Cl^- Conduction of $GABA_A$ Receptor Complex on Synaptic Membranes in Rat Cortex at the Early Stage of Chronic Cerebral Epileptization

I. G. Rebrov, M. N. Karpova, A. A. Andreev, N. Yu. Klishina, M. V. Kalinina, O. S. Kuzina, and L. V. Kuznetsova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 8, pp. 139-141, August, 2006
Original article submitted December 27, 2005

Experiments on Wistar rats showed that muscimol-stimulated Cl^- conduction of synaptoneurosomes, isolated from the cerebral cortex increased after 5-day systemic treatment with subconvulsive doses of pentylene tetrasole during the initial stage of pharmacological pentylene tetrasole kindling, characterized by gradually augmenting convulsive readiness of the brain. This indicates an increase in functional activity of the $GABA_A$ receptor/Cl^- ionophore complex.

Key Words: *pentylenetetrazole; $GABA_A$ receptor; Cl^- channel; synaptoneurosomes; $^{36}Cl^-$ isotope*

Electrostimulation and pharmacological kindling (chronic subthreshold electrostimulation of brain structures or injections of epileptogens in subconvulsive doses gradually increasing convulsive readiness of the brain to previously non-convulsive influences) is used in the studies of mechanisms of epileptogenesis [1,2,7]. Kindling is a model of chronic epileptization of the brain, most close to human epilepsy. However, the stages and characteristics of the development of elevated convulsive readiness of the brain in kindling remains poorly studied. One of the mechanisms of the convulsive threshold reduction in kindling is attenuation of the inhibitory antiepileptic GABAergic processes in the CNS [3,4, 7,8]. We studied the status of Cl^- conduction of the GABA receptor/Cl^- ionophore complex ($GABA_A$-RC) at the early stages of the development of elevated convulsive readiness of the brain induced by pentylenetetrazole (pharmacological kindling).

Laboratory of Epileptogenesis, Institute of Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Experiments were carried out on 30 male Wistar rats initially weighing 170-190 g. The animals were kept under standard vivarium conditions on standard rations.

Experimental animals were intraperitoneally injected with pentylenetetrazole in a subconvulsive dose of 25 mg/kg for 5 days. Controls received the same volume of saline.

Functional activity of $GABA_A$-RC was evaluated by muscimol-induced $^{36}Cl^-$ entry into synaptoneurosomes from brain cortex. This method evaluates functional activity of $GABA_A$-RC by the content of $^{36}Cl^-$ entering the synaptoplasm, which depends not only on Cl^- channel conduction, but also on transmembrane Cl^- gradient. Synaptoneurosomes from the cerebral cortex of experimental and control rats were isolated on the same day 48 h after the last injection of pentylenetetrazole or saline using previously developed method [9] with some modifications [5]. The rats were decapitated, the brain

0007-4888/06/1422-0176

cortex was isolated and homogenized at 0-4°C (5 frictions in a glass homogenizer with a Teflon pestle in Krebs—Ringer solution: 145 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES (pH 7.4) at 20°C; 1 g tissue/15 ml medium). The homogenate was filtered through a series of Nylon sieves (Rakhmanov Plant) with 300-, 99-, 60-, and 27-μ pores. The filtrate was centrifuged for 5 min at 2700*g*, the precipitate was resuspended in the same volume of Krebs—Ringer solution, and repeatedly centrifuged under the same conditions. After the second centrifugation synaptoneurosome precipitate was suspended in Krebs—Ringer solution to a final protein concentration ~4 mg/ml. Synaptoneurosomes were used directly after isolation. Functional activity of $GABA_A$-RC was evaluated by a previously described method [10]. $^{36}Cl^-$ entry in synaptoneurosomes was stimulated by $GABA_A$ receptor agonist muscimol. To this end, 100-μl aliquots of synaptoneurosome suspension (400 μg protein) were incubated for 30 min at 20°C. Krebs—Ringer solution with 0.5 μCi $^{36}Cl^-$ (Isotop) and muscimol in concentrations 10 or 30 μM was then added to the samples. After 5 sec, $^{36}Cl^-$ entry in synaptoneurosomes was stopped by filtering through GF/C fiberglass filters (Whatman). The filters were washed with cold (0-4°C) Krebs—Ringer solution (3×4 ml), dried, transferred into flasks with a scintillation fluid, and counted on a RACBETA (LKB) counter. Muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes was evaluated by the difference between $^{36}Cl^-$ entry in the presence of muscimol and its basal entry. In order to evaluate the basal entry of $^{36}Cl^-$, it was added to synaptoneurosomes without muscimol.

The significance of differences was evaluated using Student's *t* test.

RESULTS

Basal (not modulated by $GABA_A$ receptor) entry of $^{36}Cl^-$ in the synaptoneurosomes from brain cortex in the control and experimental group was 30.12±1.83 and 38.05±1.56 nmol/mg protein, respectively. Hence, injections of pentylenetetrazole in subconvulsive doses over 5 days caused no convulsive reactions, but led to a 26.36% increase ($p<0.01$) in the basal $^{36}Cl^-$ entry into synaptoneurosomes (Fig. 1).

Muscimol in concentrations of 10 and 30 μM was used for evaluating functional activity of $GABA_A$-RC, as, according to our previous data [6], the former dose was close to the concentration of muscimol half-maximum effect (EC_{50}), while in a concentration of 30 μM the drug caused an effect close to its maximum effect (B_{max}).

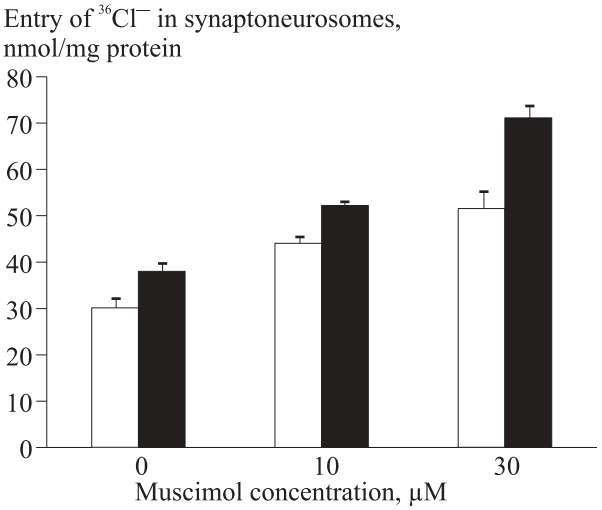

Fig. 1. Effect of muscimol in different concentrations on $^{36}Cl^-$ entry in rat cerebrocortical synaptoneurosomes. Light bars: control; dark bars: experiment.

Studies of $GABA_A$-stimulated Cl^- conduction in rat synaptoneurosomes showed that in the presence of 10 μM muscimol $^{36}Cl^-$ entry into synaptoneurosomes from control animals was 44.07±1.16 nmol/mg protein, while in the experimental group this parameter increased to 52.20±0.61 nmol/mg protein (*i.e.* by 18.45% protein compared to the control, $p<0.001$, Fig. 1).

In control group $^{36}Cl^-$ entry into synaptoneurosomes was equal to 13.95±1.17 nmol/mg protein, in experimental animals 14.15±0.61 nmol/mg protein. Hence, this concentration of $GABA_A$-RC agonist did not modify $^{36}Cl^-$ entry in the cerebrocortical synaptoneurosomes for any of the animal groups.

In the presence of 30 μM muscimol (concentration close to B_{max}) $^{36}Cl^-$ entry into the synaptoneurosomes of experimental animals was by 37.98% higher ($p<0.001$) and reached 71.10±2.58 nmol/mg protein compared to 51.53±3.52 nmol/mg protein in the control (Fig. 1). Evaluation of muscimol-stimulated $^{36}Cl^-$ entry in the cerebral synaptoneurosomes showed it to be 21.41±1.17 nmol/mg protein in the control and 33.05±2.61 nmol/mg protein in experimental group. Hence, the muscimol-stimulated entry of $^{36}Cl^-$ in the cerebral synaptoneurosomes of experimental animals increased by 54.37%; $p<0.001$.

Hence, activation of both GABA-independent and GABA-dependent Cl^- transport through the synaptic membrane was observed at the early stage of the development of increased convulsive readiness of the brain in kindling after 5-day systemic treatment with pentylenetetrazole in subconvulsive doses causing no convulsive reactions in animals:

the main process of GABAergic inhibition (Cl^- transport through the synaptic membrane) was activated in response to the convulsant (pentylenetetrazole). Therefore, the antiepileptogenic mechanism (increase in Cl^- conduction) is activated at the early stage of pentylenetetrazole-induced kindling. However, with prolongation of exposure to the convulsant and development of the pathological process, when convulsions develop, the inhibitory antiepileptic GABAergic processes are attenuated [1,2,7]. These data indicate that the development of the pathological process (epileptogenesis) is preceded by fortification of the defense sanogenetic mechanisms, and the disease develops only in case of subsequent failure of these mechanisms. If the pathological exposure to the epileptogen were not prolonged, epileptogenesis would not have developed, which is proven by experimental data: no epileptization of the brain develops after discontinuation of pentylenetetrazole treatment at the initial stage of kindling. On the whole, the data indicate that epileptogenesis is a dysregulation condition [4].

REFERENCES

1. A. S. Bazyan, V. V. Zhulin, M. N. Karpova, *et al.*, *Zh. Vyssh. Nervn. Deyat.*, **48**, No. 1, 135-142 (1998).
2. M. N. Karpova and I. G. Rebrov, *Dysregulation Pathology* [in Russian], Moscow (2002), pp. 596-604.
3. G. N. Kryzhanovskii, *Ibid.*, pp. 18-78.
4. G. N. Kryzhanovskii, A. A. Shandra, L. S. Godlevskii, *et al. Uspekhi Fiziol. Nauk*, **23**, No. 3, 53-77 (1992).
5. I. G. Rebrov, G. N. Kryzhanovskii, N. P. Belykh, *et al.*, *Byull. Eksp. Biol. Med.*, **118**, No. 8, 160-163 (1994).
6. I. G. Rebrov, G. N. Kryzhanovskii, and R. N. Glebov, *Neirokhimiya*, No. 2, 19-21 (1995).
7. A. S. Bazyan, V. V. Zhulin, M. N. Karpova, *et al.*, *Brain Res.*, **888**, 212-220 (2001).
8. H. F. Bradford, *Prog. Neurobiol.*, **47**, No. 6, 477-511 (1995).
9. E. B. Hollingsworth, E. T. McNeal, J. L. Burton, *et al.*, *J. Neurosci.*, **5**, 2240-2253 (1985).
10. R. D. Schwartz, P. D. Suzdak, and S. M. Paul, *Mol. Pharmacol.*, **30**, 419-426 (1986).