the nociceptive system. The identity of the central structures of the nociceptive system in which the GPEE is formed requires further study.

LITERATURE CITED

1. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System [in Russian], Moscow (1980).
2. G. N. Kryzhanovskii, V. K. Reshetnyak, M. L. Kukushkin, M. P. Gorizontova, and V. S. Smirnova, Patol. Fiziol. Éksp. Terap. (1991).
3. L. M. Pozhidaeva, "Neurotic changes in a long-lasting phantom pain syndrome after lower limb amputation," Author's Abstract of Dissertation for the Degree of Candidate of Medical Sciences (1976).
4. O. J. Andy, Stereotact. Funct. Neurosurg., **52**, No. 2, 176 (1989).
5. J. Gorecki, T. Hyrayama, J. O. Dostrovsky, et al., Stereotact. Funct. Neurosurg., **52**, No. 2, 219 (1989).
6. T. Hyrayama, J. O. Dostrovsky, J. Gorecki, et al., Stereotact. Funct. Neurosurg., **52**, No . 2, 120 (1989).
7. H. Markus and B. Pomeranz, Brain Res., **416**, No. 2, 315 (1987).
8. J. T. Wall and C. G.-Cusick, J. Neurosci., 4, No. 6, 1499 (1984).

EFFECT OF NEUROTROPIN ON SEIZURE ACTIVITY IN PICROTOXIN KINDLING

A. A. Shandra, L. S. Godlevskii, A. M. Mazarati, and R. S. Vast'yanov

UDC 616.8-009.24-02:615.213]-07

KEY WORDS: neurotropin; picrotoxin; kindling; cycloheximide

The study of the effect of neurotropin on models of generalized seizures, induced by various epileptogens, has demonstrated its antiepileptic activity in picrotoxin-induced seizures [5]. It was decided to study the anticonvulsant activity of neutrotropin on other models of epileptic activity (EpA). One model of progressively increasing EpA and prolonged and enhanced seizure activity is picrotoxin kindling [3]. Furthermore, to continue the study of the mechanisms of the anticonvulsant action of neurotropin, its effects were studied when protein synthesis was blocked by cycloheximide [4].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 220-320 g. Each group consisted of 10 animals. Pharmacologic kindling was produced by daily single intraperitoneal injections of picrotoxin ("Sigma," USA) in a dose of 1.0 mg/kg body weight [3]. The intensity of the seizures was judged by the use of the adopted scale and expressed in points [2]. Neurotropin ("Nippon Zoki," Japan) was injected 24 h after the 20th injection of picrotoxin. The injection was given under open ether anesthesia under stereotaxic conditions [10] into the lateral cerebral ventricles in a volume of 12.5 or 25 μl, or intraperitoneally (1.0 ml per animal). Animals of the control groups, under similar conditions received 0.9% sodium chloride solution. Starting with the day after injection of neurotropin, seizure responses to injection of a testing dose of picrotoxin (1.0 mg/kg) were investigated daily. The latent period of the first seizures, of convulsions, and the mean intensity

Department of Normal Physiology, N. I. Pirogov Odessa Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 3, pp. 236-239, March, 1992. Original article submitted June 25, 1991.

0007-4888/92/0003-0298$12.50

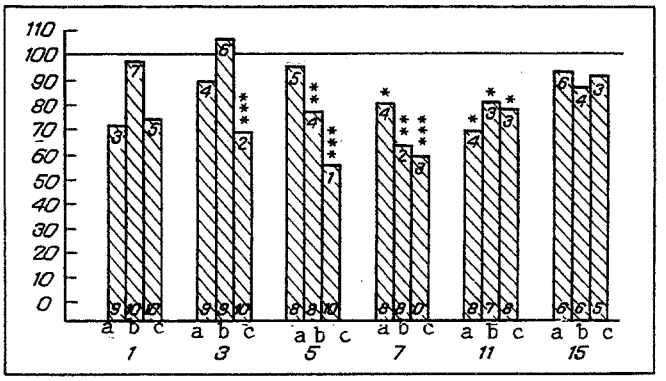

Fig. 1. Effect of neurotropin on seizure activity of animals subjected to kindling. Abscissa, 1st-15th day after injection of neurotropin; ordinate, intensity of seizures (in % of control, taken as 100%, and indicated by horizontal line). a) Intraventricular injection of neurotropin (12.5 μl); b) the same, 25 μl, intraperitoneal injection of neurotropin (1.0 ml). Asterisks indicate significant differences compared with control: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Numbers at foot of columns indicate number of animals in group; numbers at top of columns indicate number of animals with convulsions. Cross next to numbers indicates $p < 0.025$ compared with control.

of the seizure responses were estimated. To study the effect of neurotropin on EpA formation during kindling, besides kindling formation by the method described above, the animals were given daily intraperitoneal injections of neurotropin (1.0 ml) 24 h before the next injection of picrotoxin (1.0 mg/kg). For electroencephalography (EEG) monopolar constantan electrodes (diameter 0.15 mm) were implanted stereotaxically [10] into the rats, under pentobarbital anesthesia (35 mg/kg), into the ventral hippocampus, reticular part of the substantia nigra, caudate nucleus, and sensomotor cortex; the reference electrode was secured in the nasal bones. The EEG was recorded under unrestrained conditions (10 days after the operation) by means of an EEG-16-S electroencephalograph ("Medicor," Hungary). Cycloheximide ("Serva," Germany) was injected into the lateral ventricles, using coordinates taken from the atlas [10], in a dose of 30 μg/kg 1 h before intraperitoneal injection of neurotropin [4]. The experimental results were subjected to statistical analysis by Student's t test and by Fisher's exact method [1].

EXPERIMENTAL RESULTS

Repeated injection of picrotoxin led to the appearance of and to a progressive increase in the intensity of seizure responses from individual myoclonic twitches to generalized clonicotonic convulsions, which were recorded in all animals after the 20th injection. The mean intensity of the seizures was 4.2 ± 0.4 points. These results agree with those obtained in previous studies [3].

The aim of the first series of experiments was to study the effect of neurotropin when administered intracerebrally and systemically on the seizure responses in rats subjected to kindling. A test injection of picrotoxin during the 6 days after intraventricular injection of neurotropin (12.5 μl) caused the animals to develop seizure reactions in the form of repeated clonic spasms of the forelimbs, and also generalized convulsions. The mean intensity of the seizures did not differ from that in animals of the control group (Fig. 1a). When picrotoxin was given on the 7th-11th days after injection of neurotropin, the seizures were in the nature of myoclonic twitches or repeated clonic convulsions of the whole trunk. At these times the mean intensity of the seizures was significantly less than in animals of the control group ($p < 0.05$; Fig. 1a). Starting with the 12th day and until the end of testing, the parameters of intensity of seizures did not differ significantly in the experimental and control groups.

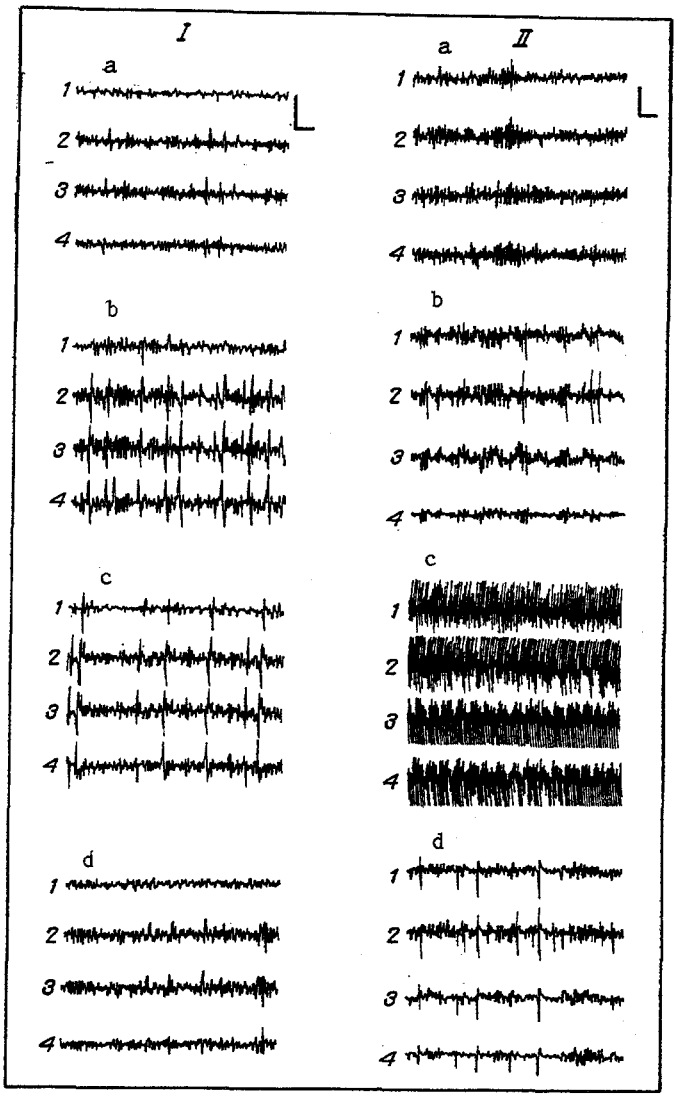

Fig. 2. Effect of neurotropin on EEG changes induced in rats with kindling by chest injection of picrotoxin. I. Picrotoxin (1.0 ml) injected on 5th day after intraperitoneal injection of neurotropin. a) Spontaneous activity, b-d) 17, 45, and 70 min respectively after injection of picrotoxin. Legend: 1) sensomotor cortex, 2) right hippocampus, 3) left hippocampus, 4) caudate nucleus. II) Picrotoxin injected into animal of control group on 5th day after intraperitoneal injection of physiological saline. a) Spontaneous activity, b-d) 10, 20, and 30 min respectively after injection of picrotoxin. Legend: 1) reticular part of substantia nigra, 2) hippocampus, 3) caudate nucleus, 4) sensomotor cortex. I and II) Calibration 300 μV, time marker 1 sec.

When neurotropin was injected into the cerebral ventricles in a larger dose (25 μl) its anticonvulsant action was expressed as a decrease in the mean intensity of the seizures and a decrease in the number of rats with generalized convulsions. The antiepileptic effect of neurotropin was observed starting from the 5th day after its administration, and it continued for 7 days (Fig. 1b). On subsequent days the intensity of the seizures in the rats increased and did not differ from that in animals of the control group.

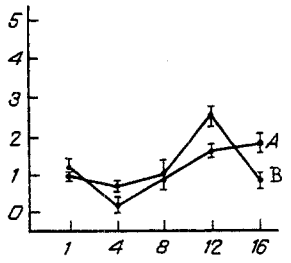

Fig. 3

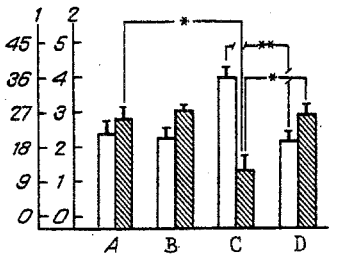

Fig. 4

Fig. 3. Effect of neurotropin on formation of seizure syndrome during kindling. Abscissa, days of injection of picrotoxin; ordinate, intensity of seizures (in points). Legend: A) experiment, B) control.

Fig. 4. Effect of cycloheximide on anticonvulsant action of neurotropin. Abscissa, injection of picrotoxin preceded by intraperitoneal and intraventricular injection of physiological saline (A), intraperitoneal injection of physiological saline and intraventricular injection of cycloheximide (B), intraperitoneal injection of neurotropin and intraventricular injection of physiological saline (C), and intraperitoneal injection of neurotropin and intraventricular injection of cycloheximide (D). Ordinate: 1) time (in min), 2) intensity of seizures (in points). Unshaded columns – latent period of 1st seizures (in min); shaded columns – intensity of seizures (in points). Asterisks indicate significant differences *$p < 0.05$, **$p < 0.01$.

Testing predisposition to seizures after intraperitoneal injection of neurotropin showed that on the 3rd day after its injection the intensity of the seizures was significantly less than in the control ($p < 0.001$, Fig. 1c). These differences in the severity of the seizures were greatest on the 5th day and they lasted 8-9 days. Starting with the 12th day and until the end of the investigations the intensity of the seizure reactions in the experimental and control groups was identical (Fig. 1c).

Electrographic correlates of the anticonvulsant action of neurotropin were investigated under conditions of maximal antiepileptic action (on the 5th-7th day after intraperitoneal injection of neurotropin). The experiments showed that injection of picrotoxin under these conditions caused the appearance of spike-wave complexes in the EEG after 10-18 min with an amplitude of 250-350 μV, and also of slow waves with a frequency of 3-5/sec and an amplitude of 200-250 μV (Fig. 2I, b). Behaviorally at this time, the rats exhibited secondary clonic convulsions of the whole trunk. These EEG changes (Fig. 2I, c) and behavioral disorders were recorded for 45-50 min after their appearance, and they then disappeared (Fig. 2I, d). Injection of picrotoxin into animals of the control group caused the appearance in the EEG after 7-14 min of spike potentials, whose maximal amplitude was observed in the hippocampus (180-300 μV, Fig. 2, II, b, zone 2), corresponding to clonic spasms of individual muscles and of the whole trunk. Fast (8-12/sec) synchronized potentials with an amplitude of 350-550 μV, were recorded in the EEG 15-30 min after injection of picrotoxin. Behaviorally at this period generalized clonicotonic convulsions were recorded. After the end of the periods of generalization (15-25 sec) spike discharges and spike-wave complexes with an amplitude of 200-350 μV were recorded in the EEG, during which the rats exhibited clonic convulsions.

In the next series of experiments the effect of neurotropin on development of epileptic activity during kindling was studied. Repeated intraperitoneal injection of neurotropin had no significant effect on the development of the seizure syndrome in the course of kindling: the intensity of the seizures did not differ from the corresponding values recorded in the control group (Fig. 3).

In a separate series of experiments the characteristics of the anticonvulsant action of neurotropin were studied in rats with generalized picrotoxin-induced seizures (2.0 mg/kg, intraperitoneally) and receiving cycloheximide. Administration of picrotoxin after intraventricular injection of cycloheximide caused the animals to develop repeated clonic convulsions of the whole trunk, and also generalized convulsions. The intensity of the seizures did not differ from that in rats receiving intraventricular injections of physiological saline (Fig. 4a, b). Neurotropin, injected intraperitoneally 24 h before picrotoxin, significantly reduced the intensity of picrotoxin-induced seizures and lengthened their latent period (Fig. 4c). Injection of neurotropin into animals receiving cycloheximide did not affect the parameters of the seizure reactions induced by picrotoxin (Fig. 4d).

The investigations thus showed that neurotropin has an anticonvulsant action under conditions of EpA formed by the picrotoxin kindling method. This anticonvulsant action is dose-dependent in character (in the case of intraventricular injection), and it peaks on the 7th day. It must also be noted that neurotropin had a stronger anticonvulsant action when injected intraperitoneally than intracerebrally. This feature of the effects of neurotropin is reminiscent of those, for example, of DSIP [6, 12], which combines ability to pass into the brain through the blood-brain barrier (BBB) with absence of systems of transport from the cerebrospinal fluid spaces into the brain. The anticonvulsant action of neurotropin when injected by the intraventricular route may perhaps also be linked with its passage into the blood, and from thence through the BBB into brain structures.

Although neurotropin effectively prevented seizure development when kindling was complete, it had no preventive action against the development of EpA during kindling. The reason for this result may be that the build-up process, i.e., EpA formation and EpA itself, formed during kindling, differ in their neurophysiological mechanisms [7].

Blocking of the anticonvulsant effects of neurotropin by cycloheximide demonstrated by the present investigation is evidence that protein (peptide) synthesis is one of the mechanisms of the antiepileptic action of neurotropin. The important role of activation of endogenous peptide systems in the control of EpA development and also in the action of certain antiepileptic preparations, must also be pointed out [8, 9]. Data obtained previously [5] and the results of the present investigations into specific inhibition of picrotoxin-induced (acute and chronic) seizure syndromes by neurotropin, compared with those induced by other convulsants, are interesting. We know that cerulein, a substance of peptide nature, is also highly specific as regards picrotoxin-induced seizures, but not seizures induced by metrazol, bicuculline, or strychnine [11]. These results, and also blocking of the effects of neurotropin by cycloheximide, suggest that the anticonvulsant action of neurotropin is due to modulation of synthesis of peptide compounds whose active principle has a structure similar to that of cerulein. Indirect confirmation of the peptide-dependent anticonvulsant action of neurotropin is given by temporary fluctuations of its activity, characteristic of peptides [8, 9].

LITERATURE CITED

1. E. V. Gubler, Computational Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Moscow (1978).
2. G. N. Kryzhanovskii. A. A. Shandra, R. F. Makul'kin, et al., Byull. Éksp. Biol. Med., No. 5, 527 (1985).
3. G. N. Kryzhanovskii, A. A. Shandra, R.F. Makul'kin, et al., Byull. Éksp. Biol. Med., No. 7, 18 (1989).
4. A. S. Spirin and L. P. Gavrilova, The Ribosome [in Russian], Moscow (1968).
5. A. A. Shandra, K. V. Sudakov, and G. N. Kryzhanovskii, Byull. Éksp. Biol. Med., No. 1, 49 (1991).
6. A. J. Kastin, C. Nissen, and D. H. Coy, Pharmacol. Biochem. Behav., **15**, No. 3, 955 (1981).
7. J. Majkowski, The Clinical Relevance of Kindling, Chichester (1989), pp. 87-103.
8. G. A. Olson, R. D. Olson, and A. J. Kastin, Peptides, **18**, No. 6, 1135 (1987).
9. G. A. Olson, R. D. Olson, and A. J. Kastin, Peptides, **10**, No. 1, 205 (1987).
10. C. Paxinos and D. Watson, The Rat Brain in Stereotaxic Coordinates, Sydney (1982).
11. G. Zetler, Eur. J. Pharmacol., No. 2-3, 297 (1980).
12. B. V. Zlocovic, M. B. Segal, H. Dawson, et al., Peptides, **9**, No. 3, 533 (1988).