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Effect of Aminooxyacetic Acid on the Antidote Activity of Diazepam Under the Action of GABA-Lytics

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Pretreatment of mice with aminooxyacetic acid enhances the antidote activity of diazepam against picrotoxin but not bicuculline. It is claimed that GABA-transaminase inhibitors may be promising candidates as an antidote in complex therapy of seizures induced by GABA-lytics blocking the chloride ionophore.

Key Words: *aminooxyacetic acid; diazepam; picrotoxin; bicuculline; toxicity*

It is known that application of anticonvulsants in combination potentiates their activity [2]. For example, the antidote activity of 1,4-benzodiazepines against GABA-lytica is enhanced by barbiturates [9], glycine [8], and aminooxyacetic acid (AOAA), which inhibits GABA-transaminase [7]. Clonazepam, injected into rats after their pretreatment with AOAA was found to have a less pronounced anticonvulsant activity in picrotoxin intoxication [7]. The effect of AOAA on the antidote efficacy of diazepam in intoxication induced by GABA-lytics has not beeen assessed, nor have the effects of AOAA on the functional state of the benzodiazepine and muscarinic receptors been examined. It is known that modulation of the M-cholinergic receptors by cholinotropic compounds alters the sensitivity of animals to picrotoxin [4].

In this study we examined the antidote activity of diazepam against the background of AOAA upon intoxication of albino mice with bicuculline or picrotoxin and assessed the effect of AOAA on the specific binding of the ligand of muscarinic receptor with synaptic membranes isolated from the brain of intact mice.

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MATERIALS AND METHODS

Experiments were performed on male albino mice weighing 25-30 g. Picrotoxin and bicuculline were suspended in normal saline with Tween-80. Aminooxyacetic acid was dissolved in normal saline and neutralized with sodium bicarbonate. The toxicity of the GABA-lytics was evaluated in probit analysis 2 h after injection of AOAA in a dose of 50 mg/kg. Diazepam (5 mg/kg) was injected 10 min prior to the toxins. All compounds used in this study were from Sigma. The preparations were injected intraperitoneally (0.2 ml solution per 10 g body weight). The effect of AOAA (10^{-10}-10^{-5} M) on the specific binding of [N-methyl-^{3}H]methyl-

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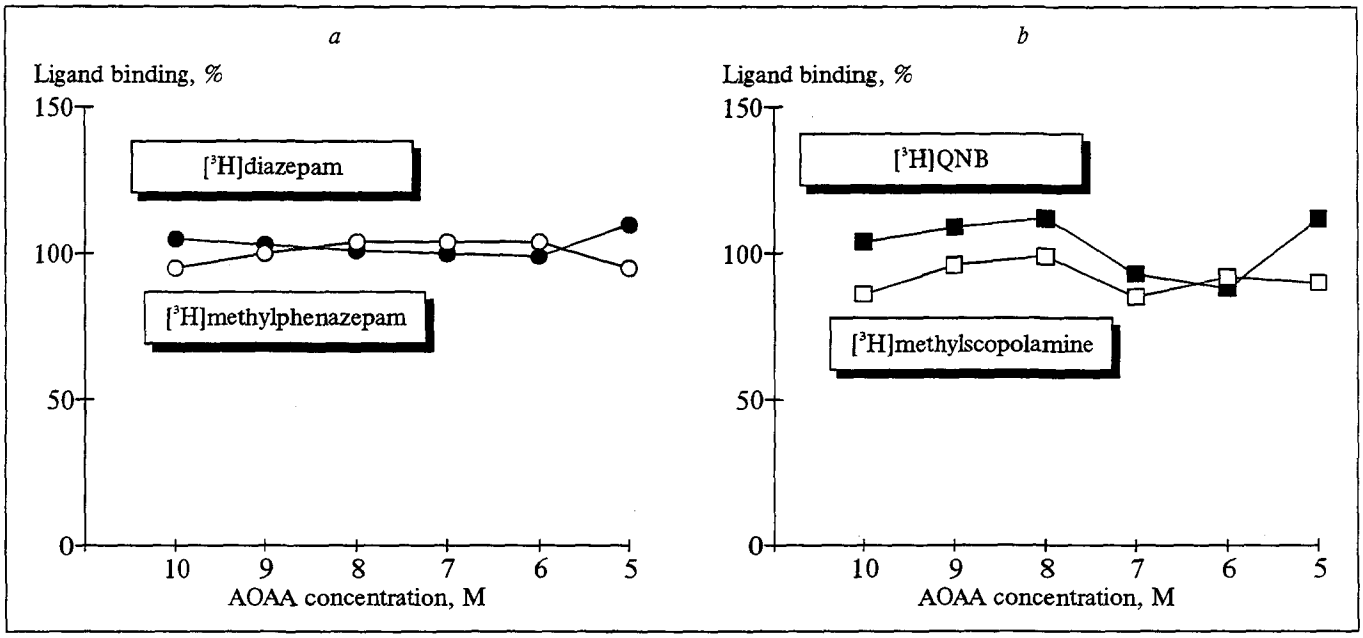

Fig. 1. Effect of AOAA on the binding of benzodiazepine (*a*) and muscarinic (*b*) receptors with synaptic membranes isolated from the brains of intact mice. Ligand binding in control experiments: [³H]diazepam: 156±38 fM/mg protein, [³H]methylphenazepam: 684±97 fM/mg protein, [³H]QNB: 1213±714 fM/mg protein, and [³H]methylscopolamine: 926±74 fM/mg protein.

phenazepam (2.4 TBq/mM, 3 nM, V. G. Khlopin Radium Institute, St. Petersburg), [N-methyl-³H]diazepam, 2.5 TBq/mM, 3 nM, V. G. Khlopin Radium Institute), 1-[N-methyl-³H]scopolamine (3.5 TBq/mM, 1 nM, Amersham), and [³H]quinuclidyl benzylate (QNB, 1.2 TBq/mM, 1 nM, NEN) with synaptic membranes isolated from the whole brain of intact mice (*in vitro* experiments) was studied. The membrane preparation was obtained as described previously [1]. The incubation mixture contained 90-120 μg membranes, 50 mM potassium phosphate buffer (pH 7.4), radioactive ligand, and AOAA. The volume of the probe was 1 ml. The effect of AOAA (1 μM) on [³H]diazepam (3 nM) binding stimulated by GABA (10 mM, Reanal, Hungary) was studied in a separate series of experiments. Nonspecific binding was determined in the presence of 30 μM diazepam. The incubation medium was supplemented with 150 mM NaCl. Vials with labeled benzodiazepines were incubated for 1 h at 0-3°C. Vials with muscarinic receptors were incubated for 2 h at 20°C. Unbound label was separated by filtration through GF/B filters (Whatman). Radioactivity was measured in 1214 Rackbeta counter. Binding parameters were determined from the results of 3-5 experiments performed in triplicate.

RESULTS

Table 1 shows the antidote efficacy of diazepam in AOAA-treated mice upon intoxication with GABA-lytics.

By itself, AOAA significantly (47%) increased the resistance of mice to picrotoxin and had small effect on bicuculline activity. The protection coefficients of diazepam against bicuculline and picrotoxin was 2.04 and 1.73, respectively.

The antidote activity of benzodiazepine increased only in picrotoxin intoxication (the protection coefficient increased to 3.49). These findings agree with the reports of others. For example, the inhibitors of GABA-transaminase AOAA and vigabatrine increased the resistance of rodents to picrotoxin [3,6,7]. At the same time, when injected prior to intoxication, AOAA had a weak effect on

TABLE 1. Effect of AOAA and Diazepam on the Toxicity of GABA-lytics in Albino Mice

Substance	Control	AOAA 2 h prior to toxins	Diazepam 10 min prior to toxins	AOAA 2 h prior to toxins + diazepam 10 min prior to toxins
Picrotoxin	5.1±0.5	7.5±0.7*	10.4±0.8**	17.8±2.2**
Bicuculline	9.8±1.7	11.3±1.6	17.0±2.8*	18.7±2.7*

Note. One and two asterisks, respectively, denote $p<0.05$ and $p<0.001$ vs. the control. The survival was assessed during 2 h.

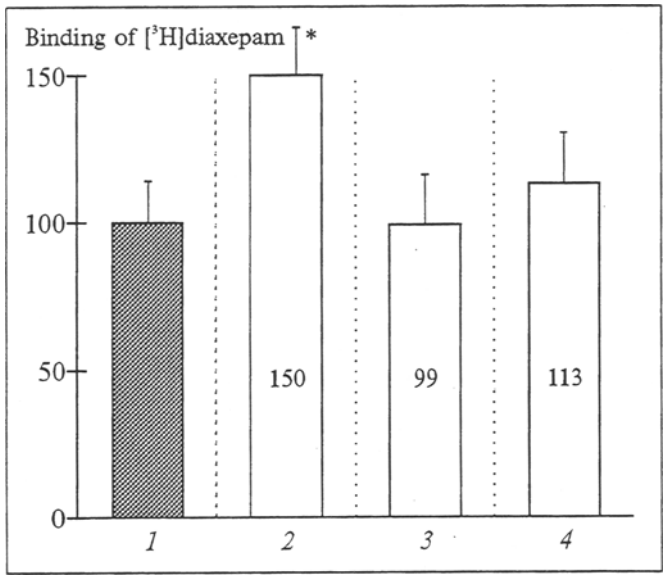

Fig. 2. Effect of AOAA on GABA (10 μM)-stimulated binding of [3H]diazepam with synaptic membranes isolated from the brain of intact mice. *1*) control, binding of [3H]diazepam (3 nM) in the absence of GABA and AOAA (194±22 fM/gm protein); *2*) binding of [3H]diazepam in the presence of GABA; *3*) ligand binding in the presence 1 μM AOAA; *4*) ligand binding in the presence of GABA and AOAA. Asterisk indicates $p<0.01$ compared with the control. Numbers over the bars indicate % compared with the control.

the resistance of albino rats to bicuculline [6]. It was found that the anticonvulsant activity of clozepam increased against the background of AOAA [7]. Our results indicate that this GABA inhibitor enhances the antidote activity of diazepam in picrotoxin-intoxicated mice. A potentiating effect was not observed in the experiments with bicuculline.

It is known that binding of 1,4-benzodiazepines with the corresponding receptors increases in the presence of GABA, i.e. a "GABA shift" occurs [5]. this phenomenon may account for the increased antiseizure activity of diazepam caused by AOAA, which elevates the GABA content of the brain [6]. The possibility that AOAA has direct effects on the benzodiazepine and M-cholinergic receptors, since, as already mentioned, modulation of cholinergic structures by cholinotropic compounds leads to changes in the toxicity of picrotoxin [4]. In our study AOAA had no effect on the binding of [3H]diazepam, [3H]methylphenazepam, [3H]QNB, and [3H]methylscopolamine (Fig. 1) but weakened the GABA-stimulated binding of [3H]diazepam (Fig. 2). This testifies to the influence of AOAA on the functional state of the benzodiazepine receptors.

Whether this effects plays any role in the enhancement of the antidote activity of diazepam or hinders it is unclear.

Thus, pretreatment of mice with AOAA potentiated the antidote activity of diazepam in mice intoxicated with picrotoxin but not with bicuculline. We failed to reveal any effect of AOAA on the binding of [3H]diazepam, [3H]methylphenazepam, [3H]QNB, and [3H]methylscopolamine to synaptic membranes isolated from the mouse brain. The GABA-stimulated binding of [3H]diazepam decreased in the presence of AOAA. Inhibitors of GABA-transaminase may be promising antidotes for complex therapy of seizures induced by GABA-lytics, which inhibit the chloride ionophore.

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