Cl^- Conduction of $GABA_A$ Receptor Complex of Synaptic Membranes in the Cortex of Rats at the Middle Stage of Chronic Cerebral Epileptization (Pharmacological Kindling)

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Experiments on Wistar rats showed a decrease in basal and muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes isolated from the cerebral cortex during the middle stage of kindling (30 mg/kg pentylenetetrazole intraperitoneally for 14 days) characterized by the development of convulsions of higher (2 points) severity in comparison with the previous stage.

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

Kindling (electrostimulation and pharmacological) as a model of chronic epileptization of the brain is most similar to human epilepsy and reflects important aspects of the pathogenesis of the epileptic syndrome approximating it to clinical development of the disease. The early stage of kindling largely reflects the mechanisms of the absence epilepsy form, while the final stage presents as generalized tonic clonic convulsions. The procedure of pharmacological kindling consists in repeated injections of the convulsant in subconvulsive doses, which increases seizure readiness of the brain manifesting in the appearance of convulsions; the severity of convulsions gradually increases during subsequent treatment with the convulsant [1,2,9]. Kindling manifests in a decrease in seizure threshold in response to the testing dose of the convulsant. Kindling as a process of gradually increasing readiness of the brain develops in certain stages. Classification of these stages is arbitrary. On the other hand, each stage is characterized by peculiar features resulting from changes which developed during the previous stage. The development of kindling is associated with dysfunctions of many systems, including the GABAergic system; various sites of the $GABA_A$ receptor/Cl-ionophore complex ($GABA_A$-RC) are involved in the process [2,9,10].

We previously detected changes in the $GABA_A$-RC Cl^- conduction after a single dose of the convulsant (pentylenetetrazole; PTZ) in the subconvulsive dose and at the early stage of kindling development (before appearance of convulsions) [6,7].

Here we studied further possible changes in Cl^- conduction of $GABA_A$-RC during the middle stage of kindling development characterized by manifestation of convulsions and increase in their severity.

MATERIALS AND METHODS

Experiments were carried out on 30 male Wistar rats (170-190 g). The animals were kept under common vivarium conditions on standard ration.

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Pharmacological kindling was realized by daily intraperitoneal injection of PTZ in the subconvulsive dose (30 mg/kg). The severity of convulsive reaction in response to the convulsant was daily scored using a common 5-point scale. Controls were injected with the same volume of saline under the same conditions.

Functional activity of $GABA_A$-RC was evaluated after 14-day treatment with PTZ (middle stage of kindling) by muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes from brain cortex. The advantage of this method is the possibility of evaluating activity of $GABA_A$-RC by the content of $^{36}Cl^-$ which entered the synaptoplasm, which depends not only on the conductivity of Cl^- channels, but also on transmembrane Cl^- gradient. Synaptoneurosomes were isolated 48 h after the last injection of PTZ or saline from the cerebral cortex of experimental and control rats on the same day as described previously [14] with minor modifications [5] and used directly after isolation. Functional activity of $GABA_A$-RC was evaluated [15]. $^{36}Cl^-$ entry into synaptoneurosomes was stimulated with muscimol ($GABA_A$-RC agonist). To this end, 100 μl synaptoneurosome suspension (400 μg protein) was preincubated for 30 min at 20°C and then Krebs—Ringer solution containing 0.5 μCi $^{36}Cl^-$ (Izotop) and muscimol (30 μM) was added to the samples. After 5 sec, $^{36}Cl^-$ entry into synaptoneurosomes was arrested by filtration through GF/C fiberglass filters (Whatman). The filters were washed 3 times with 4 ml cold (0-4°C) Krebs—Ringer solution, dried, and placed into flasks with scintillation fluid. Radioactivity was measured on a RAC-BETA scintillation counter (LKB). 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 basal entry of $^{36}Cl^-$, it was added to synaptoneurosomes without muscimol. The significance of differences was evaluated using Student's *t* test.

RESULTS

Injections of PTZ to animals for 14 days induced convulsions, their severity during this period was 2 points. Basal (not depending on $GABA_A$ receptor) entry of $^{36}Cl^-$ into synaptoneurosomes was 33.40±0.66 nmol/mg protein in control animals *vs.* 27.57±1.41 nmol/mg protein in experimental rats.

Muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes was evaluated at muscimol concentrations of 2-100 μM. Curves reflecting the relationship between $^{36}Cl^-$ entry into synaptoneurosomes and muscimol concentration are typical saturation curves (Fig. 1, *a*). The following concentration relationships were obtained: EC_{50}=6.78±2.87 μM (EC_{50} is the concentration producing a half-maximum effect) and B_{max}=48.34±3.91 nm/mg protein (B_{max} is maximum effect) in the control and EC_{50}=8.47±2.33 μM and B_{max}=52.93±2.15 nm/mg protein in the experiment. Woolf—Hanes linearization [3] yielded regression coefficients r=0.9716 in control and r=0.9912 in the experiment (Fig. 1, *b*).

Basal and muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes isolated from the cerebral cortex during the middle stage of kindling characterized by development of convulsions and increase in their severity virtually did not differ from the cor-

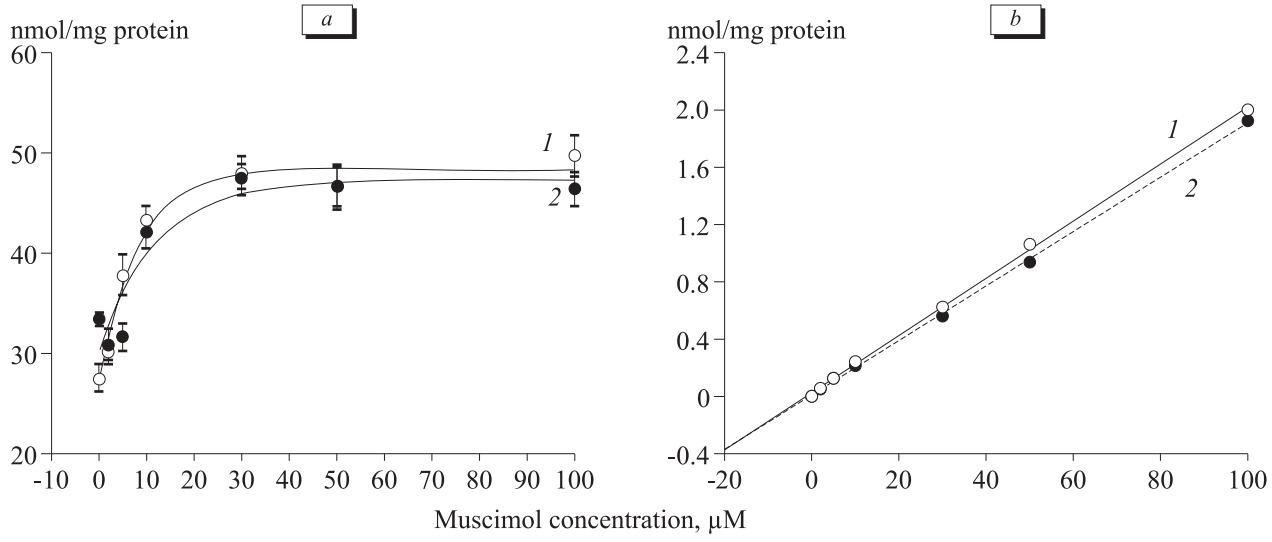

Fig. 1. Changes in $^{36}Cl^-$ entry into synaptoneurosomes isolated from rat cortex. *a*) relationship between $^{36}Cl^-$ entry and muscimol concentration; *b*) relationship in Wolff—Hanes coordinates. *1*) control; *2*) experiment.

responding values in controls. These data can be explained if we compare them with our previous results. Single injection of PTZ in the subconvulsive dose inhibited muscimol-stimulated Cl^- conduction of synaptoneurosomes isolated from the cerebral cortex of animals 15 min after PTZ injection, which attests to reduced functional activity of $GABA_A$-RC; muscimol-stimulated Cl^- conduction of synaptoneurosomes is completely restored 48 h after PTZ injection [6]. During the early stage of kindling, when convulsions have not yet develop, basal and muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes increased [7]. Hence, intensification of defense sanogenetic mechanisms (GABAergic inhibition) precedes epileptogenesis at the early stage of kindling, and while these mechanisms are effective, no convulsions develop. On the other hand, this stage is characterized by higher sensitivity of $GABA_A$-RC to classical convulsants (picrotoxin, bicucullin, and PTZ), which manifests in more intense inhibition of muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes isolated from rat cerebral cortex [8]. However, these changes are insufficient for disordering the regulation of the antiepileptic system, and the convulsions do not manifest clinically. These data are in line with previous results [9,11-13] and indicate that the CNS sensitization process, specifically, $GABA_A$-RC sensitization to convulsants injected chronically in subconvulsive doses, is one of the mechanisms of chronic epileptization of the brain in pharmacological kindling. Presumably, this increase in sensitivity is a prerequisite for further suppression of GABAergic inhibition at later stages of kindling, because in chronic treatment these changes persist in the form of traces after each PTZ dose and are summed up. This increases the convulsive readiness of the brain to a level sufficient for the development of convulsions in response to a new subconvulsive dose. Functional activity of $GABA_A$-RC detected at the early stage decreases to the control values during the middle stage of kindling. Two-point convulsive activity in response to the subconvulsive PTZ dose at this stage seems to result from hypersensitivity of $GABA_A$-RC to the convulsant. Increased sensitivity to the convulsant leads to hyperactivation of neurons — one of the mechanisms of chronic epileptization of the brain (dysregulatory pathology of neuronal mechanisms [4]).

Hence, middle stage of kindling development is characterized by attenuation of antiepileptic mechanisms and thus creates prerequisites for further suppression of functional activity of GABAergic inhibition at later stages of kindling.

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