Contribution of Protein Synthesis to Mechanisms of Antiepileptic System Activity in Kindling

M. N. Karpova, M. Yu. Karganov, N. Yu. Klishina, N. N. Khlebnikova, and G. N. Kryzhanovskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 6, pp. 629-633, June, 2002
Original article submitted February 4, 2002

We studied changes in protein composition and their effects on convulsive activity in drug-induced and electrical kindling. Injection of protein fraction of brain extract from animals subjected to metronidazole-induced kindling to intact recipients reduced their sensitivity to the convulsant (*i. e.* increased the dose of metronidazole inducing clonic convulsions and lethal seizures). Incorporation of labeled amino acids in proteins in different brain structures after appearance of stable afterdischarges in the zone of synaptic stimulation significantly decrease compared to the control. Label incorporation decreased also in the frontal and occipital cortex. Kindling-associated changes in the protein spectrum were studied by electrophoresis.

Key Words: *epileptogenesis; kindling; metronidazole; increased convulsive readiness of the brain; acute convulsions*

Isolation and identification of substances acting as endogenous pro- and antiepileptogenic factors are essential for understanding of the mechanisms of epileptogenesis. Transfer of epileptogenic effects by brain peptides and proteins from mice hereditary predisposed to audiogenic epilepsy [15] was not reproduced after administration of blockers of ribosomal protein synthesis. The appearance of a specific protein with a molecular weight of 70-71 kDa in the cerebral cortex was demonstrated on the model of cobalt epilepsy [14]. Injection of this protein into the motor cortex induced typical changes on EEG and convulsions, which could be prevented with anticonvulsants. Experiments on a model of focal cortical epileptogenesis showed that cycloheximide (protein synthesis blocker) prevented the appearance of long trace charges in the cerebral cortex [5]. We investigated changes in protein composition and their effects on convulsive activity in drug-induced and electrical kindling.

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Two experimental series on (C57Bl6×CBA)F_1 mice and random-bred rats (280-300 g) were carried out. In the first series we studied the effect of protein fraction of brain extract from mice exposed to chronic epileptization on the sensitivity to convulsant in intact recipients. Protein fraction of brain extract was obtained from donor mice receiving intraperitoneal injections of metronidazole (30 mg/kg, subconvulsive dose) for 30 days. Controls received an equivalent volume of normal saline. The severity of convulsive reaction was daily scored: 1 point — starting or head shaks; 2 points — individual clonic convulsions of the whole body; 3 points — a series of whole body clonic convulsions or forelimb clonus; 4 points — tonic-clonic convulsions with rised hindpaws (kangaroo posture); 5 points — clonic tonic convulsions with sideways fall and tonic extension phase. After the end of kindling (day 30) before collection of brain tissue the severity of convulsions was 4-5 points.

The animals were decapitated 3 days after the last injection of metronidazole. Brain specimens were

0007-4888/02/1336-0544$27.00

weighed, homogenized in Tris-HCl buffer (pH 7.4, 1:10 wet tissue-buffer ratio) in plastic tubes with a Teflon pestle, centrifuged, and protein content was measured in aliquots [7]. Protein concentrations in samples was equalized, the extracts were lyophilized and stored in a freezer until use. For identification of active substances we studied the sensitivity of the extracts to proteolysis. To this end the extracts were incubated with 0.1 mg/ml pronase (Calbiochem, specific activity 45,000 U/g) for 2 h at 37°C. The reaction was stopped by 10-min boiling in a water bath.

For evaluation of the sensitivity of recipient mice to convulsions induced by intravenous injection of 1% metronidazole at a rate of 0.01 ml/sec, the threshold of clonic and lethal tonic seizures was determined. The threshold dose of metronidazole inducing convulsions was estimated individually for each animal. Control animals were injected with normal saline for 30 days. The extract was injected intraperitoneally (10 μg/100μl normal saline) 1 h before metronidazole.

In the experimental second series incorporation of labeled amino acids in various brain structures was studied on a model of electrical kindling [4]. The label (^{14}C hydrolysate of chlorella, "UVVVR"; 1.3 MBq/rat) was injected intraperitoneally (0.5 ml) after the appearance of stable afterdischarges. After 1 h the animals were decapitated, tissue specimens were collected and homogenized in 0.5 ml 10% trichloroacetic acid. The homogenates were centrifuged at 3000 rpm for 10 min and the supernatant was discarded. The precipitate was washed two times with 0.5 ml cold isopropanol to remove lipids, resuspended in a buffer containing 2% sodium dodecylsulfate, 5% mercaptoethanol, 10% glycerol, 50 mM Tris (pH 6.8), and aliquots for electrophoresis were taken. The extract was filtered through 0.45-μ membrane filter (Millipore) and radioactivity was measured on a RackBeta scintillation counter (LKB). The samples were analyzed by vertical discontinuous polyacrylamide gel electrophoresis (15%) after Laemmli at 7 mA and 70-90 V overnight.

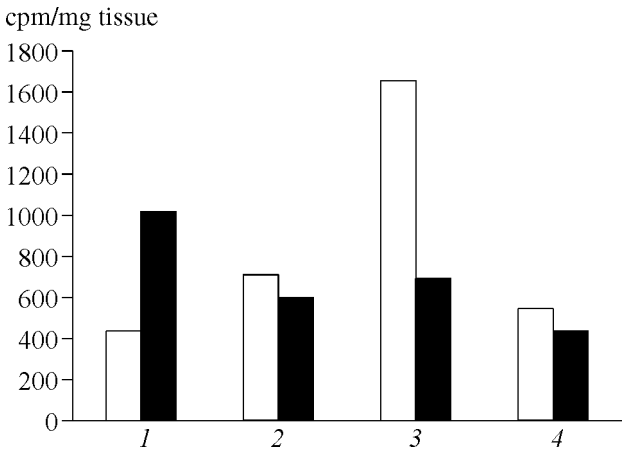

Fig. 1. Label incorporation in protein fraction of rat striatum and cortex after repeated electrical stimulation of the sensorimotor cortex. Light bars: control; dark bars: experiment. Here and in Fig. 2: *1*, *2*) left and right striatum; *3*, *4*) left and right cortex, respectively.

The significance of the effects caused by protein fractions was evaluated using Student's *t* test.

RESULTS

Injection of brain protein fraction to animals with metronidazole kindling reduced the sensitivity of recipients to the convulsant: the dose of metronidazole causing clonic and lethal tonic seizures in recipient mice increased (Table 1). Injection of brain protein fraction from the control animals and pronase-treated protein extracts from the control and kindled mice did not change mouse sensitivity to the convulsant (Table 1). The results indicate that protein fraction from animals subjected to chronic epileptization produces an antiepileptic effect and acts as an endogenous antiepileptic factor. Hence, the development of chronic epileptization is associated with the formation of protein

TABLE 1. Effect of Protein Fraction from Mouse Brain on the Development of Generalized Seizures Induced by Intravenous Injection of Metronidazole (*M*±*m*)

Experiment conditions	Convulsive dose of metronidazole, mg/kg	
	clonic	tonic
Control (normal saline; *n*=19)	26.55±1.03	39.78±1.90
Brain extract from intact mice (*n*=19)	27.22±0.94	43.81±2.00
Brain extract from mice subjected to metronidazole kindling (*n*=21)	34.87±1.24*+	55.66±2.54*+
Convulsive dose brain extract from intact mice (*n*=10)	28.13±1.67	46.18±3.06
Proteolyzed brain extract from mice subjected to metronidazole kindling (*n*=10)	28.80±1.89°	45.70±3.07°

Note. $p<0.001$: *compared to the control, +compared to brain extract from intact mice; °$p<0.02$ compared to brain extract from mice subjected to metronidazole kindling.

factors with anticonvulsive effect. This is an important pathogenetic component in the formation of antiepileptic system suppressing activity of pathological epileptic system.

In the second experimental series (electrical kindling) incorporation of the label in the zone of synaptic stimulation was significantly lower than in the control (Fig. 1). Label incorporation in the frontal and occipital cortex decreased by 25-30%. On the other hand, label incorporation in the striatum on the side of synaptic stimulation was notably higher than in the control. No appreciable changes were observed in the hippocampus and thalamus.

In further experiments we identified proteins responsible for the observed shifts. The most pronounced effects were detected in the primary and secondary foci and striatum, and therefore these structures were examined by electrophoresis. Some proteins (most

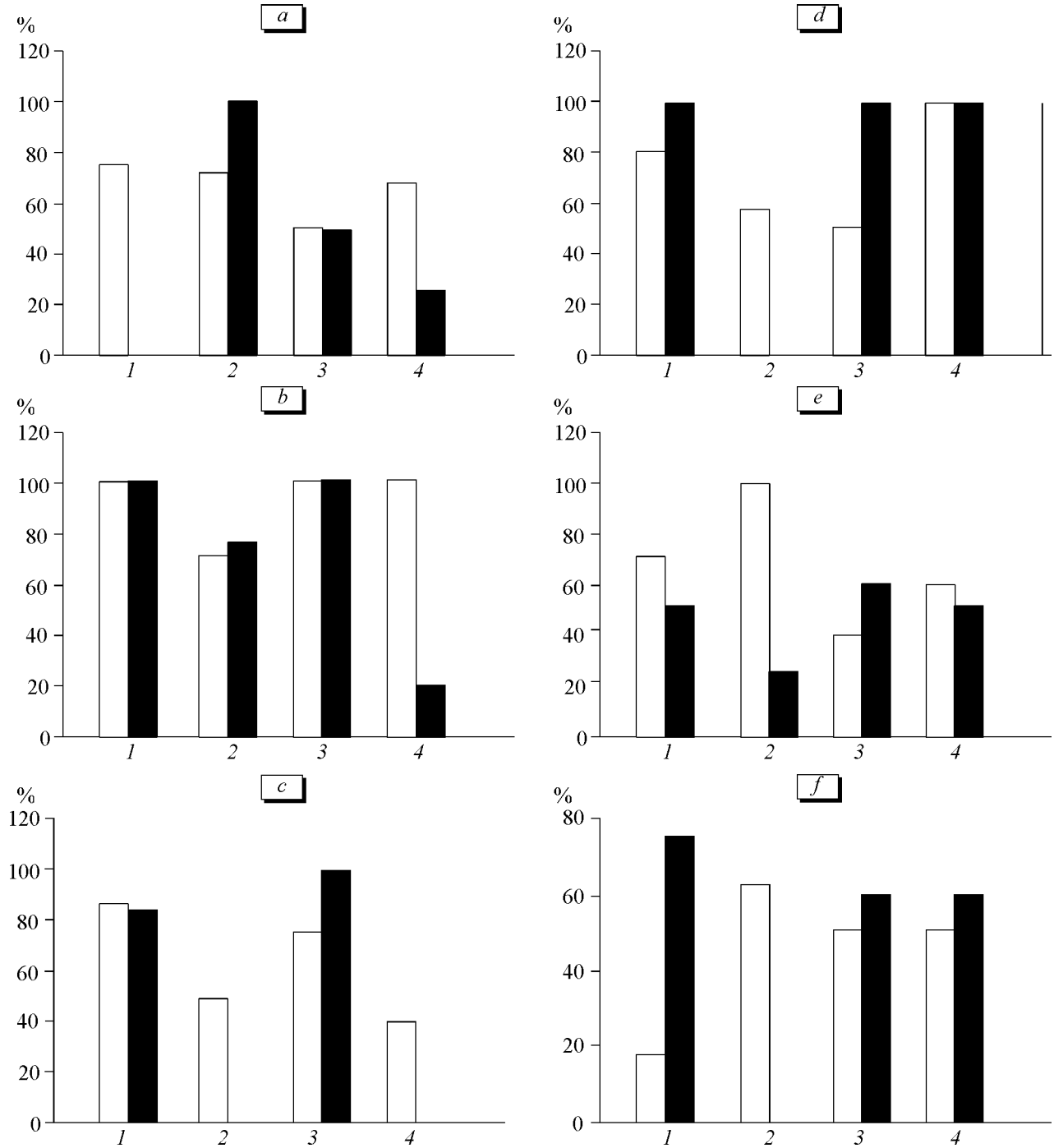

Fig. 2. Incidence (according to electrophoretic analysis) of proteins p11 (*a*), p11.8 (*b*), p25.7 (*c*), p93.9 (*d*), and p125.9 (*e*) in the control (light bars) and after repeated electrical stimulation of the sensorimotor cortex (dark bars).

likely structural proteins) were detected in all samples. Other proteins were less frequently detected in experimental animal compared to controls. Protein p20 was less frequently detected in the cortex and striatum (right and left), p4 and p21 proteins were less frequently detected in the zone of electrical stimulation, p41 was less frequently detected on both sides of the striatum, p3 and p21 were less frequently found in the striatum on the side of synaptic stimulation, and p15, p39, p40, and p44 proteins were detected in the striatum on the side of electrical stimulation. The following proteins were more often detected in experimental animals: p36 in the cortex (particularly in the zone of electrical stimulation), p44 in the left striatum, and p5 in the left cortex and right striatum. Proteins p40, p44, p43, p15, p3, and p4 were the most demonstrative (Fig. 2).

The data on the involvement of protein synthesis in kindling obtained in our experiments agree with numerous reports on the appearance and enhanced production of proteins in epileptogenesis. Protein synthesis in mice predisposed to seizures upon throwing up increased before and after convulsions; during tonic seizures protein synthesis was suppressed [12] and the content of 67 and 120 kDa proteins. Our experiments showed that incorporation of labeled amino acid in the cortex considerably decreased on the side of synaptic stimulation. Increased incorporation of labeled amino acids in the striatum in electrical cortical kindling agrees with published data [11] on long-term corticostriatal potentiation induced by low-frequency stimulation of the cortex. These plastic rearrangements involve corticostriatal glutamatergic synapses [8-10].

Hence, these findings confirm accumulation of proteins acting as inductors and activators of the antiepileptic system in the brain of kindled animals [1]. Intraventricular injection of these substances to recipient animals suppressed generalized seizures and prevented the development of clonic-tonic seizures. We previously showed accumulation of peptides possessing antiepileptic activity in the liquor and ventral mesencephalon during metronidazole-induced kindling [3]. During activation of the antiepileptic system [3] peptide substances exerting protective antiepileptic effect were detected in the liquor of animals [6]. On the other hand, peptides with proepileptic effects were detected in the brain [2]. It can be hypothesized that the observed changes in protein synthesis and composition reflect processing of peptide factors with pro- and antiepileptic effects. At the initial stages of epileptic activity activation of cerebral peptidergic systems can prevent the formation of a pathological system and development of the epileptic syndrome [13]. However, at later stages long-term activity of the pathological determinant impairing inhibitory regulation and forming numerous vicious circles induces enhanced production of endogenous factors maintaining the development of the pathological system. These antagonistic effects are characteristic of many peptides in health and disease.

Peptides act as effectors of pathological systems and antisystems [1], and therefore intense production and proteolysis of precursor proteins are important components of the development and suppression of epileptic activity.

REFERENCES

1. G. N. Kryzhanovskii, *General Pathophysiology of the Nervous System. Manual* [in Russian], Moscow (1997).
2. G. N. Kryzhanovskii, M. Yu. Karganov, A. A. Shandra, *et al.*, *Byull. Eksp. Biol. Med.*, **107**, No. 3, 271-274 (1989).
3. G. N. Kryzhanovskii, R. F. Makul'kin, A. A. Shandra, and L. S. Godlevskii, *Ibid.*, **95**, No. 3, 26-29 (1983).
4. G. N. Kryzhanovskii, V. K. Lutsenko, N. N. Khlebnikova, and M. Yu. Karganov, *Ibid.*, **112**, No. 9, 234-237 (1991).
5. G. N. Kryzhanovskii, V. K. Lutsenko, and N. N. Khlebnikova, *Ibid.*, **119**, No. 6, 610-615 (1995).
6. G. N. Kryzhanovskii, A. A. Shandra, and L. S. Godlevskii, *Ibid.*, **106**, No. 8, 146-149 (1988).
7. M. M. Bradford, *Anal. Biochem.*, **72**, 248-254 (1976).
8. P. Calabresi, A. Pisani, N. B. Mercuri, and G. Bernardi, *Trends Neurosci.*, **19**, No. 1, 19-24 (1996).
9. S. Charpier, J. P. Bourgeois, and J. Deniau, *Soc. Neurosci. Abstr.*, **23**, No. 3, 1963 (1997).
10. S. Charpier and J.-M. Deniau, *Proc. Natl. Acad. Sci. USA*, **94**, No. 5, 7036-7040 (1997).
11. S. Charpier, S. Mahon, and J.-M. Deniau, *Neuroscience*, **91**, No. 4, 1209-1222 (1999).
12. D. Furutsuka, A. Yamagami, and Y. Kawakita, *Neurosci. Res.*, **9**, Suppl., 63 (1989).
13. G. N. Kryzhanovsky, A. A. Shandra, L. S. Godlevsky, and M. Yu. Karganov, *Epilepsia*, **30**, No. 5, 631-635 (1989).
14. M. Onozuka and S. Imai, *Brain Res.*, **507**, No. 1, 143-145 (1990).
15. R. A. Schriber, *Soc. Neurosci. Abstr.*, **3**, No. 2, 144 (1977).