex.

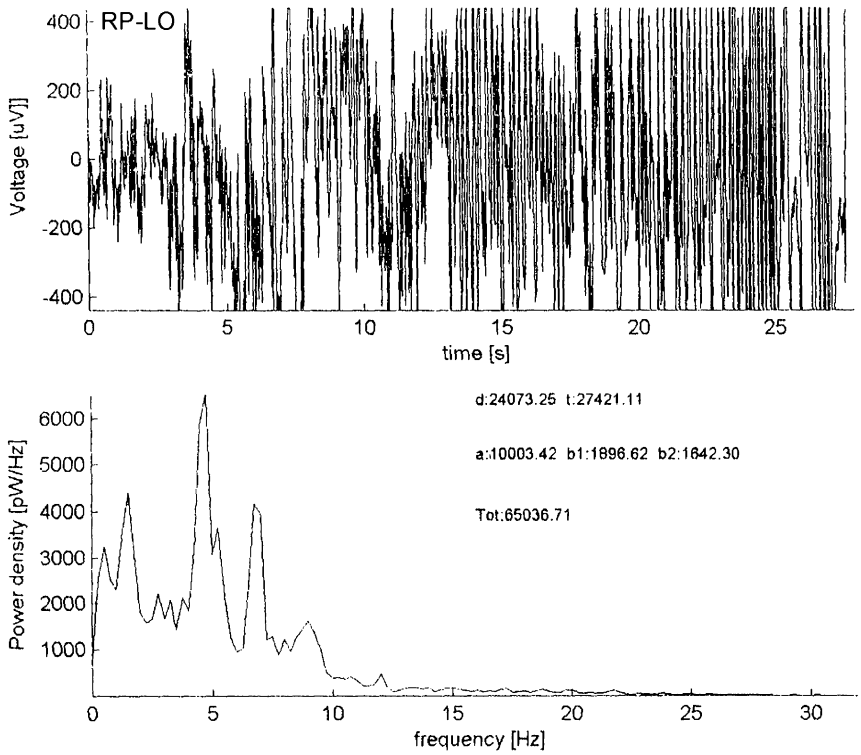

Fig. 3. Continuous spiking activity, often culminating in status epilepticus in EEG recordings and a very intense power spectrum (absolute values, pW/Hz) in a metaphit-treated rat during sound stimulation. RP-LO: right parietal-left occipital cortex.

teristic EEG changes such as burst of spike and wave complexes, EEG seizure responses and EEG effects of DSIP, varying broadly from 7 to 45 s. Selected EEG power spectra were analyzed visually and by Matlab software. Frequency range was defined by the time constant (0.3 s, lower and upper limit frequencies of 0.5 and 30 Hz, respectively). Delta frequency range is from 0.5 to 4 Hz. Such analysis provided absolute

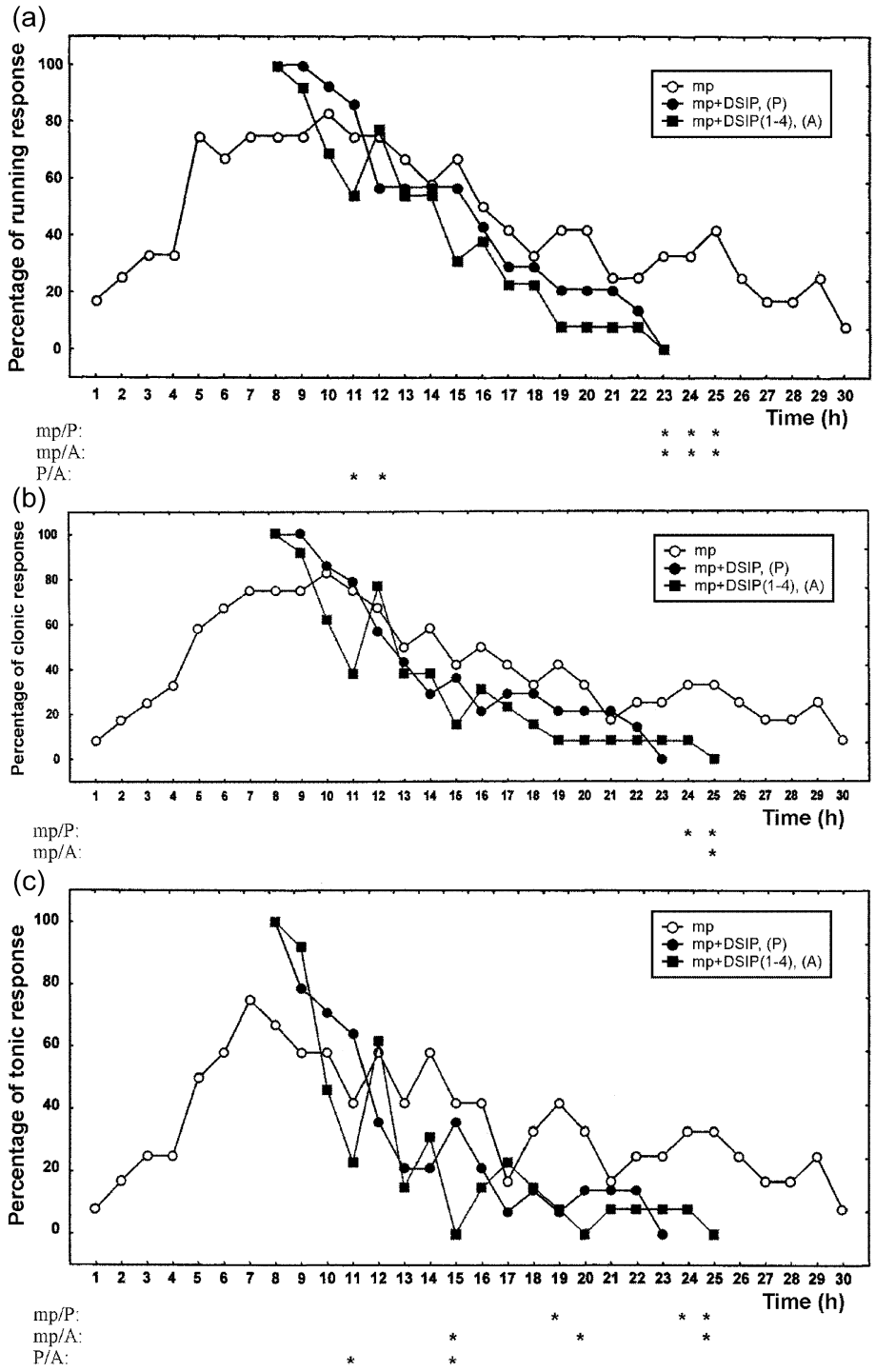

Fig. 4. (a) The incidence of running component in metaphit-induced seizures followed by DSIP and DSIP(1-4) injection after 8 h. All animals were exposed to an intense AGS at hourly intervals after metaphit injection; *y*-axis: incidence in percentages; *x*-axis: time (h). Comparisons: metaphit (mp) versus metaphit + DSIP(P), mp versus metaphit + DSIP(1-4) (A) groups and P versus A ($*P<.05$, $**P<.01$, Fisher's exact probability test). (b) The incidence of clonic component of metaphit-induced seizures followed by DSIP and DSIP(1-4) administration. For details, see caption in a. (c) The incidence of tonic component of metaphit-induced seizures followed by DSIP and DSIP(1-4)injection. For details, see caption a.

numerical values of individual EEG components. The power spectra were plotted and the integrated energy signals were expressed as pW/Hz.

Total of 51 animals were divided into the following groups: (1) control, saline injected ($n=6$); (2) metaphit administered (mp; 10 mg/kg; $n=12$); (3) DSIP injected (1 mg/kg, $n=6$); (4) metaphit (10 mg/kg) + DSIP treated (1 mg/kg; $n=14$) and (5) metaphit (10 mg/kg) + DSIP(1-4) injected (1 mg/kg; $n=13$). The rats were used only once. Metaphit-treated animals displaying seizures in eight previous tests received DSIP and DSIP(1-4) and AGS followed at hourly intervals in order to investigate their effects on fully developed seizures. Injected solutions were prepared in sterile physiological saline prior to intraperitoneal administration of 0.1 ml volume.

Statistical analysis included assessing significance of the differences between experimental groups: metaphit (mp) versus metaphit + DSIP(P), mp versus metaphit + DSIP(1-4) (A) and P versus A. Evaluation was done using Fisher's exact probability test for the incidence data, Kruskal–Wallis one-way ANOVA and Mann–Whitney U test for the differences in mean seizure grade and Kruskal–Wallis ANOVA test for the differences in mean duration of convulsive components ($*P<.05$, $**P<.01$).

DSIP and its analogue DSIP(1-4) were synthesized at the Shemyakin-Ovchinnikov Institute, Moscow, Russia. Dr. M.E.A. Reith of the University of Illinois, College of Medicine, Peoria, USA, kindly donated Metaphit methanesulfonate, produced by Sigma-Aldrich Chemical, USA.

3. Results

No convulsive response to AGS in control group was recorded.

All metaphit-treated animals expressed normal gross behavioral activity. The evolution of EEG changes in metaphit-treated animals is shown in Figs. 1–3. Metaphit-injected animals after 30 min showed initial EEG changes in form of sporadic spikes. There were desynchronizations in EEG, which overlapped latency period (period from the sound onset till running). Continually registered EEG changes were in the form of sporadic-isolated waves (corresponding to running), which progressed into series of polyspikes, synchronized spikes (clonic convulsion) and fast, high-voltage activity that represent typical seizure manifestation (tonic extension). The EEG power spectra increased and became more

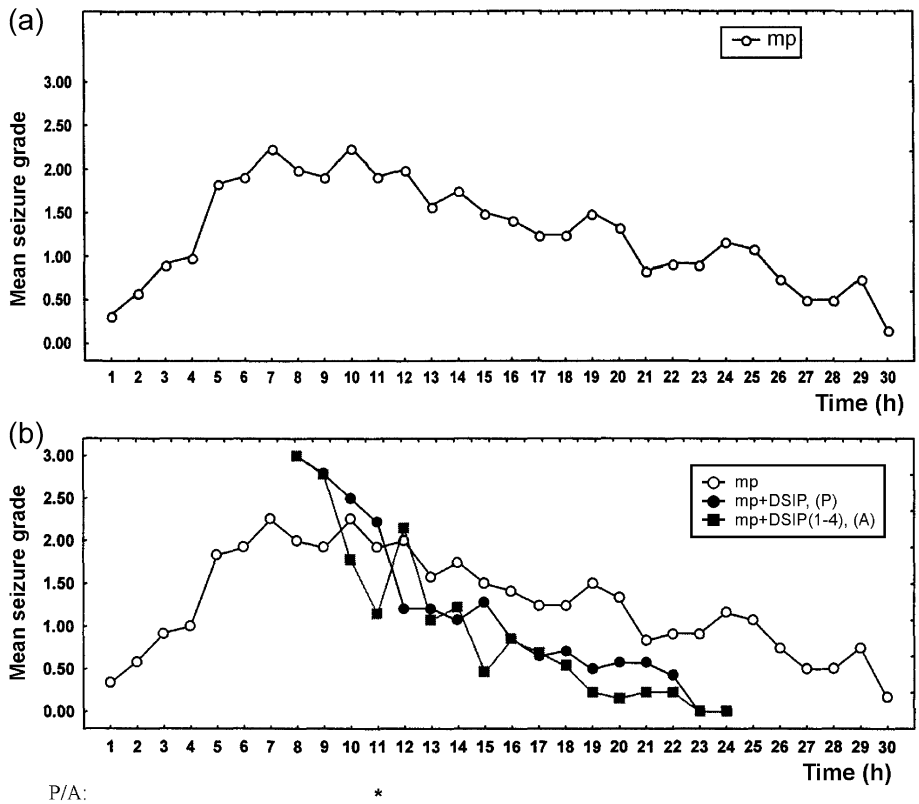

Fig. 5. (a) Time course of seizure grade in metaphit (10 mg kg $^{-1}$ ip)-induced convulsive activity. All animals were exposed to an intense AGS (100 ± 3 dB, 60 s) at hourly intervals after the injection. Severity of seizures (expressed as a seizure score) was determined by a descriptive rating scale ranging from 0 to 3 (0 = no response; 1 = wild running only; 2 = clonic seizures; 3 = tonic extension). Metaphit-treated rats—empty circles. (b) Time course of mean seizure grade upon metaphit injection followed by DSIP and DSIP(1-4) after 8 h. Comparison of mean seizure grades between groups: Kruskal–Wallis one-way ANOVA and Mann–Whitney U test ($*P<.05$, $**P<.01$).

intense in the period of sound onset and seizure events (Figs. 2 and 3). Time course studies revealed that the peak of metaphit-induced convulsive activity occurred 7–12 h after the injection, as judged by the intensity, high incidence and severity of seizures. Seizures in observed animals upon metaphit administration abated gradually and disappeared 30 h later. About 25% of metaphit-treated animals never responded to AGS and behaved normally during this critical time period. Therefore, they were excluded from further experiments.

Peak values in EEG delta power were registered during 2–11 h following DSIP administration (1 mg/kg ip). Statistically significant DSIP-related differences in EEG power spectra density of delta (δ) wave SWS episodes in comparison with the corresponding control were observed (Mann–Whitney U test) 2 h ($P<.05$), 4 h ($P<.05$), 5 h ($P<.05$), 6 h ($P<.05$), 7 h ($P<.01$) and 11 h ($P<.05$) upon DSIP injection.

Only those metaphit animals expressing epilepsy with a maximum incidence and seizure severity after the eight AGS received DSIP and DSIP(1-4). Time course studies revealed mild incidence reduction of all convulsive components occurring about 2–5 h after DSIP and DSIP(1-4) injection.

In fully developed convulsions, DSIP and DSIP(1-4) acted by decreasing seizure severity and significant reduction of running activity at the same time-points in comparison with the metaphit group [metaphit vs. DSIP; metaphit vs. DSIP(1-4) 23 h ($P<.05$), 24 h ($P<.05$) and 25 h ($P<.05$) after metaphit injection; Fig. 4a–c].

Incidence of clonic convulsions in DSIP group was significantly reduced in comparison with metaphit group in the two final time points 24 h ($P<.05$) and 25 h ($P<.05$). Also, a statistically significant reduction in the number of clonic convulsions was observed in DSIP(1-4)-treated group comparing to metaphit group 25 h ($P<.05$).

Tonic convulsion as the most severe component of seizure showed most marked reductions. Such reductions were observed in metaphit + DSIP-treated animals versus metaphit group at the following time points: 19 h ($P<.05$), 24 h ($P<.05$) and 25 h ($P<.05$). Reduction in the incidence of tonic convulsions was also recorded when metaphit + DSIP(1-4) versus metaphit group were compared at 15 h ($P<.05$), 20 h ($P<.05$) and 25 h ($P<.05$) after metaphit injection.

DSIP(1-4) analogue used in the present study expressed a more potent blocking effect than the genuine peptide. The differences in P versus A groups were statistically significant for running at 11 h ($P<.05$) and 12 h ($P<.05$) and those for tonic convulsions at 11 h ($P<.05$) and 15 h ($P<.05$) after metaphit administration (Fig. 4a–c). In metaphit + DSIP and metaphit + DSIP(1-4) groups, the mean seizure grades were reduced with the reduction being most prominent at 3 h ($P<.05$) after injection of DSIP or its analogue (Fig. 5a and b).

In addition to the differences in convulsive response, the groups also differed in the duration of several aspects of seizure components. Mean duration of running component in metaphit + DSIP rats was significantly shorter at 11

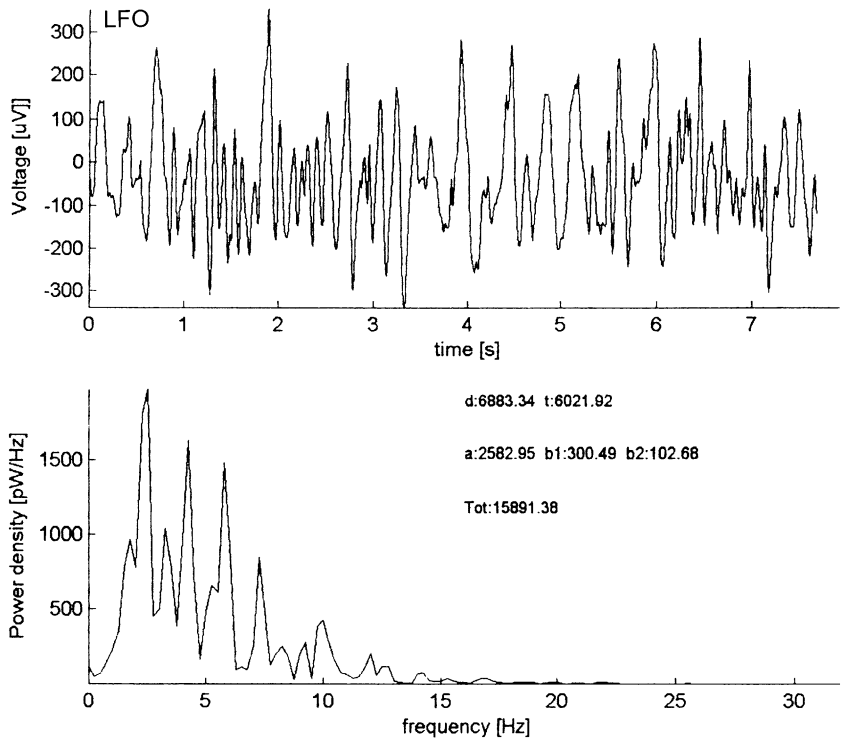

Fig. 6. Effect of DSIP (1 mg/kg bw ip) on suppression of brain excitability induced by metaphit. Note high amplitude waves in 1–6 Hz range (delta and theta) and a very intense power spectrum (pW/Hz) overlapping a sharp metaphit-induced spike-wave complex. LFO: left-frontooccipital cortex.

h [$F(8.53) = 21.05$, $P < .01$] and 14 h [$F(8.40) = 4.51$, $P < .05$] after metaphit administration when compared to the group treated with metaphit only. The same holds true for the duration of tonic component in metaphit + DSIP group 13 h after metaphit administration, in comparison with the group that received metaphit only [$F(8.29) = 6.90$, $P < .01$].

Also, duration of tonic component in metaphit + DSIP(1-4) group was significantly reduced at 12 h [$F(8.29) = 1.90$, $P < .01$] and 13 h [$F(8.29) = 6.90$, $P < .01$] in comparison with metaphit-only group.

There were also certain differences of brain activity in metaphit + DSIP rats in relation to that recorded in animals injected with metaphit alone. Polyspike and spike waves and fast EEG activity characteristic for metaphit epileptic action were overlapped with bursts of high amplitude EEG in the 1- to 9-Hz range (δ) and theta (θ) while power spectra activity was enhanced (Fig. 6).

4. Discussion

In the present paper, the effects of DSIP and its analogue DSIP(1-4) on metaphit-induced seizures upon systemic administration to adult Wistar rats have been studied, proving the decrease in incidence and duration of convulsive components, as well as severity.

We reported earlier that the dose of 1 mg DSIP/kg acted by increasing the EEG output in the δ range and significantly elevated the mean power spectra (Stanojlović et al., 2000b).

A PCP congener, metaphit, is known to induce generalized, reflex audiogenic epilepsy with typical EEG synchronization registrated in rats, guinea pigs and mice (Šušić et al., 1991), thus providing a suitable experimental model for the studies of the seizure development mechanism (Stanojlović et al., 2000a). It irreversibly binds to the ligand-gated ionic channels of NMDA/PCP receptor complex, opening it for Na^+ and Ca^{2+} influx and/or up-regulating NMDA receptor and consequently increases the receptor affinity for the binding of natural ligands, such as glutamate and aspartate (Debler et al., 1989; Lipovac et al., 1993).

To the extent of our knowledge, only one study dealing with the DSIP effect on audiogenic seizures was published (Mendzheritskii et al., 1996) and only one study evaluated the effect of the DSIP on NMDA receptors (Shandra et al., 1998). Our data presented here are in accordance with the results reported by Shandra et al. (1998), who demonstrated the existence of a connection between DSIP and NMDA excitatory receptors. The authors suggested that after DSIP administration, NMDA doses had to be increased to induce clonic and tonic seizures. DSIP decreases the liberation of glutamic acid from presynaptic terminals. This supports the view that DSIP plays a neuroprotective role against NMDA as an excitatory amino acid, which shares the same receptor with metaphit, although they bind at different binding sites.

Our results demonstrate reduction in incidence of running (in 23rd, 24th and 25th h), clonic convulsion (24th and 25th h) and tonic extension (19th, 24th and 25th h) after metaphit administration to animals in DSIP group (mp vs. P). The same holds true for the duration of running (in 11th and 14th h) and tonic activity (13th h). Speaking about the analogue group (mp vs. A), there was a reduction in the incidence of running (in 23rd, 24th and 25th h), clonic convulsion (25th h) and tonic extension (15th, 20th and 25th h) following the metaphit administration while the duration of tonic component was significantly reduced (12th and 13th h) when compared to metaphit-only group too.

It was reported earlier that DSIP administration dramatically increased the content of γ-aminobutyric acid and homocarnosine while brain glutamate and aspartate levels were decreased (Mendzheritskii et al., 1997). Also, DSIP was reported to activate GABA-decarboxylase and to decrease GABA-transaminase activity, thus increasing inhibitory and decreasing excitatory amino acid levels in brain cortex (Mendzheritskii et al., 1997; Mikhaleva et al., 1992). Taken together, this peptide creates an optimal ratio between inhibitory and excitatory amino acid neurotransmitters and may represent one of the endogenous control systems of the brain, thus exerting a protective effect against the seizures. The results obtained throughout the present study corroborate and extend the data on prolonged antiepileptic DSIP effect. It has been demonstrated that DSIP injection leads to a long-term alteration of proteolytic enzymes resulting in limited proteolysis, the change in the number of glutamate receptors and the functioning of the glutamate itself as an excitatory neuromediator (Mendzheritskii et al., 1997; Shandra et al., 1998).

Anticonvulsive DSIP action significantly decreased locomotion behavior in hypomotility state (Graf et al., 1982) after the application of large doses (1 or 3 mg/kg) (Yehuda et al., 1980).

Miller et al. (1986) observed that a DSIP analogue penetrates the blood-brain barrier more readily than DSIP itself, producing significantly lower locomotor activity. The reason for a decreased convulsive response after DSIP was suggested to originate from an enhanced threshold, so that the protection during the night period was improved (Yehuda and Mostofsky 1993). Our results suggest that DSIP(1-4) is able to antagonize the epileptic symptoms (running in 11th and 12th h; tonic convulsion: 11th and 15th h; mean seizure grade: 11th h) occurring because metaphit induced increased audiogenic seizure susceptibility more efficiently than the genuine peptide (P vs. A). Taken together with the facts that DSIP and its analogue penetrate the blood-brain barrier after intraperitoneal administration and have no harmful effects when overdosed, obtained data put them in line of promising antiepileptics. The action of DSIP analogue was obvious in all experimental points (8–30 h) being in accordance with the data of other authors who

observed seizure intensity decrease and latent period lengthening in picrotoxin-induced convulsions upon administration of DSIP and its analogues, the latter exhibiting considerably higher efficiency than DSIP itself (Schoenenberger 1984; Mendzheritskii et al., 1997). DSIP acts as a potent antiepileptic in all aforementioned experimental seizures induced by GABA blockers (corazol, picrotoxin, bicuculline and tiosemicarbazide) (Prudchenko et al., 1993; Shandra et al., 1993). Our study conveys new information about the DSIP and its analogue action on NMDA receptors.

All aforementioned data together with our results support the idea that DSIP could represent one of the factors of the endogenous stabilization of brain excitability. Structural DSIP(1-4) was shown to be a more potent antiepileptic in generalized metaphit-induced convulsive activity than genuine peptide.

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References

Anlezark G, Horton RW, Meldrum BS, Sawaya MCB. Anticonvulsant action of ethanolamine-*O*-sulphate and di-*n*-propylacetate and metabolism of γ-aminobutyric acid (GABA) in mice with audiogenic seizures. Biochem Pharmacol 1976;25:413–7.

Berger P, Jacobson AE, Rice KC, Lessor RA, Reith MAE. Metaphit, a receptor acylator, inactivates cocaine binding sites in striatum and antagonizes cocaine-induced locomotor stimulation in rodents. Neuropharmacology 1986;25:931–3.

Debler EA, Lipovac MN, Lajtha A, Zlokovic BV, Jacobson AE, Rice KC, et al. Metaphit, an isothiocyanate analog of PCP, induces audiogenic seizures in mice. Eur J Pharmacol 1989;165:155–9.

Graf MV, Zadine JE, Schoenenberger GA. Amphetamine-induced locomotor behavior of mice is influenced by DSIP. Peptides 1982;3:729–31.

Jobe PC, Dailey JW, Reigel CE. Noradrenergic and serotonergic determination of seizure susceptibility and severity in genetically epilepsy-prone rats. Life Sci 1973;39:775–82.

Khvatova EM, Rubanova NA, Prudchenko IA, Mikhaleva II. Effect of DSIP and some analogues on the activity of monoamine oxidase type A in rat brain under hypoxia stress. FEBS Lett 1995;368:367–9.

Lipovac MN, Debler EA, Zlokovic BV, Jacobson AE, Rice KC, de Costa B, et al. Metaphit-induced audiogenic seizures in mice: II. Studies on *N*-methyl-D-aspartic acid, GABA and sodium channel receptors and on the disposition of metaphapit in the brain. Epilepsia 1993;34:211–9.

Mendzheritskii AM, Uskova NI, Lysenko AB. The neuromediator mechanism of the additive action of the delta sleep-inducing peptide in experimental audiogenic epilepsy caused by hypokinesia. Eksp Klin Farmakol 1996;59:8–10.

Mendzheritskii AM, Lysenko AB, Uskova NI, Sametkii EA. Studies of the mechanism of the anticonvulsant effect of delta-sleep-inducing peptide in conditions of increased oxygen tensions. Neurosci Behav Physiol 1997;27:714–7.

Mikhaleva I, Prudchenko I, Ivanov V. Delta sleep inducing peptide (DSIP) and its analogues: sleep and extra sleep actions. Proceedings of the 22nd European Peptide Symposium; 1992. p. 663–4.

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;9:80–4.

Monnier M, Dudler J, Gachter R, Schoenenberger GA. Delta-sleep inducing peptide (DSIP): EEG and motor activity in rabbits following intravenous administration. Neurosci Lett 1977;6:9–13.

Nabeshima T, Tohyama Noda A, Maeda Y, Hiramatsu M. Effect of metaphit on phencyclidine and serotonin$_2$ receptor. Neurosci Lett 1989;102:303–8.

Prudchenko A, Stashevskaya LV, Mikhaleva II, Ivanov VT, Shandra AA, Godlevskii LS, et al. Synthesis and biological properties of new analogs of DSIP. Antiepileptic effect. Bioorg Chem (Russian) 1993;19:43–55.

Rafferty RM, Mattson M, Jacobson AE, Rice KCA. Specific acylating agent for the [^{3}H] phencyclidine receptors in rat brain. FEBS Lett 1985;181:318–22.

Sershen H, Berger P, Jacobson AE, Rice KC, Reith MEA. Metaphit prevents locomotor activation induced by various psychostimulants and interferes with the dopaminergic system in mice. Neuropharmacology 1988;27:23–30.

Shandra AA, Godlevskii RS, Mazarati AM, Oleshko AA, Mikhaleva II. The influence of the delta sleep-inducing peptide on convulsive activity. Neurosci Behav Physiol 1993;23:480–5.

Shandra AA, Godlevskii RS, Brusentsov AI, Mikhaleva II, Prudchenko IA, Zaporozhan VN. Effect of intranigral dosage with DSIP and its analogs on movement and convulsive activity in rats. Neurosci Behav Physiol 1996;26:567–71.

Shandra AA, Godlevskii LS, Brusentsov AI, Karlyuga VA. Effect of delta sleep inducing peptide on NMDA-induced convulsive activity in rats. Neurosci Behav Physiol 1998;28:694–7.

Schoenenberger GA. Characterization, properties and multivariate functions of Delta-Sleep-Inducing Petide (DSIP). Eur Neurol 1984;23:321–45.

Schoenenberger GA, Monnier M. Characterization of a delta electroencephalogram (sleep)-inducing peptide. Proc Natl Acad Sci 1977;74:1282–6.

Schoenenberger GA, Maier PF, Tobler HJ, Wilson K, Monnier M. The delta EEG (sleep)-inducing peptide: XI. Amino acid analysis, sequence, synthesis and activity of the nonapeptide. Pflugers Arch 1978;376:119–29.

Stanojlović O, Živanović D, Šušić V. *N*-methyl-D-aspartic acid and metaphit-induced audiogenic seizures in rat model of seizure. Pharmacol Res 2000a;42:247–53.

Stanojlović O, Živanović D, Šušić V. The effect of delta-sleep inducing peptide on the EEG and power spectra in rat. Indian J Physiol Pharmacol 2000b;44:428–34.

Šušić VT, Marković O. Potentiation of metaphit-induced audiogenic seizures by REM sleep deprivation in rats. Physiol Behav 1993;54:331–8.

Šušić V, Reith MEA, Zloković B, Lajtha A, Jacobson AE, Rice KC, et al. Electroencephalographic characteristics of audiogenic seizures induced in metaphit-treated small rodents. Epilepsia 1991;32:783–9.

Wang Yu, Kim MB, Palmer MR. Interactions of metaphit with phencyclidine and sigma agonist actions in rat cerebellum. Determination of specifity and selectivity. J Pharmacol Exp Ther 1987;241:321–7.

Yehuda S, Mostofsky DI. Circadian effect of β-endorphin, melatonin, DSIP and amphetamine on pentylenetetrazole-induced seizures. Peptides 1993;14:203–5.

Yehuda S, Kastin AJ, Coy H. Thermoregulatory and locomotor effects of DSIP: paradoxical interaction with d-amphetamine. Pharmacol Biochem Behav 1980;13:895–900.

Živanović D, Šušić V, Stanojlović O. The anticonvulsant potency of ±-2-amino-7-phosphoheptanoic acid and ±2-amino-phosphonovaleric acid against metaphit-induced audiogenic seizures in rats. Pharmacol Res 1998;38:347–52.

Živanović D, Šušić V, Stanojlović O. CPP blocks sound-induced seizures in metaphit-treated rats. Med Sci Res 1999;27:701–3.