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Effect of 3-Mercaptopropionic Acid on the Toxicity of GABA-Lytics in Mice

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The substances which produce an effect on the GABA level in the brain are known to alter the toxicity of GABA-lytics [3,9,13]. An increase in the level of amino acid due to inhibition of its biodegradation goes along with a decrease in the toxicity of poisons [4,12]. On the other hand, the effect of inhibitors of GABA synthesis on the sensitivity of animals to GABA-lytics has been insufficiently studied.

In this study we assessed the effects of 3-mercaptopropionic acid (3-MPA), a catalyst of reversible inhibition of glutamate decarboxylase (GD), on the toxicity of bicuculline (BC) and picrotoxin (PT) in albino mice.

MATERIALS AND METHODS

The experiments were carried out on male albino mice weighing 18-20 g. PT and BC were suspended in physiological saline using TWEEN-80. 3-MPA was dissolved in saline and injected 20, 10, and 5 min ahead of the GABA-lytics or simultaneously with them, in a dose of 14 mg/kg (0.5 LD_{50}), which does not provoke seizures in the animals. All the preparations used in the study were purchased from Sigma (USA). The substances were intraperitoneally administered in a volume of 0.2 ml of solution per 10 g animal weight. For assessment of the toxicity no less than 5 doses were used and no less than 6 animals for each dose. The LD_{50} values were calculated using regression analysis by the method of least squares.

We studied the effect of 3-MPA (10^{-9}-10^{-4} M) on the specific binding of ^{3}H-tertbutylbicycloorthobenzoate (TBOB; Amersham, England; 1.1

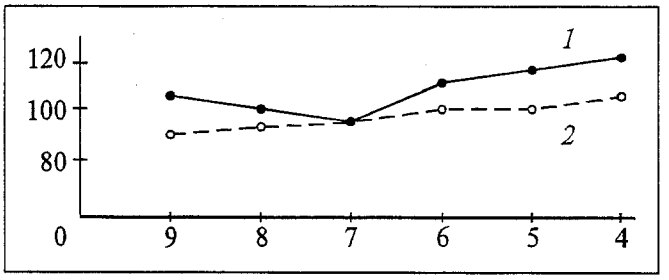

Fig. 1. Effect of 3-MPA on ^{3}H-TBOB (5 nM) and ^{3}H-GABA (100 nM) binding by synaptic membranes of the brain in intact mice. Abscissa: 3-MPA concentration, log (M); ordinate: binding of ^{3}H-GABA (*1*) and ^{3}H-TBOB (*2*), % of control. Binding of ligands in the control: 145±18 fmol/mg protein for ^{3}H-TBOB and 750±64 fmol/mg protein for ^{3}H-GABA.

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TBq/mM; 5 nM) and ^{3}H-GABA (Izotop, Russia; 1.42 TBq/mM; 100 nM) by the synaptic membranes of the intact brain in intact mice. The preparation of membranes and radioligand assay were described previously [1,2]. The reliability of differences of the parameters in question was assessed using Student's *t* test.

RESULTS

The data on the toxicity of BC and PT for simultaneous or preliminary administration of 14 mg/kg 3-MPA are presented in Table 1. It is evident that under these conditions, the toxicity of GABA-lytics increased. For instance, when 3-MPA was injected 5 min before, the LD_{50} of PT and BC dropped 26 and 31%, respectively. The toxicity of BC was also reliably higher (by 40%) after pretreatment with 3-MPA (10 min before).

The revealed effect may be associated with a drop of the GABA level when GD is inhibited by 3-MPA [7]. GABA is known to inhibit the receptor binding of chlorine ionophore ligands, and PT is such an ionophore [1,11]. It seems likely that a deficiency of the amino acid promotes enhanced binding of the poison by the Cl^- channel of the $GABA_A$-receptor, thereby increasing the toxicity of picrotoxin. Such a mechanism may account for the convulsive activity of BC under conditions of GD inhibition.

On the other hand, the possibility of a direct influence of 3-MPA on the ionophore (a target for PT) and on the low-affinity $GABA_A$-receptors (a target for BC) cannot be ruled out. The ability of 3-MPA to compete for synaptic membrane binding sites with ^{3}H-TBOB (a ligand for the Cl^- channels of the $GABA_A$-receptors) and with ^{3}H-GABA (a ligand for the low-affinity $GABA_A$-receptors) in the intact brain of intact mice was studied *in vitro*. The results of these experiments are presented in Fig. 1.

3-MPA did not affect the specific binding of either ligand. However, in the experiments with ^{3}H-GABA, a slight enhancement of radioactive label binding (by 10-15%) was noted within a micromole range of 3-MPA concentrations. Probably, this was due to an increase of nonspecific sorption of the "^{3}H-GABA-3-MPA" complex by the fiberglass filters. A similar effect was noted during a study of the effect of organochlorine pesticides on the binding of ^{3}H-diazepam and ^{35}S-TBPS [10]. Evidently, 3-MPA does not exert any direct effect on the receptor sites for PT and BC.

Potentiation of the toxicity of GABA-lytics by 3-MPA may be associated with other, not GABA-ergic, mechanisms. For instance, 3-MPA has been shown to alter the energy exchange in nervous tissue [6], to affect the metabolism of dopamine and adenosine [5,15] and the density of muscarine receptors in the cerebellum of experimental animals [14], and to facilitate aspartate release [8].

Thus, 3-MPA, a catalyst of reversible inhibition of GD, potentiates the toxic effect of the GABA-lytics PT and BC. This effect is probably due to the enhancement of poison binding under conditions of a lowered GABA level in the brain. We failed to discover any direct effect of 3-MPA on the Cl^- channels and low-affinity $GABA_A$-receptors.

TABLE 1. Toxicity of GABA–Lytics for the Simultaneous or Preliminary Administration of 3–MPA (14 mg/kg) to Mice ($M \pm m$)

Conditions of toxicity measurements	LD_{50}, mg/kg
PT	10.65±1.30
PT + 3–MPA simultaneously	8.81±1.11
PT + 3–MPA 5 min before	7.84±0.42*
PT + 3–MPA 10 min before	9.17±1.54
PT + 3–MPA 20 min before	9.89±0.93
BC	12.40±1.23
BC + 3–MPA simultaneously	10.00±1.03
BC + 3–MPA 5 min before	8.50±0.87*
BC + 3–MPA 10 min before	7.40±0.76*
BC + 3–MPA 20 min before	11.31±1.15

Note. An asterisk indicates $p<0.05$ vs. the control.

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