Effect of Single Injection of Pentylenetetrazole in a Subconvulsive Dose on Cl^- Conductance of the $GABA_A$-Receptor Complex

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Muscimol-stimulated Cl^- conductance in brain cortex synaptoneurosomes from Wistar rats decreased 15 min after single intraperitoneal injection of pentylenetetrazole in a subconvulsive dose. These changes reflected a decrease in functional activity of the $GABA_A$-receptor/Cl^--ionophore complex. Muscimol-stimulated Cl^- conductance in synaptoneurosomes returned to normal 48 h after pentylenetetrazole administration.

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

Kindling is an adequate model of chronic epileptiform activity in the brain. This phenomenon consists in a increase in convulsive readiness of the brain to non-convulsive factors under the effect of repeated subthreshold electrical stimulation of brain structures or chronic systemic administration of convulsants [5]. These influences do not produce clinical reaction, but induce changes that are summarized during kindling and cause epileptiform activity in the brain. Here we studied functional changes in the $GABA_A$-receptor/Cl^--ionophore complex [6] playing a role in the antiepileptic system, which prevents and suppresses epileptiform activity [2].

MATERIALS AND METHODS

Two series of experiments were performed on 28 male Wistar rats weighing 180-200 g. Pentylenetetrazole (PTZ) in subconvulsive doses of 25-30 mg/kg was injected intraperitoneally. Control animals received an equivalent volume of physiological saline.

In series I and II synaptoneurosomes were isolated 15 min and 48 h after intraperitoneal injection of PTZ, respectively. Synaptoneurosomes were isolated from the cerebral cortex of experimental and control rats by the method of Hollingsworth [7] with modifications [3] in the same day. The animals were decapitated. The cerebral cortex was isolated and homogenized in a glass homogenizer with a Teflon pestle (5 frictions at 0-4°C) in Krebs—Ringer medium containing 145 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, and 10 mM HEPES (pH 7.4, 20°C, 1 g tissue per 15 ml medium). The homogenate was consecutively filtered through nylon sieves (Rakhmanovsky Factory) with a cell size of 300, 99, 60, or 27 μ. The filtrate was centrifuged at 2700*g* for 5 min. The pellet was resuspended in Krebs—Ringer medium and centrifuged under similar conditions. After repeated centrifugation the pellet was suspended in Krebs—Ringer medium. The final concentration of synaptoneurosomes was 4 mg protein/ml. Synaptoneurosomes were studied immediately after isolation. Functional activity of the $GABA_A$-receptor/Cl^--ionophore complex was estimated by the method of Shwartz [8].

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$^{36}Cl^-$ influx into synaptoneurosomes was stimulated with the $GABA_A$ receptor agonist muscimol. The suspension of synaptoneurosomes (100 μl aliquots, 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 (2-100 μM) was added to samples. $^{36}Cl^-$ influx into synaptoneurosomes was stopped by filtering through GF/C fiberglass filters (Whatman) after 5 sec. The filters were washed 3 times with 4 ml cold Krebs—Ringer solution (0-4°C), dried, placed in flasks with a scintillation fluid, and studied on a Racbeta counter (LKB). Muscimol-stimulated $^{36}Cl^-$ influx into synaptoneurosomes was determined by the difference between $^{36}Cl^-$ influx in the presence of muscimol and basal influx of the isotope. The results were analyzed by Student's *t* test.

RESULTS

In series I the dependence of $^{36}Cl^-$ influx into synaptoneurosomes on muscimol concentration was described by typical saturation curves (Fig. 1, *a*). In the presence of muscimol (2-100 μM) $^{36}Cl^-$ influx into brain synaptoneurosomes from experimental rats was lower than in control animals. This effect was most prono-

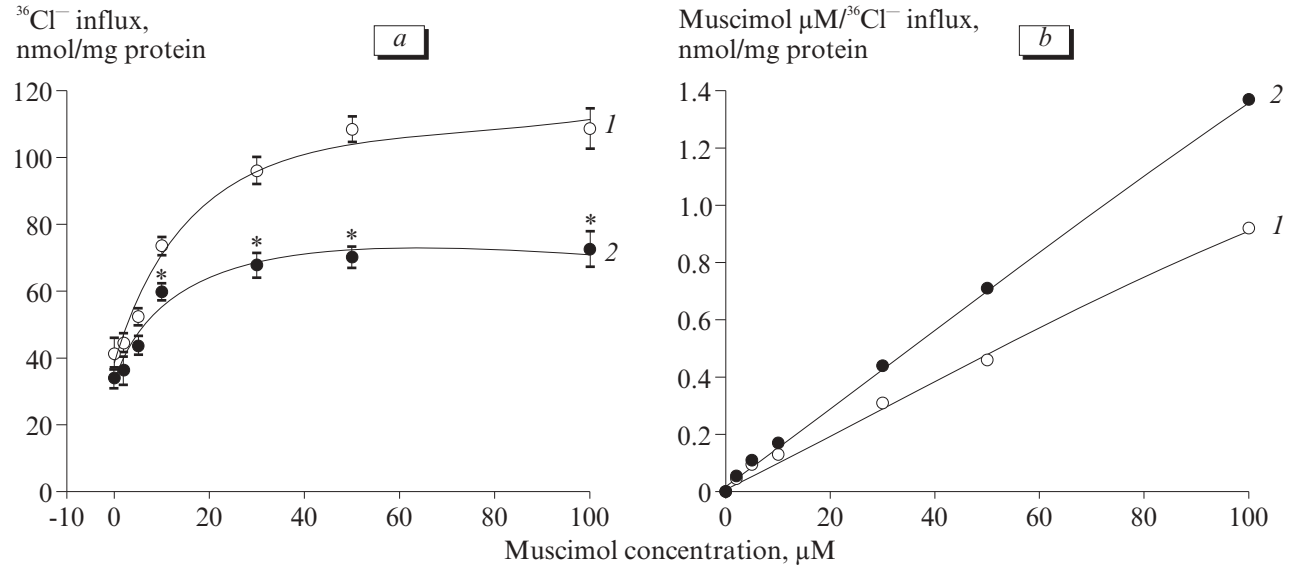

Fig. 1. Effect of muscimol on $^{36}Cl^-$ influx into synaptoneurosomes isolated from rat brain cortex 15 min after administration of physiological saline (*1*) or pentylenetetrazole (PTZ, *2*). Here and in Fig. 2: muscimol-stimulated $^{36}Cl^-$ influx into synaptoneurosomes (nmol/mg protein, *a*); linearization of curves for $^{36}Cl^-$ influx into synaptoneurosomes by the method of Hanes—Woolf (*b*). *$p<0.02$ compared to the control.

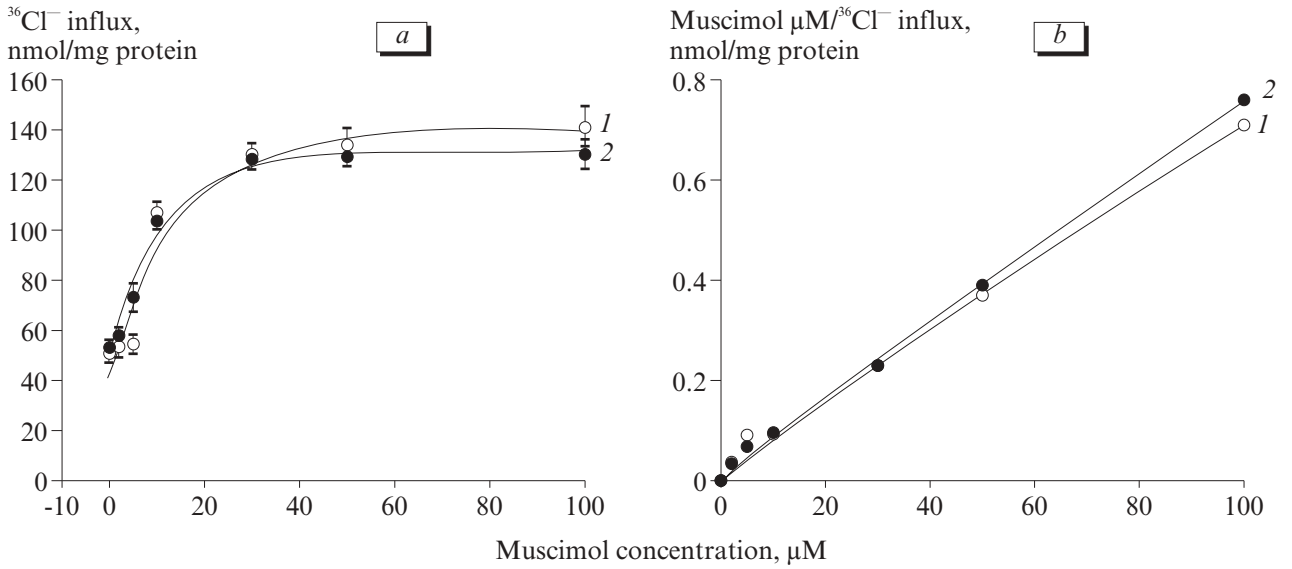

Fig. 2. Effect of muscimol on $^{36}Cl^-$ influx into synaptoneurosomes isolated from rat brain cortex 48 h after administration of physiological saline (*1*) or PTZ (*2*).

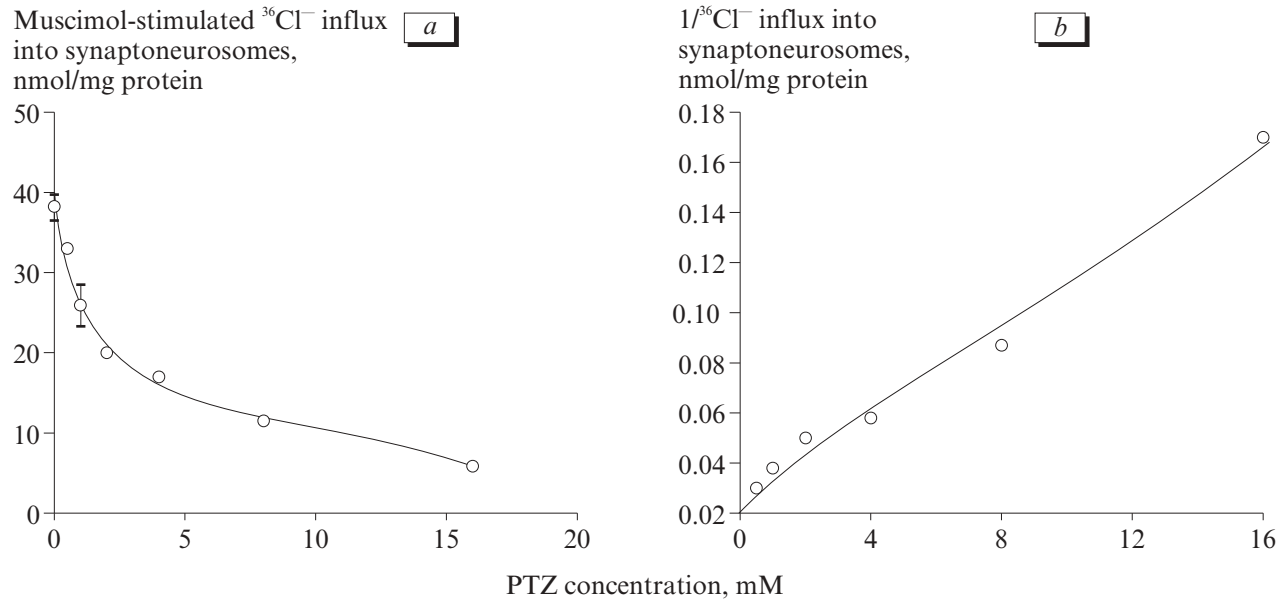

Fig. 3. Effect of PTZ on muscimol-stimulated $^{36}Cl^-$ influx into synaptoneurosomes from rat brain cortex. Muscimol-stimulated $^{36}Cl^-$ influx into synaptoneurosomes (nmol/mg protein, *a*); linearization of the curve for $^{36}Cl^-$ influx into synaptoneurosomes (*b*).

unced in the presence of high concentrations of muscimol (30-100 μM). Under the direct influence of PTZ on synaptoneurosomes *in vitro* [4], muscimol in low concentrations (1-10 μM) most significantly inhibited the $GABA_A$-receptor/Cl^--ionophore complex. Maximum inhibition (67%) was observed after treatment with muscimol in a concentration of 5 μM. Muscimol in high concentrations (50-100 μM) did not produce this effect.

Kinetic parameters of concentration dependences were calculated in series I. In control rats the half-maximum concentration producing the effect (EC_{50}) and the maximum effect (B_{max}) were 11.4±4.2 μM and 113.7±6.9 nmol Cl/mg protein, respectively. In experimental animals these indexes were 8.8±1.3 μM and 76.6±2.9 nmol Cl/mg protein, respectively. Therefore, in brain synaptoneurosomes from experimental animals B_{max} decreased more significantly than EC_{50}. Hanes—Woolf linearization [1] yielded regression coefficients r=0.9986 and r=0.9996 in control and experimental rats, respectively (Fig. 1, *b*). Intersection of linearized concentration dependence curves (x=0.4, y=0.06) attests to noncompetitive inhibition of muscimol-stimulated Cl^- flux in brain synaptoneurosomes from experimental rats.

In series II the dependence for $^{36}Cl^-$ influx into synaptoneurosomes on muscimol concentration was presented by typical saturation curves (Fig. 2, *a*). In Hanes—Woolf coordinates the plots for control and experimental rats were linear and had regression coefficients of 0.9978 and 0.9993, respectively (Fig. 2, *b*). Kinetic parameters of concentration dependences were calculated. In control rats EC_{50} and B_{max} were 9.1±2.3 μM and 140±11 nmol Cl/mg protein, respectively. In experimental animals these indexes were 9.5±1.6 μM and 136±12 nmol Cl/mg protein, respectively, no differences between the control and experimental groups were revealed in the whole muscomol concentration range. Thus, muscimol-stimulated Cl^- flux in synaptoneurosomes returned to normal 48 h after PTZ administration.

PTZ concentration in the brain was measured 15 min after administration. In our previous *in vitro* experiments we obtained concentration dependence of PTZ-produced changes in Cl^- flux in synaptoneurosomes stimulated by 10 μM muscimol (IC_{50}=2.11± 0.25 mM, Fig. 3) was constructed previously [4]. We assumed that intraperitoneal injection and addition of PTZ to the incubation medium for synaptoneurosomes produces similar inhibitory effects on the $GABA_A$-receptor/Cl^--ionophore complex. In PTZ-treated rats Cl^- conductance of synaptoneurosomes stimulated by 10 μM muscimol was 19% lower than in control animals (Fig. 1, *a*). PTZ in a concentration of 0.5 mM decreased $^{36}Cl^-$ influx into synaptoneurosomes (Fig. 3).

Our experiments show that single treatment with convulsant PTZ in a subconvulsive dose decreases Cl^- flux in the $GABA_A$-receptor/Cl^--ionophore complex. These changes are insufficient to cause dysregulation in the antiepileptic system and, therefore, have no clinical manifestations [2]. The observed trace changes are summarized after chronic administration of the convulsant. It increases convulsive readiness of the brain. In this instance, convulsions can develop spontaneously or in response to the influence of subconvulsive factors.

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