Effect of Antibodies to Serotonin on the Development of Alcohol Withdrawal Syndrome in CBA Mice

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In previous reports we published data indicating a possible protective role of antibodies to neurotransmitters in the pathogenesis of chronic alcoholism. We demonstrated in animals experiments a suppression of congenital alcohol motivation and of a developed craving for alcohol during active production of antibodies to serotonin [2,4] and passive immunization of mice with these antibodies [3]. The possibility of eliminating alcohol withdrawal syndrome by means of antiserotonin antibodies is still to be researched. The present study was aimed at elucidating the effect of passive immunization of animals with antiserotonin antibodies on the development of the withdrawal syndrome.

MATERIALS AND METHODS

A total of 100 male CBA mice weighing 18-20 g were used in two series of experiments. Antibodies to serotonin were obtained by immunization of rabbits with serotonin conjugate with bovine serum albumin (S/BSA) using standard schemes. The conjugate was synthesized using a modified Mannich method of formaldehyde condensation [5]. The resultant conjugate contained 10 to 15 serotonin molecules covalently bound to one molecule of bovine serum albumin. Antibodies to serotonin were measured by solid-phase ELISA enzyme immunoassay using conjugate on horse γ-globulin, a heterologous protein carrier, as antigen. The mean titer of antiserotonin antibodies was 1:1000. The g-globulin fraction from sera of immunized and control animals was isolated by readsorption with ammonium sulfate, freeze-dried, and stored at 4°C. For control γ-globulins of rabbits immunized with BSA and of intact animals were used. Protein concentrations in the isolated fractions were measured spectrophotometrically after Lowry. Withdrawal syndrome was induced in mice by forced alcoholization with an alcohol aerosol using special chambers [1], the alcohol concentration in a chamber being 14 mg/liter. The animals were kept in these chambers for 10 days and given a 15% alcohol solution instead of water. Withdrawal symptoms were recorded 6 and 24 h after cessation of the combined alcoholization. The following clinical withdrawal symptoms were assessed: muscle twitching, tremor, starting at a knocking sound, tail rigidity (Staub's syndrome), and convulsions if held by the tail. Motor activity was tested 24 h after alcoholization cessation in the open field for 3 min by recording square crossing and upright postures.

For passive immunization antibodies to serotonin in doses of 5 and 10 mg by protein were used which were injected intraperitoneally in a single volume of 0.4 ml a day before the cessation of alcoholization or immediately after ethanol

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TABLE 1. Withdrawal Syndrome Manifestations in CBA Mice after Injection of Antibodies to Serotonin

Group of animals	Duration of withdrawal syndrome, h	Number of animals per group	Number of animals with withdrawal syndrome	Clinical signs of withdrawal syndrome				
				tremor	starting at noise	tail rigidity	muscle twitching	convulsions if held by the tail
ABS in a dose of 5 mg 24 h	6	6	5	0*	2	4	1*	3
before alcoholization discontinued	24	6	2	0	1	1	0	0
ABS in a dose of 10 mg 24 h	6	12	5	0*	4*	5	0*	0*
before alcoholization discontinued	24	12	2	0	1	1	0	0
ABS in a dose of 10 mg after	6	6	6*	1*	2	6	2	4
alcohol discontinued	24	6	2	0	1	1	0	0
AB to BSA in a dose of 10 mg 24 h	6	10	8	4	3	8	7	8
before alcoholization discontinued	24	10	4	1	1	2	2	2
Rabbit γ–globulin in a dose of								
10 mg 24 h before alcoholization	6	6	6	1*	3	6	2	6
discontinued	24	6	1	0	0	1	0	0
Normal saline 24 h before	6	12	10	9	9	9	10	9
alcoholization discontinued	24	12	3	1	2	3	2	2
Intact mice		10	0	0	0	0	0	0

Note. ABS: antibodies to serotonin; AB: antibodies.

discontinuation. The data were processed using the Student t test and the χ^2 test.

RESULTS

All the animals were divided into seven groups: in group 1 antiserotonin antibodies in a dose of 5 mg by protein were injected 24 h before alcoholization was discontinued; in group 2 antibodies to serotonin were injected in a dose of 10 mg by protein; in group 3 these antibodies were injected in a dose of 10 mg by protein immediately after alcohol was discontinued; groups 4 and 5 were controls for antibody specificity and were administered, respectively, antibodies to BSA and rabbit γ-globulin in a dose of 10 mg by protein 24 h before the cessation of alcoholization; group 6 animals were injected with normal saline, and group 7 were intact controls.

The experimental results are summarized in Table 1. Injection of antiserotonin antibodies 24 h before alcoholization is discontinued reliably decreases the number of animals with clinical manifestations of withdrawal syndrome recorded 6 h after alcohol is discontinued. The number of animals with convulsions if held by the tail is reduced, and tremor, rigidity, and starting at knocking are less frequent. The effect of an injection of antibodies to serotonin is increased with the dose increase from 5 to 10 mg. The reduction of such withdrawal symptoms as convulsions and tremor is particularly significant. The total number of animals with withdrawal syndrome in groups 1 and 2 was 50% lower than in the nonimmunized groups. The number of animals with signs of the syndrome was also reduced in the group administered antibodies to serotonin after the cessation of alcoholization: tremor was seen in only one of these animals. Rabbit γ-globulin injected 24 h before alcohol was discontinued had a similar nonspecific protective effect.

Alcohol withdrawal was attended by an increase in the overall motor activity of the animals, which was arrested, as shown in Fig. 1, only by injection of antiserotonin antibodies in a low dose and

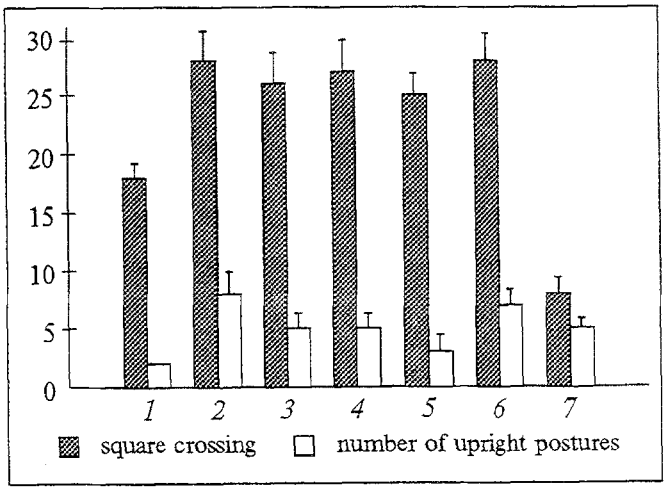

Fig. 1. Changes in motor activity of mice with withdrawal syndrome after injection of antibodies to serotonin. *1*) ABS in a dose of 5 mg 24 h before alcohol discontinued; *2*) ABS in a dose of 10 mg 24 h before alcohol discontinued; *3*) ABS in a dose of 10 mg after alcohol discontinued; *4*) AB to BSA in a dose of 10 mg 24 h before alcohol discontinued; *5*) rabbit γ–globulin in a dose of 10 mg 24 h before alcohol discontinued; *6*) normal saline 24 h before alcohol discontinued; *7*) intact mice. ABS: antibodies to serotonin; AB: antibodies.

only 24 h before alcohol was discontinued. Anti-BSA antibodies had no effect on the manifestation of withdrawal. Our experiments demonstrated that antiserotonin antibodies alleviate the manifestations of withdrawal syndrome in mice when injected 24 h before or after alcohol is discontinued. The efficacy is higher in the former case. The weakly pronounced immunomodulating antiwithdrawal effect of rabbit γ-globulin may be related to the presence of normal antibodies to serotonin. The inefficacy of anti-BSA antibodies is in line with the results of their administration with the aim of eliminating alcohol motivation [2]. On the whole, our results confirm the data of our previous research, in which the possibility of passive immunization with antibodies to serotonin for the treatment of alcoholism was demonstrated [3,4].

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Blood Rheology in Experimental Diabetes Mellitus

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One of the major causes of unsuccessful prevention and treatment of diabetic angiopathies is lack of knowledge about the mechanisms of their development [1,11].

The objective of the present study was to explore the membrane disturbances in the formed elements of the blood and their effect on the blood rheology in the early stages of experimental diabetes mellitus.

MATERIALS AND METHODS

The experiments were carried out on male albino rats. A model of type I diabetes mellitus was created by administering a 5% solution of alloxan hydrate (Chemapol, Czechoslovakia) in a dose of 11 mg/100 g, i.p. The severity of diabetes was judged by the blood sugar level, which was assayed by the orthotoluidine method. The assays were performed on days 7 and 14 of the experiment. The electrical breakdown of the erythrocyte membrane, the electrophoretic mobility and ξ-potential of the erythrocyte membrane, the shear velocity, and the dynamic viscosity of the blood were determined. The electrical breakdown of the erythrocyte membrane was determined after Putvinskii [4] by placing erythrocytes in media with low concentrations of Cl^-. The electrophoretic mobility and ξ-potential were determined by Stolyar's micromethod [6]. The ξ-potential of the erythrocyte membrane was calculated by the formula:

$$\xi = \frac{4\pi U}{HD},$$

where U is the erythrocyte mobility; H is the potential gradient; D is the dielectric constant of the medium; and 4π is a coefficient. The vis-

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