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ANTICONVULSANT EFFECTS OF NEUROTROPIN

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The possibility of suppressing epileptic activity by substances of endogenous origin has now been demonstrated [1, 2, 5, 6]. One preparation, obtained from rabbit skin inoculated with cowpox virus, is neurotropin, produced by the firm "Nippon Zoki Pharmaceutical Limited" [7]. Neurotropin possesses a broad spectrum of biological activity. Besides its antiallergic and anti-inflammatory action, neurotropin also affects brain function. The antistressor action of neurotropin and its beneficial effect in experimental cerebral edema in mice have been established [11]. Neurotropin acts selectively on brain electrical activity [6].

The aim of this investigation was to study the effects of neurotropin on generalized and local forms of epileptic activity induced in animals of different species (rats, mice, cats).

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats (270-320 g), C5BF6 mice (18-22 g), and cats (2.5-3.2 kg) under acute conditions. Seizures were induced by intraperitoneal injection of picrotoxin ("Serva," West Germany) 4.0 mg/kg, bicuculline ("Serva") 4.0 mg/kg, metrazol 60 mg/kg, or kainic acid ("Sigma," USA) 15 mg/kg. Seizures were recorded visually for 1 h after the injection of the convulsants. The latent period of the first seizures, their maximal intensity, and the number of animals developing seizures were determined. The intensity of the seizures was estimated by the use of a five-point scale [4]. Foci of epileptic activity were induced by application of a piece of filter paper (2 × 2 mm), soaked in 0.1% strychnine nitrate, to the cerebral

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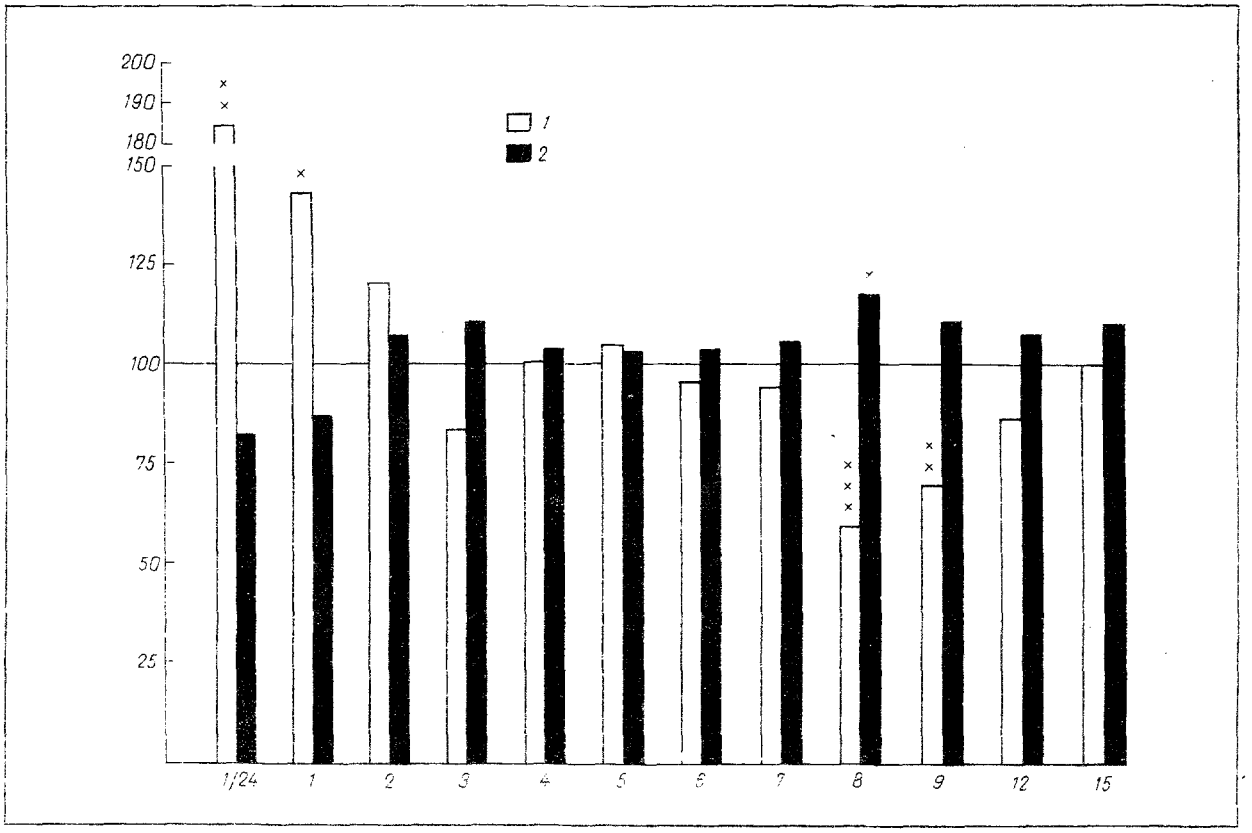

Fig. 1. Effect of intraventricular injection of neurotropin (25 μl) on generalized seizures induced in rats by injection of picrotoxin (2 mg/kg). Abscissa, time after injection of neurotropin (in days); ordinate, latent period of first seizures (1) and average severity of seizures (2) (in per cent relative to control, taken as 100%); *) $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

cortex of cats anesthetized with pentobarbital (40 mg/kg, intraperitoneally). Electrical activity in the foci was recorded by a monopolar method on a 16-channel electroencephalograph ("Medicor," Hungary). Neurotropin was injected into the rats and cats in a volume of 25 μl, into the lateral ventricles of the brain, taking coordinates from atlases [9, 10] and with the aid of a microinjector (SGE Ply Ltd., Australia). Animals of the control group received an injection of 25 μl of 0.9% NaCl solution under analogous conditions. Neurotropin was injected intraperitoneally into the mice in a volume of 0.2 and 0.5 ml of the preparation per animal. Animals of the control group were given a similar volume of physiological saline.

The experimental results were subjected to statistical analysis by Student's t test, Wilcoxon's test, and Fisher's exact test [3].

EXPERIMENTAL RESULTS

The aim of the first series of experiments was to study the effect of intraventricular injection of neurotropin into rats on the intensity of acute generalized seizures induced by injection of picrotoxin. Neurotropin, injected 1 h before picrotoxin, increased the latent period of the seizures to 28.4 ± 4.1 min, significantly longer than in animals of the control group (15.5 ± 0.4 min, $p < 0.01$) (Fig. 1). Neurotropin had no significant effect on the severity of the seizures or on mortality after the use of picrotoxin (Fig. 1).

Neurotropin, if injected 24 h before picrotoxin, also increased the latent period of the first seizures (from 16.5 ± 1.8 min in the control to 22.3 ± 1.4 min in the experiment, $p < 0.05$) and had no effect on the severity of the seizures (Fig. 1). Neurotropin, if injected 2-7 days before picrotoxin, did net affect the intensity of the seizure responses (Fig. 1). Under the influence of neurotropin, injected 8 days before picrotoxin, the latent period of the seizures was shortened to 11.8 ± 0.9 min ($p < 0.001$) compared with 16.3 ± 0.9 min in the control (Fig. 1). Under these circumstances, all the animals (20 rats) developed marked chronic convulsions of the whole trunk, which changed in the course of 15-25 min into generalized clonicotonic convulsions. The

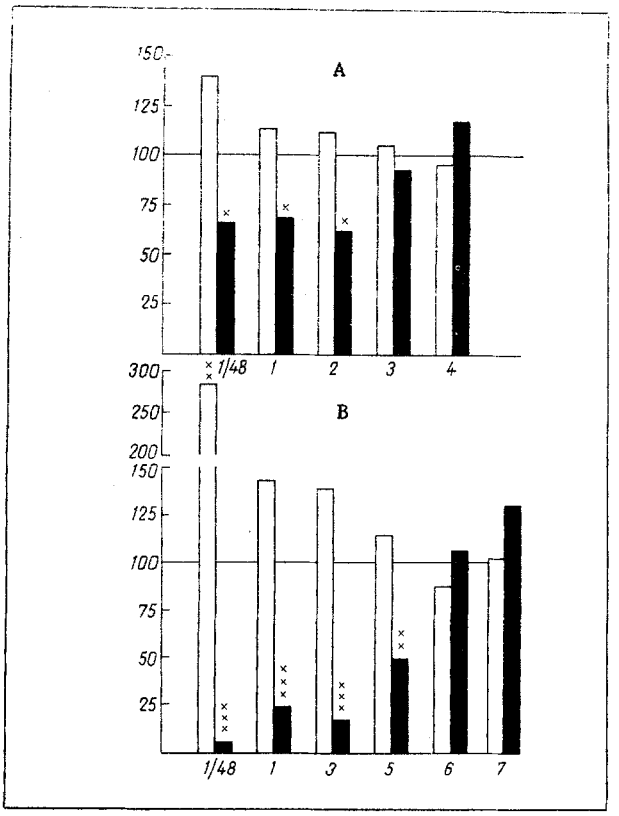

Fig. 2. Effect of various doses of neurotropin on generalized seizures induced in mice by injection of picrotoxin. A and B: action of neurotropin in doses of 0.2 and 0.5 ml per animal respectively on generalized seizures induced by intraperitoneal injection of picrotoxin in a dose of 4 mg/kg. Legend as to Fig. 1.

ratio of the number of animals with generalized seizures to the total number of animals in the group was greater than in the control group. In rats of the control group, after injection of picrotoxin seizures were observed in only five of 11 animals ($p < 0.025$). The average severity of the seizures when neurotropin was injected 8 days previously was 4.2 ± 0.1 point and was greater than in the control (3.44 ± 0.3 point; $p < 0.05$; Fig. 1).

Shortening of the latent period of the first seizures in animals receiving neurotropin also was observed on the 9th day after its injection (Fig. 1). Later (after 10-15 days) no difference was observed between the groups with respect to these parameters (Fig. 1).

In another series of experiments on mice the effect of intraperitoneal injection of various doses of neurotropin (0.2 and 0.5 ml per animal 24 h before injection of the convulsants) was studied on generalized seizures induced by injection of various epileptogens. The results of these experiments are given in Fig. 2 and Table 1. As will be clear from the results (Fig. 2), under the influence of neurotropin the severity of the seizures induced by intraperitoneal injection of picrotoxin (4.0 mg/kg) was reduced. A more marked and lasting decrease in the intensity of the seizures was observed when neurotropin was injected in a dose of 0.5 ml (Fig. 2B). Neurotropin had no significant effect on seizures induced by metrazol, strychnine, bicuculline, or kainic acid (Table 1).

In the next series of experiments the effect of neurotropin (25 μl) was studied on activity of epileptic foci induced in the cat cerebral cortex. Characteristic strychnine potentials with an amplitude of 50-200 μV appeared at the site of application 5-12 min after application of strychnine solution to the posterior sigmoid gyros, and in the course of 5-10 min they increased in amplitude to 250-350 μV. The frequency of their generation varied from 20 to 35 potentials per minute (Fig. 3b, zone I). Control experiments (seven observations) showed that epileptic activity, stable with respect to frequency and amplitude was recorded for 12-20 min, after which, for the next 10-50 min, the frequency and amplitude gradually decreased, and the discharges in the zone of strychnine application were completely inhibited. The focus of seizure activity created in this manner continued to

TABLE 1. Effect of Neurotropin (0.5 ml/animal) on Generalized Seizures Induced by Picrotoxin, and also on Seizures Induced by Other Epileptogens, 24 h After Injection of Neurotropin (M ± m)

Experimental conditions	Dose of preparation, mg/kg	Latent period of first seizures, min	Number of animals with generalized clonico-tonic convulsions	Mean severity of seizures, points
Picrotoxin	4,0 (10)	11,85±1,75	7	3,8±0,4
Picrotoxin + neurotropin	4,0 (7)	16,5±5,0*		0,43±0,3***
Metrazol	60 (10)	1,6±0,2	8	3,9±0,3
Metrazol + neurotropin	60 (10)	1,9±0,3	4	3,3±0,3
Strychnine	1,0 (8)	5,6±0,5	6	4,1±0,3
Strychnine + neurotropin	1,0 (10)	5,2±0,3	6	3,6±0,3
Bicuculline	4,0 (7)	3,2±0,6	4	2,9±0,8
Bicuculline + neurotropin	4,0 (8)	3,1±0,8	5	3,0±0,5
Kainic acid	15 (10)	22,3±4,2	2	2,0±0,5
Kainic acid + neurotropin	15 (10)	18,9±2,5	3	1,6±0,5

Legend. Number of animals in group given in parentheses. *) $p < 0.05$, **) $p < 0.025$, ***) $p < 0.001$ compared with picrotoxin.

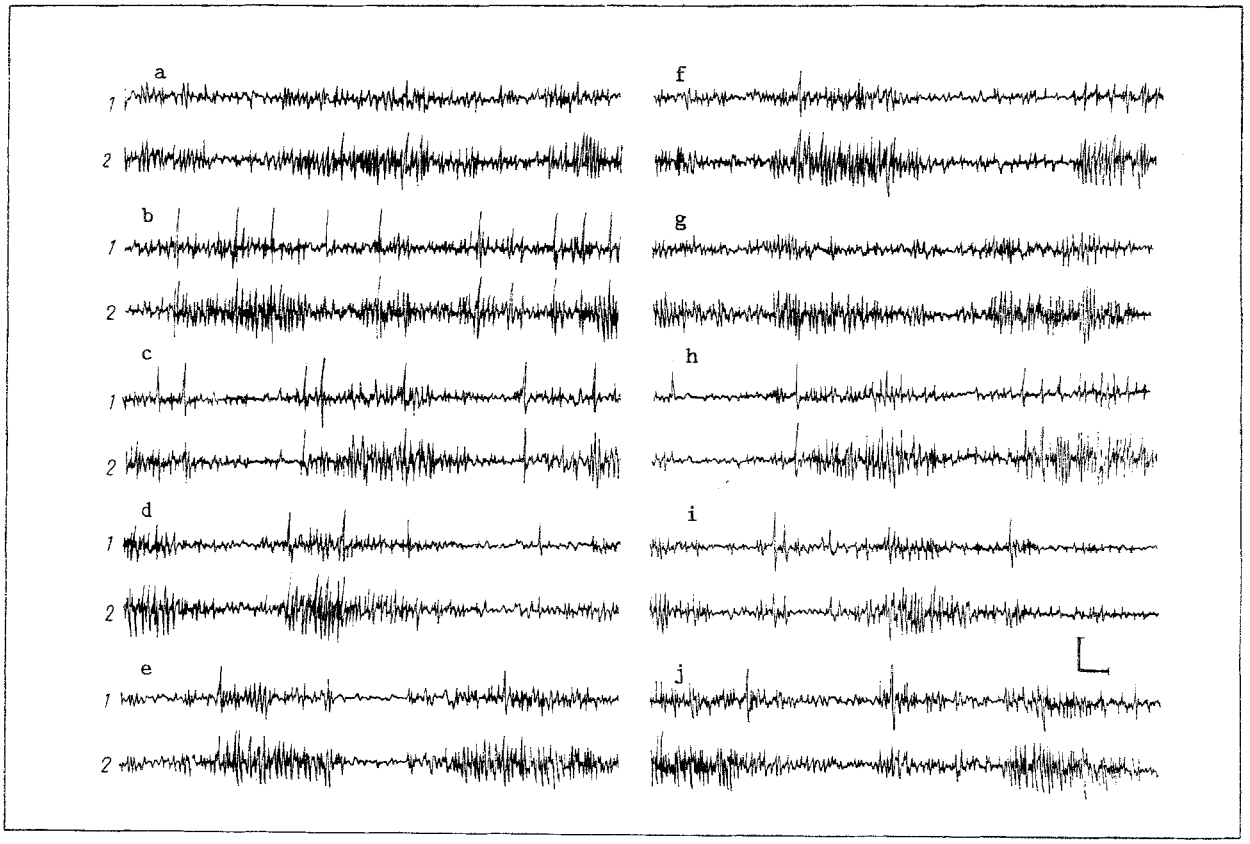

Fig. 3. Effect of intraventricular injection of neurotropin on focus of epileptic activity created in the cat cerebral cortex by strychnine. a) Spontaneous activity (ECOG) before strychnine application, b) 10 min after application of 0.1% strychnine solution to zone I (application continuing); c) 4 min after b and 3.5 min after intraventricular injection of neurotropin (25 μl); d, e, f, g) 6, 11, 15.5, and 18 min respectively after c; h) 22 min after g and 15 min after application of 0.1% strychnine solution to zone I (application continuing); i, j) 5 and 10 min respectively after h (application continuing). Legend: 1) middle and 2) posterior sigmoid gyri. Calibration: 250 μV, 1 sec.

generate spike potentials for 20-40 min. Intraventricular injection of neurotropin (25 μl), given during generation of spike discharges stable in amplitude and frequency (Fig. 3c, zone I) induced a marked decrease of frequency and amplitude of the seizure discharges in the focus (Fig. 3d-f) in six of the seven animals after 10-20 min, followed by their complete disappearance (Fig. 3g). The total duration of existence of the epileptic focus after injection of neurotropin was 15-27 min, less ($p < 0.05$) than in the control. Repeated application of 0.1% strychnine solution to the cerebral cortex caused the appearance of discharges only 10-12 min after application of the convulsant. The amplitude of individual discharges in the period of their appearance varied from 180 to 250 μV (Fig. 3h), and the frequency of generation varied from 5-10 to 15-25 discharges/min. The epileptic potentials had the same frequency of appearance, and also the same amplitude of individual discharges during repeated and prolonged applications of the convulsant (Fig. 3i, j).

The investigations thus show that neurotropin, if injected intraventricularly, suppresses seizure activity induced by a single injection of a convulsive dose of picrotoxin. This effect was manifested as lengthening of the latent period of the first seizures, and it continued for 24 h after administration of neurotropin. Later (on the 8th-9th day) intensification of the seizures was observed in the animals compared with rats of the control group: there was a marked decrease in the latent period and facilitation of the appearance of generalized clonicotonic convulsions. This dual time-dependant effect on epileptic activity is also characteristic of opioid compounds [6]. Neurotropin may perhaps have an effect on the predisposition to seizures observed in animals to synthesis of endogenous peptides. However, the fact that the use of a cyclic phosphate, inhibiting protein and peptide synthesis, did not affect the anticonvulsant properties of neurotropin, did not confirm this hypothesis. The mechanisms of the anti- and proepileptic action of neurotropin and their possible linkage with the peptidergic mechanisms of the brain are subjects which require further research.

In experiments on mice with a model of acute seizures induced by picrotoxin, intraperitoneal injection of neurotropin was followed by a marked anticonvulsant effect, which was dose-dependant in character and lasted for 2 to 5 days after injection of the preparation; intensification of seizure activity did not arise in the late period after administration of neurotropin. The results of these experiments demonstrate the importance of the mode of administration of neurotropin for the realization of its antiepileptic action. The absence of an effect of neurotropin on epileptic activity induced by other epileptogens also was demonstrated. It can be tentatively suggested that the anticonvulsant action of neurotropin is connected with its interaction with specific picrotoxin binding sites, modifying the properties of the chloride channel of the membrane [12].

The study of the effect of neurotropin on foci of epileptic activity showed that after intraventricular injection of the preparation activity of a relatively weak strychnine-induced focus of epileptogenesis was suppressed, and its repeated formation was difficult. This result demonstrates the species nonspecific antiepileptic action of neurotropin and also points to the possibility of rapid development of the antiepileptic action of the preparation.

The experiments thus demonstrated the antiepileptic action of neurotropin, manifested in animals of different species under conditions of acute generalized and localized forms of epileptic activity.

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