EFFECT OF δ-SLEEP-INDUCING PEPTIDE, ANTICONVULSANTS, AND NICOTINAMIDE ON GENERALIZED SEIZURE ACTIVITY

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The study of the effect of δ-sleep-inducing peptide (DSIP) on focal forms of seizure activity, and also on seizure activity formed during metrazol-induced kindling has shown that DSIP may be effective against epilepsy [2, 3]. The aim of the present investigation was to study the anticonvulsant effects of DSIP on models of generalized seizures induced by various epileptogens, to determine how the effects depend on the intensity of the seizures on the dose of DSIP, and also to observe particular features of the effects of DSIP when used in conjunction with phenobarbital, relanium,* carbamazepine, diphenylhydantoin, and nicotinamide.

EXPERIMENTAL METHOD

Experiments were carried out on 750 (CBA × C57BL/6)F_1 mice weighing 18-24 g. Clonic seizures were induced by intraperitoneal injection of metrazol in doses of 40 and 60 mg/kg, and clonicotonic seizures by injection of metrazol in doses of 80 and 100 mg/kg, bicuculline (3.5 mg/kg), and thiosemicarbazide (15 mg/kg). The effects observed were recorded visually for 15-45 min after injection of metrazol, strychnine, bicuculline, and picrotoxin, and 2 h after injection of thiosemicarbazide. The intensity of the seizures was expressed in points on a scale described previously [1]. The latent periods of the first convulsive manifestations and of marked seizures (the animals falling on their side), and the mortality were determined also. DSIP (synthesized at the M. M. Shemyakin Institute of Bio-Organic Chemistry) was injected intraperitoneally in 0.1-0.3 ml of physiological saline 30 min before injection of metrazol, strychine, bicuculline, and picrotoxin and 30 min after injection of thiosemicarbazide. Phenobarbital, diphenylhydantoin, and carbamazepine (all from "Serva," West Germany) were injected 1 h, and relanium ("Polfa," Poland) 30 min and nicotinamide 15 min before injection of metrazol. Animals of the control group received an injection of the same volume of physiological saline.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of DSIP (100 μg/kg) was studied on seizures induced by injection of the various epileptogens. Administration of DSIP to animals with generalized seizures induced by metrazol (60 mg/kg) led to a considerable increase in the latent period of the first seizure responses, to a decrease in the number of animals with generalized clonicotonic seizures, and also to a decrease in severity of the seizures (Table 1). Under the influence of DSIP there was a significant increase in the latent period of the first seizure manifestations and a decrease in severity of the seizures induced by bicuculline. Administration of DSIP also was followed by lengthening of the latent period of the first seizures induced by picrotoxin (Table 1).

In the next series of experiments the effect of different doses of DSIP was studied on seizure activity induced by injection of metrazol in a dose of 60 mg/kg. Administration of DSIP in a dose as low as 1 μg/kg was followed by a significant increase in the latent period of the first seizures, but their intensity was unchanged (Fig. 1). An increase in the dose of DISP (10, 50, and 100 μg/kg) increased the intensity of the antiepileptic action of

*Diazepam.

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TABLE 1. Effect of DSIP on Generalized Seizure Activity of Differing Nature (M ± m)

Experimental conditions	Dose of drug, mg/kg	Number of animals	Latent period, min		Number of animals with generalized clonicotonic seizures	Average severity of seizures, points
			of first seizures	of marked seizures		
Metrazol	60	15	1,6±0,1	3,9±0,6	8	2,3±0,2
Metrazol + DSIP	60 0,1	10	2,7±0,3***	—	1**	1,5±0,2*
Bicuculline	3,5	10	4,7±0,3	7,2±1,0	8	2,9±0,2
Bicuculline + DSIP	3,5 0,1	10	11,2±1,1***	12,8±2,3	4	1,8±0,3*
Picrotoxin	4,0	9	12,0±0,3	16,1±0,8	9	3,6±0,2
Picrotoxin + DSIP	4,0 0,1	10	15,0±0,5***	16,8±0,9	10	3,3±0,2
Strychnine	1,5	26	3,4±0,3	4,6±0,4	18	3,4±0,2
Strychnine + DSIP	1,5 0,1	23	3,8±0,2	5,8±0,4	17	3,5±0,2
Thiosemicarbazide	15	21	73,4±2,4	75,3±2,3	18	3,2±0,2
Thiosemicarbazide + DSIP	15 0,1	20	69,4±1,8	69,7±2,1	16	3,1±0,2

Legend. Significance of differences from control: *$p < 0.05$, **$p < 0.025$, ***$p < 0.01$.

the drug, as shown by an increase in the latent period of the first seizures and a more marked decrease in the intensity of the seizures. The maximal decrease in severity of the seizures was observed after injection of DSIP in a dose of 100 μg/kg. Under the influence of DSIP in doses of 10-100 μg/kg the number of animals with generalized clonicotonic seizures was reduced by 4-6 times compared with the control ($p < 0.025$). With a further increase in the dose of DSIP (250 and 500 μg/kg) no significant effect of the drug on seizure activity was observed (Fig. 1). Injection of DSIP in a dose of 1000 μg/kg led to a considerable increase in the latent period of the first seizures and to a smaller change in their intensity (Fig. 1).

The aim of the next series of experiments was to study the effect of DSIP, in a dose of 100 μg/kg, on generalized seizures of differing severity, induced by injection of different doses of metrazol (40, 60, 80, 100 mg/kg). Depending on the dose of the epileptogen, the seizures in the animals differed in character – from single myoclonic spasms of the trunk following injection of metrazol in a dose of 40 mg/kg reduction of activity (intensity of seizures 1-2 points) to clonicotonic seizures with a lethal outcome following injection of metrazol in a dose of 100 mg/kg (severity of seizures 4 points). Injection of metrazol in a dose of 60 mg/kg led to the development of clonicotonic seizures with a severity of 2-3 points in some animals (60%), whereas with a dose of 80 mg/kg, half of the animals developed lethal seizures with a severity of 4 points. DSIP gave rise to a significant increase in the latent period of the first seizures, induced by injection of metrazol in doses of 40 and 60 mg/kg (Fig. 2). Under these circumstances there was also a decrease in the severity of the seizures, which was significant compared with the control when metrazol was given in a dose of 60 mg/kg (Fig. 2).

Injection of DSIP (100 μg/mg) together with phenobarbital (10 mg/kg) led to a marked increase in the latent period of the first seizures induced by metrazol (60 mg/kg) and to a decrease in their severity (Fig. 3a). The severity of the seizures was significantly less than when the two drugs were given separately ($p < 0.05$). After combined administration of DSIP (100 μg/kg) and diphenylhydantoin (100 mg/kg) there was a significant increase in the latent period of the first seizures, as well as a marked decrease in their severity (Fig. 3a). Carbamazepine (50 mg/kg) together with DSIP led to a more marked increase in the latent period of the seizures than when the drugs were given separately (Fig. 3a). Combined administration of DSIP (100 μg/kg) and nicotinamide (250 mg/kg) had a more marked anticonvulsant effect than injection of nicotinamide in a dose of 500 mg/kg or of DSIP alone (Fig. 3b). The anticonvulsant action also was stronger when DSIP in a dose of 500 mg/kg was given together with nicotinamide in a dose of 1000 mg/kg. In this case generalized seizures were completely prevented (Fig. 3b).

DSIP thus depresses seizure activity induced by a single injection of a convulsive dose of metrazol, and also by bicuculline and picrotoxin. These observations confirm the anti-epileptic effects of DSIP found previously in rats and cats in which epileptic foci had been created in the cerebral cortex by application of penicillin and strychnine solutions [2], and also of generalized seizure activity formed by repeated injections of subthreshold doses of metrazol [3].

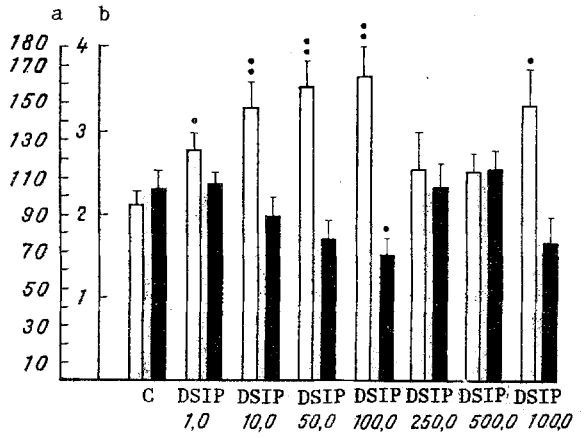

Fig. 1

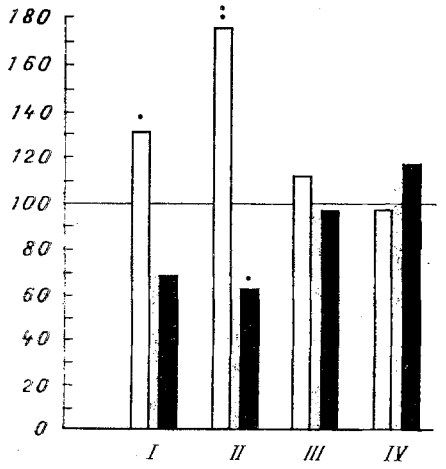

Fig. 2

Fig. 1. Effect of various doses of DSIP on seizure activity induced in mice by injection of metrazol. Abscissa: C) control (60 mg/kg), followed in order by effects of different doses of DSIP (in μg/kg) on seizures induced by metrazol in dose of 60 mg/kg; ordinate: I) latent period of first seizures (in sec); II) intensity of seizures (in points). Unshaded columns show latent period of first seizures; black columns – severity of seizures. Here and in Fig. 2, one dot indicates $p < 0.05$, two dots $p < 0.01$ compared with control.

Fig. 2. Effect of DSIP on seizures induced by different doses of metrazol in mice. Abscissa, effects of DSIP (100 μg/kg) on seizures induced by metrazol in doses of: I) 40 mg/kg, II) 60 mg/kg, III) 80 mg/kg, IV) 100 mg/kg. Ordinate: latent period of seizures and their severity (in % compared with controls, indicated by horizontal line). Legend as to Fig. 1.

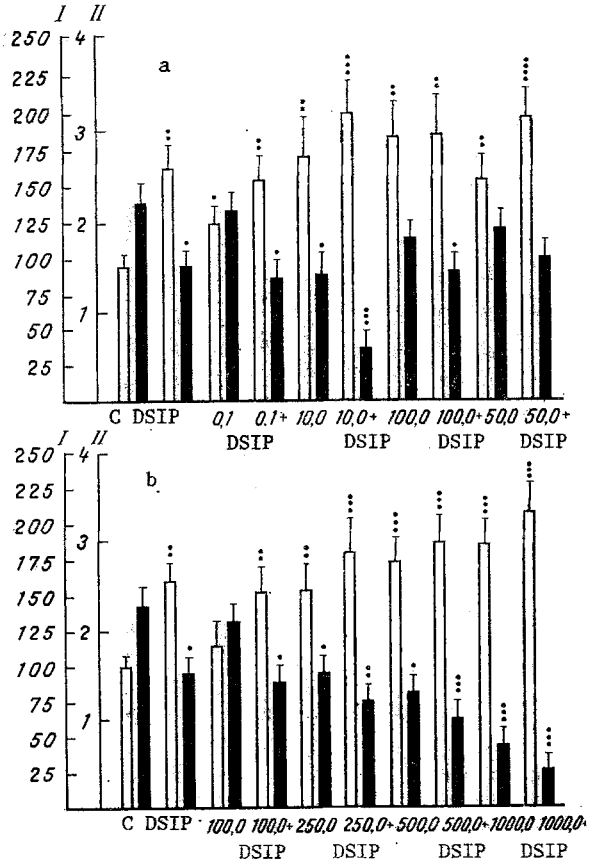

Fig. 3. Effect of DSIP alone and together with anticonvulsant agents and nicotinamide on seizure activity induced in mice by injection of metrazol. Fragment a. Abscissa: C) control (60 mg/kg metrazol), followed in order by effects of drugs used separately and in combination with DSIP (100 μg/kg): 0.1) relanimum, 1 mg/kg; 10.0) phenobarbital, 10 mg/kg; 100.0) diphenylhydantoin, 100 mg/kg; 50.0) carbamazepine, 50 mg/kg. Ordinate: I) latent period of seizures (in sec); II) severity of seizures (in points). Fragment b. Abscissa: C and DSIP as in fragment a, followed by effects of various doses of nicotinamide (100.0, 250.0, 500.0, and 1000.0 mg/kg), given separately or in combination with DSIP (100 mg/kg). Ordinate, the same as in fragment a. Legend as to Figs. 1 and 2; three dots $p < 0.001$ compared with control.

The data given above show that the action of DSIP depends on the severity of generalized seizure activity observed when seizures with an average level of severity (2-3 points) have been created. Under the influence of DSIP complete inhibition of generalized seizure activity does not take place. In a study of dose-dependent effects of DSIP, antiepileptic activity was strongest when DSIP was given in doses of 10-100 μg/kg, and an increase in the dose of DSIP did not cause any increase in the anticonvulsant action of the peptide. This feature, namely the absence of a linear dose-dependent character of the antiepileptic action of DSIP over a wide range of doses of the preparation, characteristic of substances of peptide nature [6], is evidence of the specificity of the antiepileptic action of DSIP.

Experiments to study effects of DSIP on seizures induced by the different epileptogens showed that under the influence of the peptide, seizure manifestations induced by bicuculline and, to a lesser degree, by picrotoxin, also were inhibited, whereas seizures induced by thiosemicarbazide and strychnine were not sensitive to the action of DSIP. One of the common mechanisms of the epileptogenic effect of metrazol, bicuculline, and picrotoxin is blocking of GABA receptors [7]. It can therefore be tentatively suggested that the antiepileptic action of DSIP is realized through its effect on GABA-receptor regions of the neuron membrane. Meanwhile seizures induced by thiosemicarbazide and strychnine, which exert their epileptogenic action through a decrease in GABA synthesis and blocking of glycine receptors, respectively [7], was unaffected by DSIP.

Enhancement of the antiepileptic effects observed in this investigation during the use of a combination of anticonvulsant agents and nicotinamide with DSIP can be explained by interaction of these substances at the level of the receptor complex, which includes binding sites for barbiturates, benzodiazepines, and GABA [5], and it is also evidence that the anticonvulsant action of DSIP can be realized through activation of GABA-ergic brain mechanisms.

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