# Effect of Picrotoxin and Bicuculline on Desensitization of the GABA Receptor/$Cl^-$ Ionophore Complex

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A study is performed of the effect of the convulsants picrotoxin and bicuculline, blockers of GABA-dependent $Cl^-$-conductivity, on the rate of desensitization of muscimol-induced $^{36}Cl^-$ entry into synaptoneurosomes isolated from rat cortex. Both picrotoxin and bicuculline, despite the difference in the mechanisms of inhibition of the GABA receptor/$Cl^-$ ionophore complex, markedly reduce the rate of desensitization. However, the initial moment of the action of both convulsants is characterized by inhibition of $Cl^-$ transport alone, without a drop of the rate of desensitization.

**Key Words:** *desensitization; GABA-receptor complex; picrotoxin; bicuculline; $^{36}Cl^-$ transport; synaptoneurosomes*

The GABA receptor/$Cl^-$ ionophore complex is one of the most important molecular structures of pre- and postsynaptic membranes. Binding of GABA or its agonists (in particular, muscimol) with the GABA receptor opens up the coupled $Cl^-$ channel and leads to a transient increase of the $Cl^-$ permeability of neuronal membranes and, correspondingly, to spike inhibition. Long-term action of the transmitter results in desensitization of the receptor/ionophore complex, i.e., in a decrease of GABA-dependent $Cl^-$ permeability [2,3].

In our previous study we showed that pentylenetetrazole inhibition of muscimol-dependent $^{36}Cl^-$ entry into synaptoneurosomes is accompanied by a considerable delay of desensitization. Here we report on experiments with the classic blockers of the GABA receptor/$Cl^-$ ionophore complex bicuculline and picrotoxin, which act through different mechanisms. Bicuculline inhibits binding of GABA with the GABA receptor in a competitive manner [7], while picrotoxin inhibits $Cl^-$ transport via the $Cl^-$ channel by binding with the so-called picrotoxin center, which is situated either directly on the $Cl^-$ channel or close to it [5]. The inhibiting effect of pentylenetetrazole is thought to be mediated via its binding with the picrotoxin center of the receptor/ionophore complex [6].

## MATERIALS AND METHODS

In our experiments we used the methods described earlier [1] with some modifications.

White random-bred male rats weighing 180-200 g were decapitated, and the cerebral cortex was removed and homogenized manually (5 frictions) at 0-4°C in a glass homogenizer with a Teflon pestle in Krebs-Ringer medium containing (in mM): 145 NaCl, 5 KCl, 1 $MgSO_4$, 1 $CaCl_2$, 10 glucose, and 10 HEPES, pH 7.4 (20°C, 15 ml/g tissue). The homogenate was sequentially filtered through 300-, 99-, 60-, and 27-μ capron sieves (Rakhmanov plant, Russia). The filtrate was centrifuged at 2700 g for 5 min, resuspended in the same volume of Krebs-Ringer medium, and recentrifuged under the same conditions. After the second centrifugation the pellet was suspended in Krebs-Ringer solution so that the final concentration of synaptoneurosomes was 4

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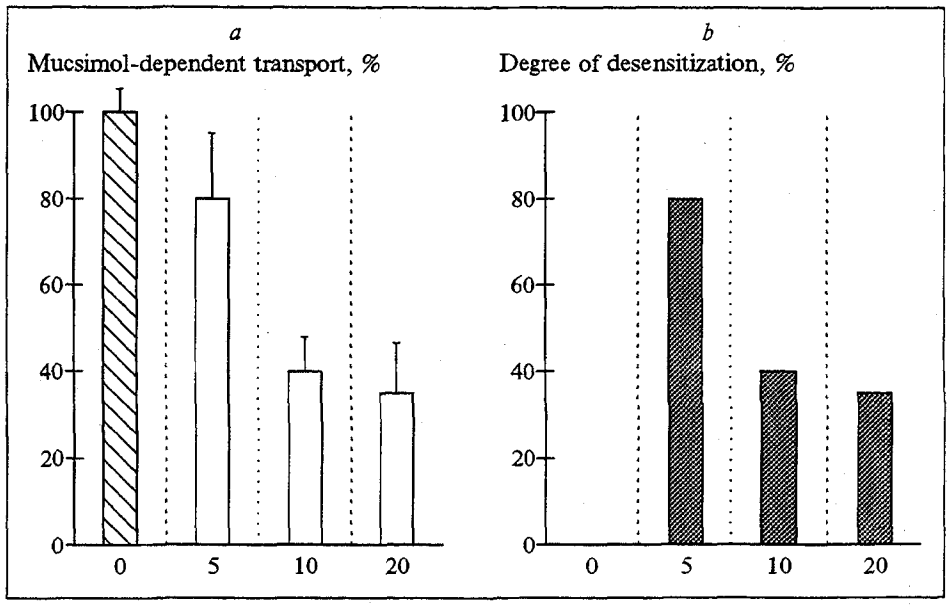


**Fig. 1.** Desensitization of GABA receptor/$Cl^-$ ionophore complex as a function of time of preincubation of synaptoneurosomes with muscimol. Here and in Figs. 2–4: shaded bars represent muscimol–dependent $^{36}Cl^-$ entry after simultaneous addition of muscimol and $^{36}Cl^-$ to synaptoneurosomes; white bars represent muscimol–dependent $^{36}Cl^-$ entry after successive addition of muscimol and $^{36}Cl^-$ to synaptoneurosomes; dark bars represent degree of desensitization. *a*) muscimol–dependent $^{36}Cl^-$ entry as a function of time of incubation with muscimol (2.5 μM); *b*) degree of desensitization of GABA receptor/$Cl^-$ ionophore complex as a function of time of incubation with muscimol.

mg protein/ml. The synaptoneurosomes were used immediately after isolation.

The $Cl^-$ permeability of the membrane regulated by the GABA receptor/$Cl^-$ ionophore complex was assayed by $^{36}Cl^-$ entry into synaptoneurosomes. The synaptoneurosome suspension was aliquoted 100 μl per tube (=400 μg protein) and the test preparations were added in the necessary concentrations, and incubated at 20°C during 30 min. Then 100 μl Krebs-Ringer solution containing 0.5 μCi $^{36}Cl^-$ (Izotop, Russia) and muscimol in an appropriate concentration were added to the samples and vigorously agitated. The entry of $^{36}Cl^-$ into synaptoneurosomes was terminated after 5 or 10 sec by vacuum filtration through GF/C fiberglass filters (Whatman, UK) and the filters were washed 3 times with 4 ml Krebs-Ringer solution (0-4°C) containing 100 μM picrotoxin. For determination of the baseline entry of $^{36}Cl^-$ the radioactive label was added to synaptoneurosomes without muscimol. The muscimol-stimulated $^{36}Cl^-$ entry into synaptoneurosomes, characterizing the functional activity of the $Cl^-$ channel of the GABA receptor complex, was defined as the difference between the baseline and muscimol-stimulated $^{36}Cl^-$ uptake in synaptoneurosomes. For the study of the desensitization of the GABA receptor complex, the synaptoneurosomes were preincubated with muscimol before the addition of $^{36}Cl^-$. The radioactivity was measured on a 1219 Rackbeta counter (LKB).

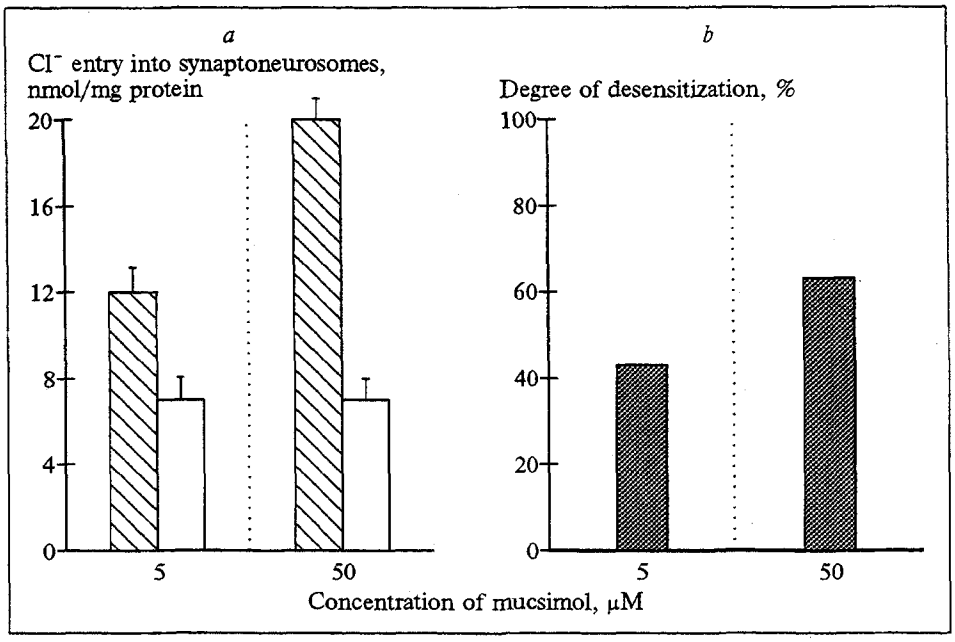


**Fig. 2.** Effect of concentration of muscimol on desensitization of GABA receptor/$Cl^-$ ionophore complex. *a*) muscimol–dependent $^{36}Cl^-$ entry as a function of muscimol concentration; *b*) degree of desensitization of GABA receptor/$Cl^-$ ionophore complex as a function of muscimol concentration.

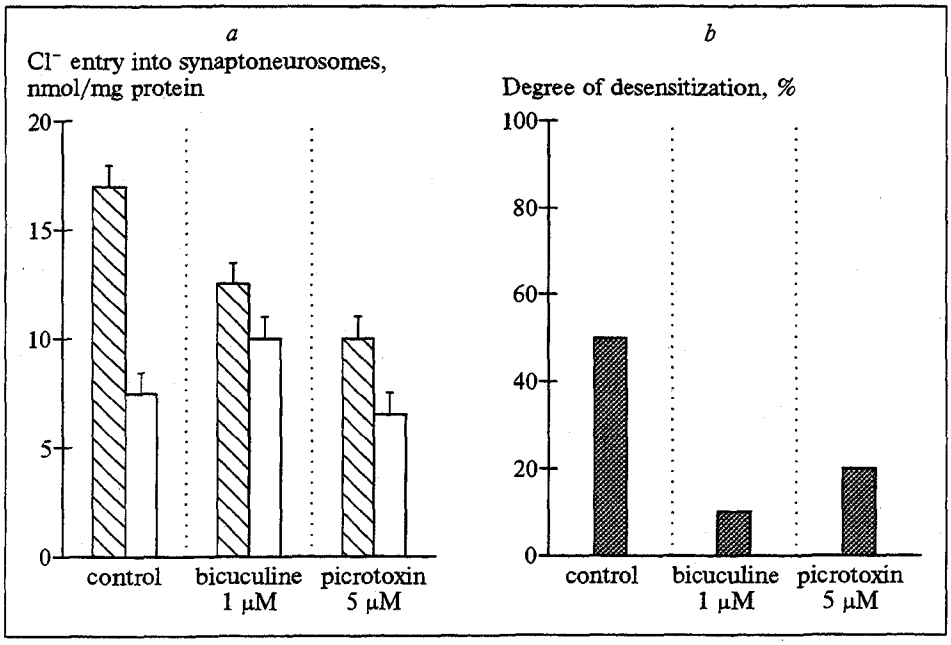


**Fig. 3.** Bicuculline and picrotoxin–induced inhibition of desensitization of GABA receptor/$Cl^-$ ionophore complex. *a*) muscimol–dependent $^{36}Cl^-$ entry in control and in the presence of bicuculline and picrotoxin; *b*) degree of desensitization of GABA receptor/$Cl^-$ ionophore complex in the presence of bicuculline and picrotoxin; concentration of muscimol 20 μM; time of incubation of synaptoneurosomes with bicuculline and picrotoxin 30 min.

## RESULTS

The rate of desensitization of the GABA receptor/$Cl^-$ ionophore complex was assessed by the degree of desensitization, which was determined as the decrease (in %) of the muscimol-stimulated transport of $^{36}Cl^-$ after a 5-sec incubation of synaptoneurosomes with muscimol. The entry of $^{36}Cl^-$ into synaptoneurosomes after the simultaneous addition of muscimol and $^{36}Cl^-$ served as the control.

The rate of desensitization increased with the length of time of that the synaptoneurosomes were incubated with muscimol (Fig. 1, *a, b*) and with the increase of the concentration of muscimol (Fig. 2, *a, b*). Muscimol-stimulated $^{36}Cl^-$ entry decreased 62.1% after 20 sec at a concentration of muscimol of 2.5 μM. The half-maximal value of muscimol-stimulated $^{36}Cl^-$ entry under these conditions was attained for $T_{1/2}$=12.2 sec. Increasing the concentration of muscimol from 5 to 50 μM increased the degree of desensitization from 44.7 to 68.82% (1.54-fold), the muscimol-stimulated $^{36}Cl^-$ entry being increased from 11.6 to 20.1 nmol $Cl^-$/mg protein, i.e., by 73.3%. The concentration dependence of the muscimol-stimulated $^{36}Cl^-$ entry is nonlinear, since the dose-response curve in the chosen concentration range is close to saturation.

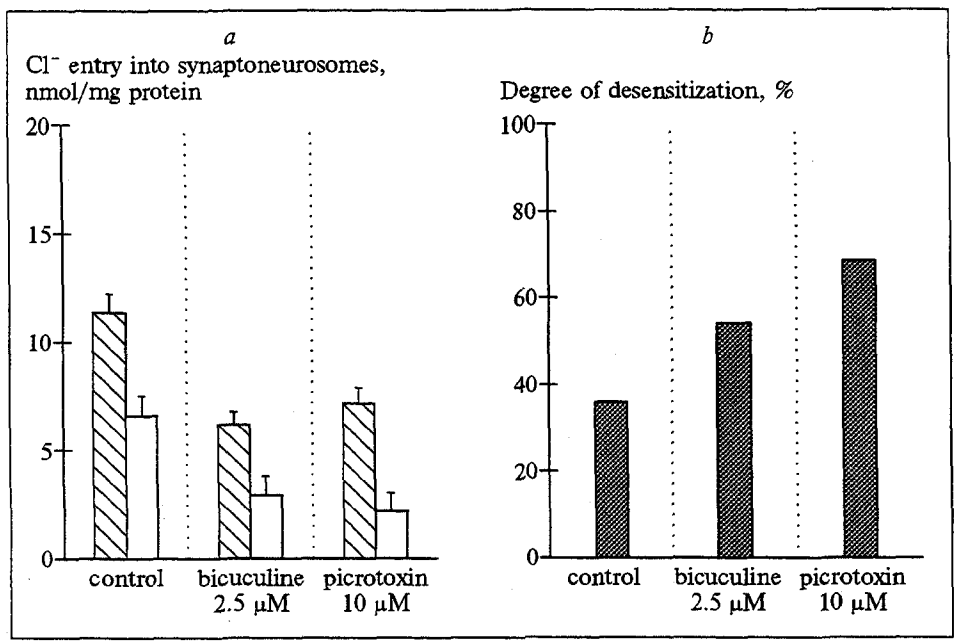


**Fig. 4.** Desensitization of GABA receptor/$Cl^-$ ionophore complex in the early moments of action of bicuculline and picrotoxin. *a*) muscimol–dependent $^{36}Cl^-$ entry in control and in the presence of bicuculline and picrotoxin; *b*) degree of desensitization of GABA receptor/$Cl^-$ ionophore complex in the presence of bicuculline and picrotoxin; concentration of muscimol 10 μM; bicuculline and picrotoxin were added simultaneously with muscimol.

A 30-min incubation of synaptoneurosomes with picrotoxin or bicuculline results both in the inhibition of muscimol-dependent $^{36}Cl^-$ entry and in the delay of desensitization of the receptor/ionophore complex (Fig. 3, *a, b*). Picrotoxin (5 mM) against the background of 20 μM muscimol reduced the degree of desensitization from 51.3 to 16.9% and bicuculline (1 μM) reduced it to 11.9%. It is interesting to note the absence of a correlation between the inhibition of muscimol-dependent $^{36}Cl^-$ entry and the degree of desensitization for different inhibitors. For instance, bicuculline reduced the muscimol-dependent transport of $^{36}Cl^-$ and the degree of desensitization by 30.3% and 4.1-fold, respectively, while picrotoxin reduced them by 39.2% and 2.8-fold.

When picrotoxin and bicuculline were added to synaptoneurosomes simultaneously with muscimol (without preincubation), the inhibition of muscimol–dependent $^{36}Cl^-$ entry was the only phenomenon observed, while the rate of desensitization remained unchanged (Fig. 4, *a, b*). Thus, the effect of the convulsants on desensitization may be assumed to develop more slowly than the effect on $Cl^-$ transport. Moreover, the convulsants increased the rate of desensitization even higher than in the control: the degree of desensitization comprised 69% for picrotoxin, 56.3% for bicuculline, and 36.8% in the control. This effect was apparently due to different times of exposure to the convulsants during the measurements of inhibition of $^{36}Cl^-$ transport and desensitization. Since the convulsants were added simultaneously with muscimol, the exposure lasted for 5 sec during the determination of $^{36}Cl^-$ transport inhibition and for 10 sec (including preincubation) during the determination of desensitization.

Desensitization of the GABA receptor/$Cl^-$ ionophore complex is one of the autoregulatory mechanisms of GABA-dependent postsynaptic feedback inhibition. An increased concentration of the transmitter or its prolonged action results in greater desensitization. The necessity of rapid inactivation of GABA-induced $Cl^-$ permeability as well as its mechanism remain poorly understood. In particular, it is unclear which functional element - receptor or ionophore - undergoes changes which are responsible for desensitization. The assumption that desensitization is caused by a reduced affinity of agonists for GABA receptors is confirmed by the time and concentration dependences of the effect of muscimol on desensitization. The inhibition of desensitization by bicuculline, a competitive inhibitor of the GABA receptor, also argues in favor of this assumption. However, the data presented here on the inhibitory effect of picrotoxin, a blocker of $Cl^-$ channels, on desensitization together with the analogous effect of pentylenetetrazole reported earlier [1] are inconsistent with this hypothesis. If the mechanism of desensitization is located in the ionophore domain of the complex, the rate of desensitization may be expected to depend on the number of the $Cl^-$ ions transported through the $Cl^-$ channel. However, electrophysiological studies performed on cultured cortical neurons have not observed any relationship between desensitization and the absolute $Cl^-$ current or transferred charge [4]. Nor have these experiments revealed any acceleration of desensitization with an increase of the intracellular concentration of $Cl^-$ (up to 149 mM). Although the authors established the inhibition of desensitization by bicuculline, they have failed to find any effect of picrotoxin on desensitization.

Thus, an analysis of the effect of convulsants with different mechanisms of action leads us to the conclusion that desensitization of the GABA receptor/$Cl^-$ ionophore complex is a complex process which includes changes of both receptor and $Cl^-$ ionophore.

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