Effect of Classic Convulsants on Cl^- Conductance of the $GABA_A$ Receptor Complex in Membranes of Cerebral Cortex Cells at the Early Stage of Kindling

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Muscimol-stimulated Cl^- conductance of synaptoneurosomes from the cerebral cortex of Wistar rats increased during the early stage of pharmacological kindling not inducing the seizure response in animals. Picrotoxin, bicuculline, and pentylenetetrazole potentiated inhibition of muscimol-dependent $^{36}Cl^-$ entry into synaptoneurosomes, which attested to increased sensitivity of the $GABA_A$ receptor/Cl^- ionophore complex to classic convulsants.

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

Electrostimulation-induced and pharmacological kindling serves as a model of chronic epileptic stimulation of the brain, due to which subconvulsive influences become convulsive with time [1-3,6]. Kindling develops in several stages and is accompanied by dysfunction of various systems, including the GABAergic system. This process involves various sites of the $GABA_A$ receptor/Cl^- ionophore complex ($GABA_A$-RC) and is probably accompanied by changes in GABA synthesis. This phenomenon manifested in a decrease in the seizure threshold. Since the decrease in seizure threshold during pharmacological kindling can be determined by increased sensitivity to convulsants, we studied the effect of classic convulsants pentylenetetrazole, picrotoxin, and bicuculline on muscimol-stimulated $^{36}Cl^-$ entry into cerebral cortex synaptoneurosomes from animal at the early stage of kindling.

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MATERIALS AND METHODS

Experiments were performed on 35 male Wistar rats weighing 170-190 g. The animals daily received intraperitoneal injections of pentylenetetrazole in a subconvulsive dose of 30 mg/kg for 5 days. The study was conducted on rats not exhibiting the seizure response to convulsant administration. Control animals received an equivalent volume of physiological saline under similar experimental conditions.

Functional activity of $GABA_A$-RC was estimated by muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes from the cerebral cortex of animals. The advantage of this method is that it allows us to evaluate functional activity of $GABA_A$-RC from the amount of $^{36}Cl^-$ entering the synaptoplasm. This value depends not only on Cl^- channel conductance, but also on the transmembrane Cl^- gradient. Synaptoneurosomes were isolated from rat cerebral cortex 48 h after the last injection of pentylenetetrazole or physiological saline by the method of Hol-

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lingsworth [10] with our modifications [5]. The study was performed immediately after isolation of synaptoneurosomes. Functional activity of $GABA_A$-RC was evaluated [11]. A $GABA_A$-RC agonist muscimol was used to stimulate $^{36}Cl^-$ entry into synaptoneurosomes. To this end, aliquots of the synaptoneurosome suspension (100 µl, 400 µg protein) was placed in tubes and preincubated at 20°C for 30 min. Krebs—Ringer solution (100 µl) containing 0.5 µCi $^{36}Cl^-$ (Izotop) and muscimol (30 µM) was added to samples. After 5 sec $^{36}Cl^-$ entry into synaptoneurosomes was stopped by filtering through GF/C fiberglass filters (Whatman). The filters were washed 3 times with 4 ml cold Krebs—Ringer solution (0-4°C), dried, and placed in flasks with a scintillator. Radioactivity of $^{36}Cl^-$ was measured on a RACBETA counter (LKB). Muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes was determined by the difference between $^{36}Cl^-$ entry in the presence of muscimol and basal $^{36}Cl^-$ entry. $^{36}Cl^-$ was added to synaptoneurosomes without muscimol treatment to estimate basal $^{36}Cl^-$ entry. Aliquots of synaptoneurosomes (100 µl) were preincubated with a convulsant at the corresponding concentration (20 min) and treated with the isotope and muscimol to study the effect of convulsants on muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes. The inhibition of muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes (%) was determined as the difference between $^{36}Cl^-$ entry in the presence and absence of convulsants. The results were analyzed by Student's *t* test.

RESULTS

Basal $^{36}Cl^-$ entry (not associated with $GABA_A$-RC) into synaptoneurosomes from control animals increased by 26.33% ($p<0.01$) at the early stage of increased seizure readiness of the brain (Fig. 1). This parameter in control and treated rats was 30.12±1.83 and 38.05±1.56 nmol/mg protein, respectively.

Muscimol in a concentration of 30 µM was used to study functional activity of $GABA_A$-RC. Our previous studies showed that muscimol in this concentration produced a near-maximal effect (B_{max}) [4]. Therefore, this value reflects the number of $GABA_A$ receptors coupled to a Cl^- channel. In the presence of muscimol, $^{36}Cl^-$ entry into brain synaptoneurosomes of treated animals was 37.98% higher compared to the control (71.10±2.58 and 51.53±3.52 nmol/mg protein, respectively, Fig. 1). Muscimol-dependent $^{36}Cl^-$ entry into synaptoneurosomes of control and treated rats was 21.41±1.17 and 33.05±2.61 nmol/mg protein, respectively. Hence,

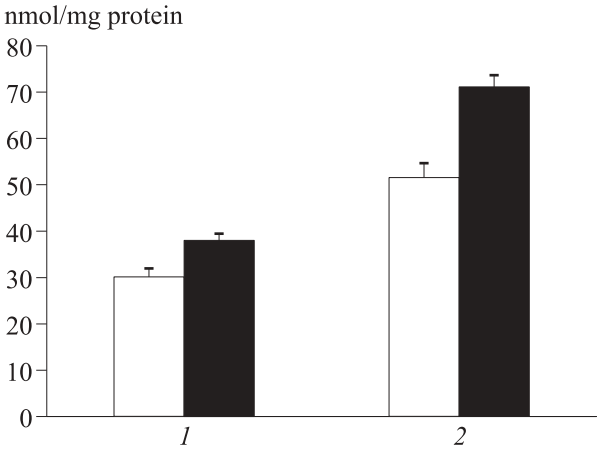

Fig. 1. Basal (*1*) and muscimol-stimulated $^{36}Cl^-$ entry (*2*) in synaptoneurosomes from rat cerebral cortex. Here and in Fig. 2: light bars, control; dark bars, treatment.

muscimol-dependent $^{36}Cl^-$ entry into synaptoneurosomes from treated rats increased by 54.37% ($p<0.001$).

Our results show that GABA-independent and GABA-dependent $^{36}Cl^-$ entry into brain synaptoneurosomes of animals increases at the early stage of kindling not accompanied by seizures.

For evaluation of the effect of convulsants on $^{36}Cl^-$ entry, synaptosomes were preincubated in the presence of 5 µM picrotoxin, 1 µM bicuculline, and 1 mM pentylenetetrazole, *i.e.* in concentrations producing a half-maximal effect (IC_{50}). All convulsants at the specified concentrations had little effect on the basal $^{36}Cl^-$ entry. The effects of convulsants on Cl^- conductance of synaptoneurosomes in the presence of 30 µM muscimol and muscimol-dependent $^{36}Cl^-$ entry into synaptoneurosomes from the cerebral cortex are presented in Tables 1 and 2. The inhibitory effects of various convulsants on muscimol-dependent $^{36}Cl^-$ entry were compared (Fig. 2). The animals of the kindling group were characterized by more pronounced inhibition with picrotoxin (95.1 *vs.* 80.2% in the control), bicuculline (99.84 *vs.* 87.5% in the control group), and pentylenetetrazole (91.4 *vs.* 66.7% in the control group).

TABLE 1. Effect of Convulsants on Cl^- Conductance of Synaptoneurosomes from Rat Cerebral Cortex under the Influence of Muscimol in a Concentration of 30 µM (nmol/mg, *M*±*m*)

Convulsant	Control group	Treatment group
Picrotoxin	34.352.01	39.67±2.14
Bicuculline	32.78±1.84	38.10±0.67
Pentylenetetrazole	37.25±2.83	40.89±3.17

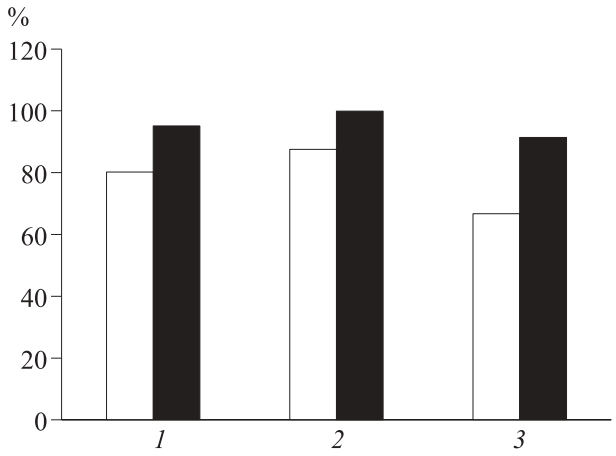

Fig. 2. Inhibition of muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes from rat cerebral cortex under the influence of picrotoxin (*1*), bicuculline (*2*), and pentylenetetrazole (*3*) at the early stage of kindling.

Therefore, the sensitivity of $GABA_A$-RC to the test convulsants increases during the early stages of kindling.

We conclude that the early stage of pentylenetetrazole-induced kindling not accompanied by seizure development results in activation of the antiepileptogenic mechanism, *i.e.* an increase in Cl^- conductance in response to treatment with a convulsant pentylenetetrazole. These findings suggest that progression of the pathological process (epileptogenesis) is preceded by activation of the protective sanogenetic mechanisms. Seizures do not develop as long as these mechanisms are active [3,4]. However, the sensitivity of $GABA_A$-RC to classic convulsants picrotoxin, bicuculline, and pentylenetetrazole increases at the early stage of kindling, which manifests in more pronounced inhibition of muscimol-dependent $^{36}Cl^-$ entry into synaptoneurosomes from rat cerebral cortex. Our results are consistent with published data [6-9] and

TABLE 2. Muscimol-Stimulated $^{36}Cl^-$ Entry into Synaptoneurosomes from Rat Cerebral Cortex in the Presence of Convulsants (*M*±*m*)

Convulsant	Control group	Treatment group
Picrotoxin	4.23	1.62
Bicuculline	2.66	0.05
Pentylenetetrazole	7.13	2.84

confirm the conclusion that sensitization of the central nervous system (in particular $GABA_A$-RC) to chronic administration of convulsants in subconvulsive doses is a mechanism of chronic epileptization of the brain during pharmacological kindling.

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