Cl^- Conduction of $GABA_A$-Receptor Complex of Synaptic Membranes of Rat Brain Cortex after Development of Chronic Epileptization of the Brain (Pharmacological Kindling)

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 3, pp. 255-258, March, 2008
Original article submitted April 27, 2007.

Experiments on Wistar rats showed that basal and muscimol-induced $^{36}Cl^-$ entry into synaptoneurosomes isolated from the brain cortex decreased after kindling (30 mg/kg pentylenetetrazole intraperitoneally for 30 days) in animals with seizure severity score 4-5. Changes in Cl^- conduction during kindling are discussed.

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

Kindling, *i.e.* gradually increasing seizure readiness of the brain, develops in several stages. The peculiarities of each stage are a result of changes taking place at the previous stage. Kindling is associated with dysfunction of various systems, including the GABAergic system. Different sites of the $GABA_A$ receptor/Cl-ionophore complex ($GABA_A$-RC) are involved in this process [1,10,13].

We determined changes in Cl^- conduction of $GABA_A$-RC after single injection of the convulsant pentylenetetrazole (PTZ) in the subconvulsive dose and at the early and medium stages of kindling development [5,7,8].

The aim of this study was to determine possible changes in Cl^- conduction of $GABA_A$-RC after completion of kindling.

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MATERIALS AND METHODS

Experiments were carried out on 40 male Wistar rats with initial body weight of 170-190 g maintained under standard vivarium conditions on standard ration.

Pharmacological kindling was induced by daily intraperitoneal injections of PTZ in a subconvulsive dose of 30 mg/kg. The severity of seizure reaction in response to administration of the convulsant was scored daily using a 5-point scale [1,10]. Control animals received physiological saline in the same volume and under similar experimental conditions. The final stage of pharmacological kindling was studied on animals demonstrating 4-5-point seizures after PTZ administration for 30 days.

Functional activity of $GABA_A$-RC was evaluated by the magnitude of muscimol-dependent $^{36}Cl^-$ entry into cortical synaptoneurosomes. This method allows evaluation of functional activity of $GABA_A$-RC by the amount of $^{36}Cl^-$ entering the synaptoplasm, which depended on not only Cl^- channel conduction, but also transmembrane Cl^- gradient.

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Synaptoneurosomes were isolated from the brain 48 h after the 30th injection of PTZ (final stage of kindling) or physiological saline [9,11]. Synaptoneurosomes from the experimental and control animals were isolated on the same day. The rats were decapitated, the brain cortex was isolated and manually homogenized in Krebs—Henseleit medium containing 45 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 10 mM glucose, 10 mM HEPES (pH 7.4 at 20°C) at 0-4°C in a glass homogenizer with Teflon pestle (1 g tissue per 15 ml medium). The homogenate was successively filtered through Nylon filters with pose size of 300, 99, 60, and 27 μ (Rakhmanovskii plant). The filtrate was centrifuged at 2700*g* for 5 min, the pellet was resuspended in the same volume of Krebs—Ringer medium and re-centrifuged under the same conditions. After recentrifugation, the pellet was suspended in Krebs—Ringer medium to a final concentration of 4 mg/ml synaptosomal protein. The synaptoneurosomes were used immediately after isolation. Functional activity of $GABA_A$-RC was determined as described elsewhere [14]. $^{36}Cl^-$ entry into synaptoneurosomes was stimulated with $GABA_A$ receptor agonist muscimol. To this end, 100-μl aliquots of synaptoneurosome suspension (400 μg protein) were preincubated for 30 min at 20°C. Then, 100 μl Krebs—Ringer solution containing 0.5 μCi $^{36}Cl^-$ (Isotop) and muscimol in concentrations of 2-100 μM were added After 5 sec, $^{36}Cl^-$ entry into synaptoneurosomes was stopped by filtering through fiberglass filters GF/C (Whatman), and the filters were washed with cold Krebs—Ringer solution (3×4 ml, 0-4°C). The filters were dried, placed into vials with scintillation fluid, and radioactivity was measured of a RACBETA counter (LKB). Muscimol-dependent $^{36}Cl^-$ entry into synaptoneurosomes was determined by the difference between $^{36}Cl^-$ entry in the absence (basal entry) and presence of muscimol. The degree of stimulation or suppression of muscimol-dependent $^{36}Cl^-$ entry into synaptoneurosomes (at muscimol concentration of 30 μM) was calculated as the ratio of the corresponding values in the control and experimental samples (expressed in %).

Significance of differences was evaluated using Student *t* test.

RESULTS

Basal (nonspecific, not mediated by $GABA_A$ receptor) $^{36}Cl^-$ entry into synaptoneurosomes decreased by 15.11% ($p<0.001$) in animals demonstrating 4-5-point seizures after kindling (Fig. 1, *a*).

The effect of muscimol depended on the concentration, the muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes in experimental animals was lower than in controls. The decrease in muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes of experimental animals was more pronounced at low and medium concentrations of muscimol, than at high concentrations: 20.40% at 10 μM ($p<0.05$), 39.13% at 30 μM ($p<0.01$), 24.64% at 50 μM ($p<0.01$), and 8.89% at 100 μM ($p<0.05$). It should be noted that in control animals the muscimol-dependent $^{36}Cl^-$ entry rapidly increased in the muscimol concentration range of 2-30 μM and remained unchanged at higher concentrations. In experimental animals, the muscimol-dependent $^{36}Cl^-$ entry into synaptoneurosomes increased with increasing

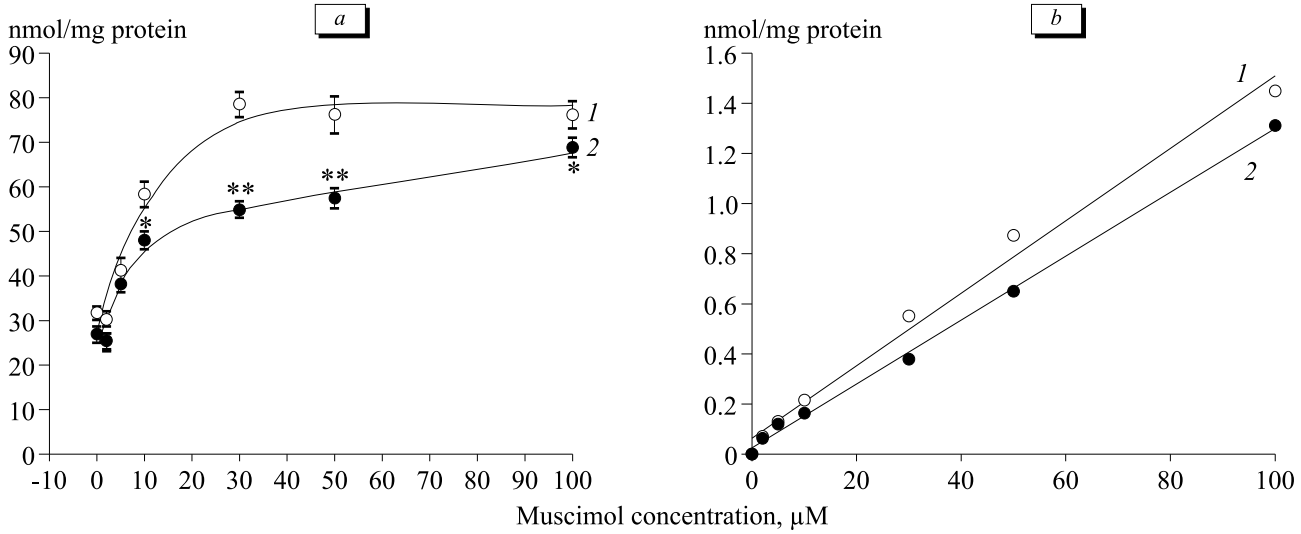

Fig. 1. Changes in $^{36}Cl^-$ entry into synaptoneurosomes isolated from rat cortex. *1*) control; *2*) experiment. *a*) relationship between $^{36}Cl^-$ entry and muscimol concentration; *b*) Wolff—Hanes linearization. *$p<0.05$ compared to the control.

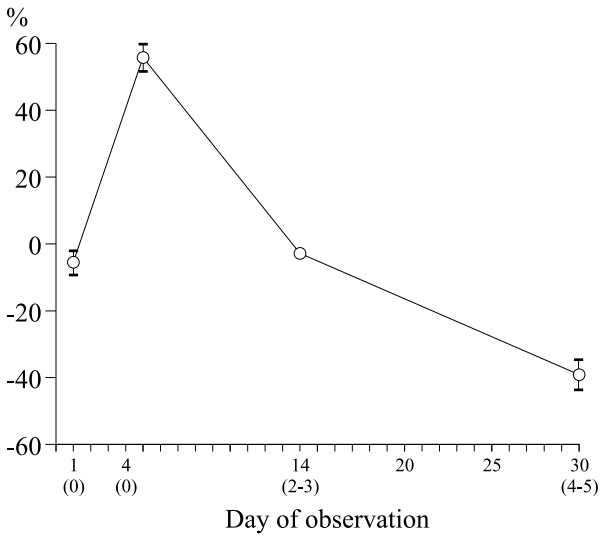

Fig. 2. Changes in functional activity (stimulation/suppression of muscimol-dependent $^{36}Cl^-$ entry) of $GABA_A$-RC in synaptoneurosomes from rat cortex at different stage of PTZ kindling at muscimol concentration of 30 μM. In parentheses: seizure score.

muscimol concentration less rapidly than in control animals, but continued to increase at muscimol concentrations ≥30 μM. It can be hypothesized that the sensitivity of $GABA_A$-RC to muscimol decreases in experimental animals. Calculation of the kinetic parameters yields the following results: the concentration corresponding to half-maximum effect and the maximum effect in the control were 11.85± 2.04 μM and 82.79±11.30 nmol/mg protein, while in experimental animals the corresponding values were 7.04±2.65 μM (p<0.001) and 75.05±9.27 nmol/mg protein (p<0.01). Hence, the number of receptors and their sensitivity decreased after kindling. Woolf—Hanes linearization [3] yielded regression coefficients r=0.9845 in the control and r=0.9859 in the experiment (Fig. 1), which suggests that the data obtained by us correspond to the theoretic binding isotherm.

These findings attest to reduction of the basal and muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes from the cortex of kindled animals, which suggests decreased functional activity of $GABA_A$-RC. The number of $GABA_A$-receptors and their sensitivity also decreased. These results are consistent with the data obtained by other authorities on the models of pharmacological and electrostimulation kindling [10,12,13,15].

Three stages can be distinguished in the development of chronic epileptization of the brain (kindling): stage 1 (early stage) is characterized by the absence of seizures; during stage 2 (middle stage) seizures appear and their severity gradually increases; stage 3 (final stage) eventuates in generalized tonic-clonic seizures and formation of the state of increased seizure readiness of the brain, which persists long time after kindling [2,10]. Changes in functional activity of $GABA_A$-RC in synaptoneurosomes of rat brain cortex at different stages of PTZ-kindling were demonstrated (including our previous results, Fig. 2) [5-8]. The basal and muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes increased at the early stage of kindling. Analysis of the observed changes from the viewpoint of dysregulation pathology [4] drove us to a conclusion that the development of the pathological process (epileptogenesis) is preceded by activation of the protective sanogenic mechanisms, in particular, enhancement of GABAergic inhibition, and the seizures do not develop while these mechanisms are active. Activation of the GABAergic system can be explained by hyperexcitation of inhibitory neurons, *i.e.* by enhanced secretion of GABA, and/or alosteric modification of $GABA_A$-RC accompanied by increased Cl^- conductance. At this stage, the antiepileptic system counteracts to neuronal hyperactivity by increasing activation threshold of inhibitory neurons. However, continued administration of the convulsant overcomes these defense mechanisms. At the same time, this stage is characterized by increased sensitivity of $GABA_A$-RC to classic convulsants (picrotoxin, bicuculline, and PTZ). But these changes are insufficient for disturbing the control of the antiepileptic system and seizures do not develop. It can be hypothesized that this sensitization is a prerequisite for further suppression of GABAergic inhibition at latter stages of kindling, because under conditions of chronic treatment with PTZ the changes are stored as traces after each injection and are summed up, which increases seizure readiness of the brain to a level sufficient for the development of seizures upon the next subconvulsive dose of PTZ. At the middle stage of kindling, the level of functional activity of $GABA_A$-RC decreases to control values. Thus, the middle stage of kindling is characterized by weakening of antiepileptic mechanisms, which creates prerequisites for further suppression of functional activity of the GABAergic inhibitory system at the later stage of kindling. The final stage of kindling is characterized by considerable impairment of antiepileptic mechanisms. Decreased functional activity of $GABA_A$-RC and increased PTZ sensitivity lead to hyperactivation of neurons, underlie chronic epileptozation of the brain, and represent dysregulation pathology of neuronal mechanisms [4] determined by weakening of regulatory inhibitory influences. Fixation of these changes due to plasticity of the nervous system can explain the maintenance

of chronic epileptization of the brain for a long time (more than one year).

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