14. P. Skolnick et al., Pharmacol. Biochem. Behav., 23, 17 (1985).
15. D. N. Stephens et al., Neuropharmacology, 23, 879 (1984).

EFFECT OF DELTA SLEEP PEPTIDE IN CORTICAL EPILEPTIC ACTIVITY IN RATS AND CATS

G. N. Kryzhanovskii,* A. A. Shandra,
L. S. Godlevskii, M. N. Karpova,
I. I. Mikhaleva, and V. T. Ivanov

UDC 615.366.8-009.836.017:615.213].015.4:
616.831-009.24-031.84-092.9

KEY WORDS: epileptic activity; delta sleep-inducing peptide (DSIP); penicillin; strychnine; epileptic focus; epileptic complex

Experimental and clinical investigations have shown the therapeutic effects of delta sleep-inducing peptide (DSIP) in several pathological states: insomnia, the withdrawal syndrome, and stress [7, 8, 10]. The next step was to study the effects of DSIP on epileptic activity (EPA).

In the investigation described below the effect of DSIP was studied on foci of EPA and on their complexes, created in the cerebral cortex of rats and cats.

EXPERIMENTAL METHOD

Experiments were carried out on 47 male Wistar rats weighing 200-220 g and on 20 cats weighing 2.5-3.5 kg. The preparatory operation on the animals was carried out in accordance with the method described previously [5]. Under hexobarbital anesthesia (150 mg/kg, intraperitoneally) a burr-hole (2 × 4 mm) was drilled in the region of the sensomotor cortex of the rats, the dura was divided, and recording electrodes were secured in the sensomotor and visual cortex. To prevent the exposed surface of the cerebral cortex from drying it was moistened with physiological saline and the burr-hole was covered with waterproof film. Next day, to create foci of EPA, the film was removed and a piece of filter paper soaked in a solution of the sodium salt of benzylpenicillin in a concentration of 12,000 or 20,000 IU/ml was applied to the surface of the cortex. In the experiments on cats, under ether anesthesia tracheotomy was performed and a burr-hole drilled in the skull to provide access to the frontal regions of the cortex. Tubocurarine (0.12-0.28 mg/kg) was injected into the animals 2.5-3 h after the administration of ether ceased and the cats were artificially ventilated. Single foci of EPA were created in different parts of the sigmoid, coronary, and orbital gyri by application of a piece of filter paper (2 × 2 mm) soaked in a 0.1% solution of strychnine nitrate. The determinant focus of the epileptic complexes was formed by application of a 3% solution of strychnine to the middle sigmoid gyrus. Electrical activity of the brain was recorded by a monopolar technique and the reference electrode was fixed in the nasal bones. Potentials were recorded on a polygraph (Nihon Kohden, Japan) and 4-ÉÉG-3 electroencephalograph. In experiments on unrestrained rats the ECoG of two animals, one of which was given DSIP intraperitoneally, the other physiological saline, was recorded simultaneously. In experiments on cats, control determinations of the effect of intravenous injection of physiological saline (1 ml) on activity of the foci and of the multifocal complexes were undertaken initially. EPS in the foci was then restored by repeated applications of solutions of the convulsants and DSIP was injected intravenously. The DSIP was diluted in physiological saline immediately before injection and was used in a dose of 100 µg/kg. The experimental results were subjected to statistical analysis by variance and parametric methods [11].

*Academician of the Academy of Medical Sciences of the USSR.

Laboratory of General Pathology of the Nervous System and Laboratory of Biochemistry, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Department of Pathophysiology, N. I. Pirogov Odessa Medical Institute. Laboratory of Peptide Chemistry, M. M. Shemyakin Institute of Bioorganic Chemistry, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 11, pp. 582-585, November, 1987. Original article submitted March 20, 1987.

 0007-4888/87/0011-1568$12.50

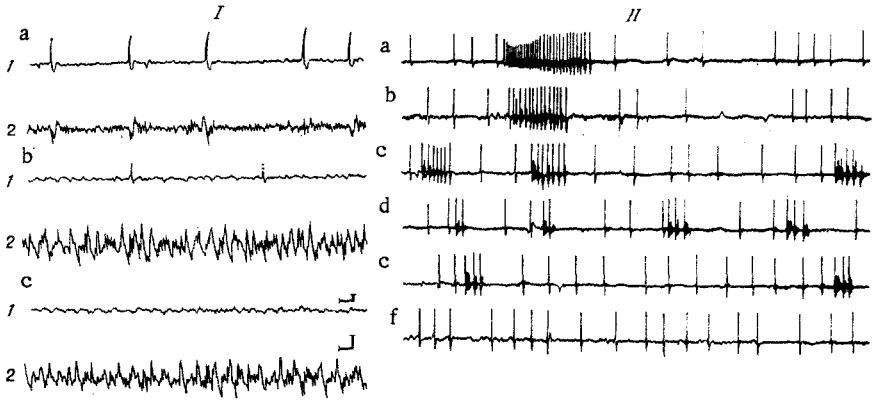

Fig. 1. Effect of DSIP on epileptic foci of different strength, created by application of penicillin solutions in the rat cerebral cortex. I) Effect of DSIP on relatively weak epileptic focus: a) 20 min after application of penicillin solution (12,000 IU/ml) to zone 1; b, c) 15 and 23 min, respectively, after intraperitoneal injection of DSIP (100 μg/kg). 1) Sensomotor, and 2) visual area of cortex. Calibration: 150 and 200 μV, 1 sec. II) Effect of DSIP on relatively strong epileptic focus. a) 25 min after application of penicillin solution (20,000 IU/ml) to sensomotor cortex; b, c, d, e, f) 5, 8, 27, 35, and 40 min, respectively after injection of DSIP (100 μg/kg). Here and in Figs. 2 and 3: calibration 500 μV, 1 sec.

EXPERIMENTAL RESULTS

In the experiments on rats the action of DSIP was studied on activity of single relatively weak (experiments of series I) and strong (series II) foci.

Experiments of Series I. Experiments in which DSIP was not injected (seven animals) showed that during creation of a relatively weak focus of EPA (by application of a solution of penicillin with 12,000 IU/ml) separate spike discharges (SD) appeared after 5-7 min, and 15-20 min later stable EPA was recorded in the form of SD with an amplitude of 0.8-1.2 mV and a frequency of 10-20/min (Fig. 1, I, a — zone 1); it was observed for 30-40 min, after which the amplitude and frequency of the discharge decreased and they were completely inhibited. The total duration of existence of the foci of EPA was 75-105 min.

In eight of 13 cases injection of DSIP 15-20 min after the beginning of penicillin application caused a decrease in the amplitude of SD after 7-20 min to 300-500 μV and reduced the frequency of spike generation to 4-10/min (Fig. 1, I, b — zone 1). During this period, in areas of the cortex to which penicillin was not applied, the appearance of irregular slow-wave potentials with an amplitude of 200-300 μV and a frequency of 1-5/sec was observed (Fig. 1, I, b — zone 2). When a further 10-40 min had elapsed after injection of DSIP, EPA in zone 1 disappeared, whereas in zone 2 slow-wave discharges with an amplitude of up to 300 μV and a frequency of 1-5/sec were recorded (Fig. 1, I, c). The total duration of existence of the foci after injection of DSIP was 45-80 min, significantly less than in the control ($p < 0.05$). In two animals injection of DSIP induced slight and transient inhibition of EPA; in three of 13 rats it had no effect on EPA. In the group of control animals (seven rats) injection of physiological saline in the same volume caused no significant changes in EPA.

Experiments of Series II. On the creation of a relatively strong focus of EPA (application of penicillin in a dose of 20,000 IU/ml; 10 animals) after 15-20 min epileptic discharges (ED) of ictal type were recorded, with a frequency of 0.5/min (Fig. 1, II, a). The total duration of the epileptic foci was 150-180 min.

Injection of DSIP 20-30 min after penicillin application caused a reduction in the duration of ED (after 5-10 min) in three of the seven animals from 10-20 sec to 5-7 sec (Fig. 1, II, b, c), and after 35-45 min the ED disappeared completely (Fig. 1, II, e). In the remaining four animals injection of DSIP led to suppression of ED for 25-35 to 60 min, after which their recovery was noted. In three animals DSIP had no effect.

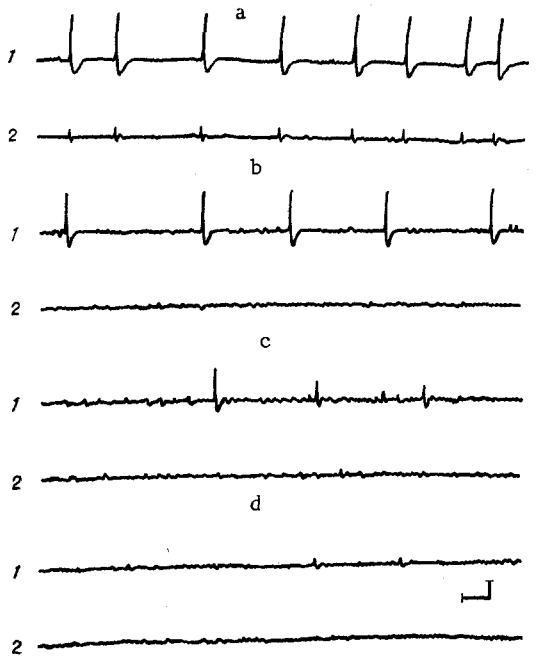

Fig. 2

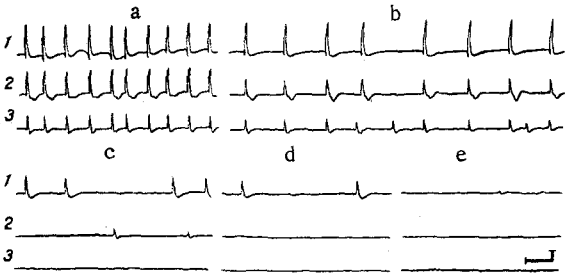

Fig. 3

Fig. 2. Effect of DSIP on epileptic focus created by strychnine application in cat cerebral cortex: a) 16 min after application of 0.1% strychnine solution to zone 1; b) 9 min after Fig. 2a and 8.5 min after intravenous injection of solution of DSIP (100 μg/kg); c, d) 7 and 14 min after b, respectively. 1) Middle sigmois; 2) coronary guri. Calibration: 500 μV, 1 sec.

Fig. 3. Effect of DSIP on complex of epileptic foci created by application of strycnine solutions in the cat cerebral cortex. a) 5 min after end of application of 3% strychnine solution to zone 1 and 0.1% strychnine solution to zones 2 and 3; b) 12 min after intravenous injection of DSIP solution (100 μg/kg); c, d, e) 8, 11.5, and 19.5 min, respectively, after b. 1) Middle; 2) posterior; 3) anterior sigmoid gyri.

In the experiments on cats the aim of series I was to study the effect of DSIP on activity of a single epileptic focus created by application of 0.1% strychnine solution. Control observations showed that 12-18 min after application of strychnine solution to zone 1, SD with an amplitude of 1.5-2.0 mV and a frequency of 20-35/min were recorded in the latter (Fig. 2a). At the same time induced discharges were recorded in zone 2, with an amplitude of 200-400 μV. The total duration of existence of EPA in the foci was 30-45 min.

In 10 of the 12 cases, 7-17 min after injection of the solution of DSIP, given 13-20 min after the beginning of strychnine application, a reduction in amplitude of the discharges to 1.2-1.6 mV and in the frequency of their generation of 14-25/min was observed in the focus (Fig. 2b, zone 1). Meanwhile virtually total inhibition of the induced potentials was discovered (Fig. 2b, zone 2). After another 5-8 min the amplitude of the discharges in the focus was 0.8-1.2 mV and the frequency of their generation was 6-10/min (Fig. 2c, zone 1). Complete inhibition of activity of the epileptic focus was observed 15-30 min after injection of DSIP (Fig. 2d, zone 1). The duration of existence of the foci was 20-35 min, significantly less than in the control, namely 30-40 min ($p < 0.01$). In two cases, when DSIP was injected no change was found in the amplitude or frequency of SD in the foci.

In the next series of experiments, the effect of DSIP on activity of a multifocal epileptic complex was investigated. The complex was created by application of a relatively weak solution of strychnine (0.1%) to zones 2 and 3 (dependent foci) and of a concentration solution (3%) to zone 1 (the determinant focus) (Fig. 3a). The amplitude of discharges in the determinant focus was 2-2.5 mV, and in the dependent foci from 1 to 2 mV; the frequency of generation of the discharges in all foci was 25-40/min. The epileptic complex thus created generated EPA with stable amplitude and frequency for 20-35 min, after which the amplitude and frequency of the discharges in the foci decreased and the complex broke up (control experiments on seven animals).

Injection of DSIP 20-30 min after application of strychnine solutions in seven of 10 animals evoked a decrease in the frequency of discharge generation in all foci after 10-15 min to 20-30/min (Fig. 3b). The amplitude of the discharges in the dependent foci fell under these circumstances to 0.7-1.5 mV (Fig. 3b, zones 2 and 3), but in the determinant focus it was unchanged. After a further 5-10 min the amplitude of SD fell in the determinant focus also to 0.8-1.5 mV, and this was accompanied by a decrease in the frequency of their generation to 10-15/min (Fig. 3c, zone 1). In this period total suppression of EPA was observed in the dependent focus furthest from the determinant focus in zone 3, together with a marked decrease in the amplitude of the discharges in the focus in zone 2. During the next 3-8 min EPA completely disappeared in the dependent foci of the complex (Fig. 3d, zones 2 and 3). In the region of the former determinant focus, separate SD with an amplitude of 0.7-1.2 mV and a frequency of 6-15/min were recorded during this period (Fig. 3d, zone 1), but later they disappeared (Fig. 3e). The duration of existence of the complex in experiments in which DSIP was injected was 30-45 min, i.e., less than in the control (50-70 min; $p < 0.05$). In two experiments DSIP had no effect on the amplitude or frequency of discharges in the foci, and in another experiment it led to a temporary (for 10 min) decrease in amplitude of discharges in the dependent foci.

The results are thus evidence that DSIP has an inhibitory action on epileptic foci in the rat and cat cerebral cortex. This effect was expressed as a reduction in the amplitude and frequency of discharges in the foci, and shortening of the total duration of their existence compared with that in control observations. It was observed both in single foci and in their complexes. The absence of any effect of DSIP on EPA observed in some cases may be attributed to the increased predisposition of these animals to seizures and also to individual sensitivity to the action of DSIP.

The results of a number of experiments (not described in this paper) give the impression that the antiepileptic effects of DSIP were more marked in relation to foci developing under the influence of strychnine than to foci formed by application of penicillin. In this connection it may be pointed out that certain antiepileptic drugs are more active against EPA induced by strychnine than against EPA induced by penicillin [3]. We know that neurochemical disturbances in foci of EPA induced by strychnine and penicillin differ [12]. However, for a final conclusion to be drawn the experimental conditions must be taken into account: different species of animals, unrestrained behavior of rats and muscle relaxation in cats, different methods of injection of DSIP — intraperitoneal (experiments on rats) and intraveous (experiments on cats), etc. This fact, like the antiepileptic effects of DSIP, also deserve attention because during delta sleep EPA may be intensified or even provoked [9]. In these cases, however, we are evidently dealing with so-called "nocturnal epilepsy" [1, 6]. Meanwhile, the absence of a facilitatory effect of slow-wave activity on EPA has been demonstrated [2, 4].

These results indicate the desirability of further study of the antiepileptic properties of compounds of the DSIP type.

LITERATURE CITED

1. R. G. Biniaurishvili, A. M. Vein, B. G. Gafurov, and A. A. Rakhimdzhanov, Epilepsy and Functional States of the Brain [in Russian], Tashkent (1985).
2. M. G. Koridze, M. M. Mgalobashvili, and M. G. Kavkasidze, Fiziol. Zh. SSSR, 62, No. 10, 1422 (1976).
3. G. N. Kryzhanovskii and A. A. Shandra, Zh. Nevropatol. Psikhiat., No. 6, 801 (1981).
4. T. N. Oniani, M. G. Koridze, M. M. Mgalobashvili, et al., The Neurophysiological Mechanisms of Epilepsy [in Russian], Tbilisi (1980), p. 39.
5. N. A. Samsonova, M. B. Rekhtman, R. N. Glebov, and G. N. Kryzhanovskii, Byull. Eksp. Biol. Med., No. 12, 655 (1979).
6. P. M. Saradzhishvili and G. Sh. Geladze, Epilepsy [in Russian], Moscow (1977).
7. D. Sepetliev, Statistical Methods in Scientific Medical Research [Russian translation], Moscow (1968).
8. O. Pompeiano, Basic Mechanisms of Epilepsies, ed. by H. Jasper et al., Boston (1969), p. 453.
9. D. Schneider-Helmert, F. Gniess, M. Monnier, et al., Int. J. Clin. Charmacol., 19, 341 (1981).
10. K. V. Sudakov, V. T. Ivanov, E. V. Koplik, et al., Pavlov. J. Biol. Sci., 18, No. 1, 1 (1983).
11. D. M. Woodbury, Antiepileptic Drugs: Mechanisms of Action, ed. by F. H. Glaser et al., New York (1980), p. 249.