Anxiolytic Effect of Proproten under Conditions of Punished and Unpunished Behavior

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The anxiolytic effect of Proproten containing potentiated antibodies to brain-specific S100 protein (2.5 ml/kg) in male outbred albino rats was studied under conditions of punished (Vogel's conflict situation with pain stimulation) and unpunished behavior (elevated plus-maze and open-field tests). Proproten significantly increased the incidence of punished drinking in the conflict situation with pain stimulation, number of entries, and time spent in the open arms of the elevated plus-maze and decreased the rate of defecation and urination. In the open-field test Proproten induced entries of rats into the center of an illuminated area. Proproten was efficient after single administration and course of treatment (2 times a day, 5 days). These results show that Proproten produces the anxiolytic effect under conditions of punished and unpunished behavior.

Key Words: *Proproten; brain-specific S100 protein; potentiated antibodies to S100 protein; anxiolytic; tranquilizer; diazepam; stress; conflict situation; elevated plus-maze; open field*

The original preparation Proproten contains potentiated antibodies to brain-specific S100 protein [7,8], which acts as the major regulator of integrative activity in the brain and play a role in synaptic processes (similarly to other Ca^{2+}-binding proteins) [6,11,12]. Proproten is approved for the use in medical practice. The preparation relieves somatovegetative and psychopathological symptoms and reduces emotional strain in patients with the alcohol withdrawal syndrome (AWS) [1].

Here we compared anxiolytic properties of Proproten and standard tranquilizer diazepam.

MATERIALS AND METHODS

The effects of Proproten and diazepam were studied on adult male outbred albino rats weighing 230-250 g. The anxiolytic and antistress effects were evaluated in standard tests of punished (conflict situation, CS) and unpunished behavior (elevated plus-maze, EPM; and open field, OF) [4]. CS was modeled by the method of Vogel, which is based on the conflict between drinking and defensive motivations [5]. 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 [5].

The EPM test is based on the fear of open spaces and heights. The device consisted of 4 arms set at right angle to each other. Two opposite arms were closed, while other arms were open. The maze was elevated above floor level (0.5 m). The animals were placed in the central area. The latency of the first entry into the open arms, number of complete and incomplete entries, time spent in the arms, and rates of defecation and urination were determined over 3 min.

Emotional stress was produced in the OF test. The rat was placed in the center of an illuminated area (1×1 m). The number of crossed squares (horizontal activity), rearing postures (vertical activity), entries into the center of OF, explored objects (exploratory activity), and grooming and scratching episodes was recorded on a Pentium 2000 MMX computer with special software over 3 min. The possible myorelaxant side effect was evaluated in the test of rotating rod. The methods are described elsewhere [4].

Potentiated antibodies to brain-specific S100 protein (Proproten) were synthesized at the "Materia Medica Holding" Research-and-Production Company and administered in a dose of 2.5 ml/kg (0.25 ml per 100 g body weight). Diazepam (2 mg/kg) served as the reference preparation. Test substances were administered intragastrically 30 min before the experiment

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(single treatment) or 2 times a day for 5 days (9.00 and 18.00).

The results were analyzed by dispersion test, Mann-Whitney *U* test, Student's *t* test, Litchfield test, and Wilcoxon test [3].

RESULTS

Proproten in a dose of 2.5 ml/kg produced a strong anxiolytic effect in CS, reduced the fear of pain stimuli, and increased the incidence of punished drinking compared to the control (Table 1). Despite pain stimulation the number of punished drinking episodes in Proproten-receiving rats increased by 1.6 times compared to the control. Diazepam in a dose of 2 mg/kg produced similar changes and increased the number of punished drinking by 1.5 times. The course of treatment with Proproten and diazepam also produced the anxiolytic effect (Table 1).

Anxiety and fear of novelty, height, and open illuminated space in EPM disappeared after administration of Proproten. The number of entries into and time spent in the open arms increased by 1.9 and 5.4 times, respectively. Besides this, Proproten 4.8-fold increased the incidence of overhanging from the end of the open arms (Fig. 1). Diazepam produced more pronounced changes compared to Proproten. This preparation increased the number of entries into and time spent in the open arms by 2.3 and 7 times, respectively. Anxiety in EPM is manifested in high rates of defecation and urination (compared to intact animals in normal situation). Proproten reduced the rates of defecation and urination in EPM ($p<0.05$, Fig. 1).

Proproten had no effect on locomotor activity of rats in OF. However, Proproten and diazepam induced entries of rats into the center of an illuminated area, which reflected a decrease in the degree of anxiety and fear. As differentiated from Proproten, diazepam produced a strong sedative effect and markedly decreased horizontal activity of animals (Table 2).

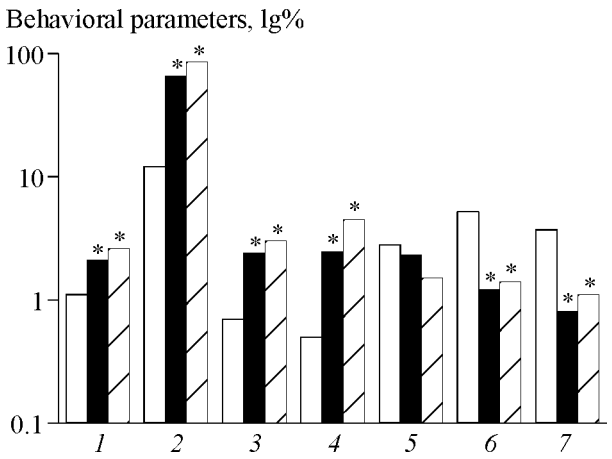

Fig. 1. Anxiolytic effect of Proproten in the elevated plus-maze. Light bars: control. Dark bars: Proproten (2.5 ml/kg). Shaded bars: diazepam (2 mg/kg). Number of entries into the open arms (*1*), time spent in the open arms (sec, *2*), number of incomplete entries into the open arms (*3*), number of overhanging (*4*), number of entries into the closed arms (*5*), and rates of defecation (*6*) and urination (*7*). **p*<0.05 compared to the control.

Proproten did not impair coordinated movements in the test of rotating rod and had no myorelaxant activity. However, diazepam produced a serious myorelaxant effect. The effective dose of diazepam causing disturbances in 50% animals (ED_{50}) was 2.65 mg/kg (2.2:3.1 mg/kg).

Standard benzodiazepine and non-benzodiazepine tranquilizers primarily affect limbic structures of the brain, hypothalamus, and cortex. These areas are characterized by long-term activation of neurons in response to exogenous stimuli. The process is followed by changes in the excitability threshold for the hippocampus and amygdaloid complex [2,4]. Proproten produces a strong effect on these brain structures, decreases the frequency of lateral hypothalamus self-

TABLE 1. Anxiolytic Effect of Proproten after Single and Repeated Administration in EPM (*M*±*m*)

Administration	Control	Proproten	Diazepam
Single	303.7±84.8	471.9±92.7*	456.3±86.1*
Repeated (5 days)	247.8±38.9	348.5±68.7*	401.7±92.1*

Note. Here and in Table 2: **p*<0.05 compared to the control.

TABLE 2. Effect of Proproten on Behavior of Rats in OF

Parameter		Control	Proproten	Diazepam
Activity	horizontal	18.2±2.4	15.8±2.1	12.5±1.8*
	vertical	8.2±3.3	5.8±2.6	6.2±1.4
	exploratory	11.1±3.1	8.9±1.6	8.7±1.5
Entries into the center		0±0	2.4±0.7*	1.8±0.9*

stimulation, and suppresses neuronal activity in limbic structures [7,8,10]. Brain-specific S100 protein is involved in general functions of neurons, including generation and conduction of nerve pulses [6,11,12]. This Ca^{2+}-binding protein provides long-term post-tetanic potentiation that plays an important role in synaptic processes. Published data show that native antiserum to S100 protein inhibits the induction of long-term post-tetanic potentiation in hippocampal slices [7,913], while Proproten abolishes this effect [7,9].

Our results suggest that the effects of Proproten are associated with its ability to modify functional activity of brain-specific S100 protein coordinating informational and metabolic processes in the brain. The anxiolytic effect of Proproten is related to modulation of synaptic transmission in limbic structures of the brain, including the hippocampus.

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