GABAergic System in the Anxiolytic Effect of Proproten: Experimental Study

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The medicinal preparation Proproten contains ultralow doses of antibodies to S100 protein that acts as an important regulator of integrative activity in the brain and synaptic processes. Intracerebroventricular administration of Proproten, diazepam, and mexidol in doses of 2.5 ml/kg, 2 mg/kg, and 100 mg/kg, respectively, produced a strong anxiolytic effect on male outbred rats in the conflict situation and markedly increased the incidence of punished drinking. Antagonists of GABAergic transmission bicuculline ($GABA_A$ receptor blocker) and picrotoxin (chlorine channel blocker) produced the pro-conflict anxiogenic effect, which was accompanied by a decrease in the number of punished drinking in control animals. The anti-conflict effect of Proproten was less pronounced during blockade of $GABA_A$ receptors or chlorine channels. Bicuculline and picrotoxin similarly modulated the anxiolytic effect of diazepam and mexidol. Our results suggest that the GABAergic system plays a role in the anxiolytic effect of diazepam, mexidol, and Proproten.

Key Words: *Proproten; S100 protein; ultralow doses; antibodies to S100 protein; anxiolytic; tranquilizer; diazepam; mexidol; GABAergic transmission*

γ-Aminobutyric acid (GABA) is a major inhibitory mediator in the central nervous system (CNS) that plays an important role in the pathogenesis of anxiety, convulsions, and other neurotic and neurological disorders. The effects of most anxiolytic preparations are associated with potentiation of GABAergic inhibition [1].

Proproten containing homeopathically potentiated antibodies to S100 protein in ultralow doses (PAB-S100) was synthesized at the "Materia Medica Holding" Research-and-Production Company [6,7]. This protein acts as the major regulator of integrative activity in the brain and is involved in synaptic processes (similarly to other Ca^{2+}-binding proteins) [5,12]. Proproten possesses a variety of pharmacological properties, produces the anxiolytic and antistress effects [11], reduces the frequency of lateral hypothalamus self-stimulation, and suppresses neuronal activity in the limbic system [9].

Here we studied the possible role of the GABAergic system in anxiolytic activity of Proproten. The benzodiazepine tranquilizer diazepam and diurnal tranquilizer mexidol served as reference preparations.

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MATERIALS AND METHODS

Experiments were performed on adult male outbred albino rats weighing 230-250 g. The animals were divided into 12 groups of 10 specimens each. The anxiolytic effects of Proproten, diazepam, and mexidol were studied in the Vogel's conflict situation. This method is based on the conflict between drinking and defensive motivations. Each episode of drinking was punished with electrical stimulation [2,3]. The animals with a strong sense of thirst were trained to take water from a special drinking bowl. On day 3 direct current (0.2 mA) was applied to an electrode floor of the chamber 10 sec after the first drinking episode. Then each episode of drinking was punished. To satisfy a sense of thirst the rats should overcome the fear of punishment. We recorded the number of punished drinking episodes over 10 min.

Proproten was administered in a dose of 2.5 ml/kg (0.25 ml per 100 g body weight). Diazepam and mexidol in doses of 2 and 100 mg/kg, respectively, served as reference preparations. Control animals received 2.5 ml/kg distilled water. Test substances were administered intragastrically 30 min before the experiment.

The $GABA_A$ receptor blocker bicuculline (ICN Biomedicals Inc.) and chlorine channel blocker picro-

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toxin (ICN Biomedicals Inc.) were injected intraperitoneally in a dose of 1 mg/kg. These compounds were injected intraperitoneally simultaneously with administration of Proproten, diazepam, mexidol, or water.

The results were analyzed by Mann-Whitney *U* test and Student's *t* test [2].

RESULTS

Proproten, diazepam, and mexidol in doses of 2.5 ml/kg, 2 mg/kg, and 100 mg/kg, respectively, produced a strong anxiolytic effect in the conflict situation when each episode of drinking was punished with pain stimuli. Proproten, diazepam, and mexidol increased the number of punished drinking episodes by 1.6, 1.5, and 1.47 times, respectively, compared to the control (Table 1). Our results show that test preparations in these doses produced a similar effect.

The $GABA_A$ receptor blocker bicuculline and chlorine channel blocker picrotoxin produced the pro-conflict anxiogenic effect. It was manifested in a decrease in the number of punished drinking by 1.6 and 1.8 times, respectively, compared to control animals (Table 1).

During combination treatment bicuculline attenuated the anti-conflict effect of Proproten. Under these conditions the number of punished drinking decreased by 1.76 compared to that observed after administration of Proproten. Bicuculline similarly modulated the anxiolytic effect of diazepam and mexidol and decreased the number of punished drinking in the conflict situation by 2 and 1.9 times, respectively (Table 1).

TABLE 1. Effects of Antagonists of the GABA-Benzodiazepine Receptor Complex on Anxiolytic Activity of Proproten, Diazepam, and Mexidol ($M \pm m$, n=10)

Substance	Number of punished drinking
Control	528.1±73.4
Proproten	740.0±108.4*
Diazepam	721.0±84.3*
Mexidol	748.2±101.4*
Bicuculline	
+water	327.3±115.4*
+Proproten	419.6±107.4$^+$
+diazepam	353.6±74.1$^+$
+mexidol	389.5±68.4$^+$
Picrotoxin	
+water	296.9±125.1*
+Proproten	469.3±85.5$^+$
+diazepam	304.8±95.4$^+$
+mexidol	358.3±87.8$^+$

Note. p<0.05: *compared to the control; $^+$compared to treatment with Proproten, diazepam, and mexidol without administration of antagonists. n, number of animals in each group.

Therefore, anti-conflict activity of Proproten, diazepam, and mexidol markedly decreased during $GABA_A$ receptor blockade induced by bicuculline.

The chlorine channel blocker picrotoxin possessed anxiogenic activity in the conflict situation (similarly to bicuculline). During combination treatment picrotoxin attenuated the tranquilizing effect of Proproten by 1.67 times. Administration of picrotoxin in combination with diazepam or mexidol also reduced the incidence of punished drinking. Therefore, picrotoxin abolished the anxiolytic effect produced by test preparations.

Our results indicate that anti-conflict activity of diazepam, mexidol, and Proproten decreases during blockade of $GABA_A$ receptors and chlorine channels with bicuculline and picrotoxin, respectively. Probably, these subunits of the GABA-benzodiazepine receptor chloride ionophore complex are involved in anxiolytic activity of test preparations. However, the mechanisms of changes in the GABA system produced by anxiolytics are different.

The effects of benzodiazepine tranquilizers, including diazepam, are associated with their ability to bind to benzodiazepine receptors, change conformation of allosterically bound $GABA_A$ receptors, and increase the incidence and duration of chlorine channel opening, which reduces neuronal excitability and modifies synaptic transmission [1,4].

As differentiated from diazepam, mexidol does not act as the direct agonist of benzodiazepine receptors. This membrane modulator of the GABA-benzodiazepine receptor complex directionally regulates its activity via membrane-receptor interactions and promotes binding of direct agonists to receptors [3].

Brain-specific S100 protein is involved in generation and transmission of nerve impulses and provides long-term postsynaptic potentiation that plays an important role in synaptic processes [5,6,8]. Native antiserum to S100 protein inhibits the induction of long-term post-tetanic potentiation in hippocampal slices, while Proproten abolishes these changes [6, 8,13]. Experiments with snail giant neurons showed that Proproten produces the direct membranotropic effect and decreases the amplitude of the action potential and maximal conductivity [10].

Our results suggest that the anxiolytic effect of Proproten is associated with its ability to modify functional activity of brain-specific S100 protein and change synaptic transmission in brain limbic structures via modulation of membrane-receptor interactions in the GABA-benzodiazepine receptor chloride ionophore complex.

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