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EFFECT OF PASSIVE INJECTION OF ANTIBODIES TO SEROTONIN AND TO CATECHOLAMINES ON ALCOHOL CONSUMPTION BY C57BL/6 MICE WITH EXPERIMENTAL ALCOHOLISM

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A particularly important role in the pathogenesis of alcoholism is played by lasting disturbances of activity of the brain dopaminergic and serotoninergic systems [1, 3]. We showed [5] for the first time that antibody formation to serotonin and catecholamines is enhanced during chronic alcoholic poisoning of animals and observed negative correlation between the height of the titer of antibodies to neurotransmitters and the level of alcohol consumption. This finding was directly confirmed in experiments with immunization of C57BL/6 mice, predisposed to alcohol consumption, by serotonin — bovine serum albumin (BSA) and noradrenalin — BSA conjugates [6, 8]. The possibility that the level of alcohol dependence may be lowered as a result of active immunization of animals chronically consuming alcohol with the serotonin conjugate also has been demonstrated [4].

The aim of this investigation was to study the possibility of using a method of passive immunization (parenteral injection of antibodies to neurotransmitters) in the experimental treatment of alcoholism.

EXPERIMENTAL METHOD

Experiments (two series) were carried out on 280 male C57BL/6 mice weighing initially 18-20 g. Antibodies to neurotransmitters were obtained by immunizing rabbits with serotonin — BSA (S — BSA), noradrenalin — BSA (NA — BSA), and dopamine — BSA (DA — BSA) conjugates by the standard program. The S — BSA conjugate was synthesized by a modified Mannich's method of formaldehyde condensation [10], the NA — BSA conjugate with the aid of the bifunctional reagent glutaraldehyde [7], and the DA — BSA conjugate was obtained in the reaction with p-aminophenylalanine [9]. The conjugates thus obtained contained 10-15 molecules of biologically active substances, covalently bound with one BSA molecule. Levels of antibodies to neurotransmitters were determined by ELISA using as the test antigen a conjugate on a heterologous protein carrier, namely horse gamma-globulin. The antibody titer averaged 1:1000. The gamma-globulin

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TABLE 1. Effect of Passive Injection of Antibodies (AB) to Serotonin and Catecholamines on Alcohol Consumption by C57BL/6 Mice

Antibody	Dose of AB, mg a	Consumption of 15% alcohol solution, g/kg of 96° alcohol				Water consumption, ml/kg			
		bef. inj.	1-3 days	4-6 days	7th day	bef. inj.	1-3 days	4-6 days	7th day
To S–BSA	5	12,5±1,2	7,3±0,4*	10,7±0,6	11,3±0,7	3,3±0,2	6,6±0,3*	6,6±0,4*	6,6±0,4*
	10	13,5±0,8	5,7±0,4*	10,7±0,6*	11,0±0,6	4,0±0,2	9,9±0,6*	6,0±0,3*	6,0±0,3*
	100	10,0±0,6	2,0±0,2*	7,5±0,4*	7,5±0,5	13,2±0,8	21,1±1,2*	13,2±0,8	13,2±0,9
To NA–BSA	5	13,0±1,3	6,7±0,7*	14,0±0,8	14,0±0,8	3,0±0,3	8,6±0,8	9,8±0,6	9,8±0,6
	10	13,5±1,2	8,3±0,9*	13,5±0,8	14,5±0,8	9,0±0,5	13,2±0,5	19,8±0,6*	9,8±0,5
	100	15,0±1,5	6,0±0,6*	13,0±1,3	12,5±1,3	3,0±0,3	9,9±1,0	14,6±1,5*	13,2±1,4
To DA–BSA	5	14,0±1,4	15,0±0,9	12,5±0,7	11,5±1,2	5,4±0,3	3,0±0,2	3,3±0,2	3,3±0,2
	10	12,0±1,2	12,5±0,7	12,5±0,7	11,5±0,7	14,6±0,9	12,2±0,7	9,0±0,5	9,8±0,6
	100	13,0±1,3	11,8±0,7	13,5±0,8	18,5±1,1	6,0±0,4	4,9±0,3	3,0±0,2	3,3±0,2
To BSA	5	12,1±1,2	11,3±0,7	12,6±0,6	14,6±0,7	14,6±0,7	13,9±0,6	14,6±0,7	14,6±0,6
	10	11,1±1,1	8,2±0,6	11,8±0,7	12,3±0,8	9,8±0,4	9,4±0,4	13,2±0,6	9,8±0,4
Gamma-globulin of unimmunized rabbits	5	16,5±1,7	13,2±1,4	16,5±1,7	17,5±1,8	19,8±2,0	17,2±1,7	22,4±2,3	19,8±2,04
	10	14,5±1,5	9,0±0,9*	11,5±11,2	12,0±1,1	13,2±1,3	11,5±1,1	13,2±1,4	13,2±1,3
	100	14,0±1,4	8,5±0,8*	13,5±1,4	16,0±1,7	4,0±0,4	6,6±0,7	13,2±1,3	6,0±0,6
To tetanus toxoid	10	8,5±0,8	8,5±0,9	8,0±0,8	9,0±0,9	14,6±1,5	13,9±1,4	13,2±1,4	14,6±1,6
Control (physiological saline)	—	11,5±1,2	10,1±0,4	14,5±0,6	14,2±0,5	19,8±0,7	14,6±0,6	19,8±0,7	19,8±0,8

Legend. *$p < 0.05$ compared with initial level of consumption.

fraction was isolated from sera of the immunized and control animals by reprecipitation with ammonium sulfate, freeze-dried, and kept at 4°C. Gamma-globulin from rabbits immunized with BSA and from intact rabbits was used as the control. The protein concentration in the isolated fractions was determined spectrophotometrically by Lowry's method. The C57BL/6 mice received a solution of 15% alcohol and water for 1 month under free choice conditions. The mice were then divided into seven groups: groups 1, 2, and 3 received antibodies to serotonin, noradrenalin, and dopamine respectively intraperitoneally, mice of group 4 received antibodies to BSA by the same method, mice of group 5 received gamma-globulin from an unimmunized rabbit, mice of group 6 received antibodies to tetanus toxoid (produced by the Pasteur Research Institute of Epidemiology and Microbiology, Leningrad), and the intact mice of group 7 received the same volume of physiological saline intraperitoneally. Thus the animals of groups 4-7 served as the control of specificity of action of the antibodies to neurotransmitters and of the physical effects of the procedure itself. Each of the five types of antibodies were injected once into three subgroups of mice in a dose of 5, 10, and 100 mg as protein respectively. The level of free consumption of 15% alcohol solution and water by the animals was measured starting with the 1st day after injection of antibodies and continuing for 2 weeks. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

The experiments revealed a dose-dependent effect of intraperitoneal injection of antibodies to neurotransmitters on alcohol consumption by the animals (Table 1). It was greatest in mice receiving antibodies in a dose of 100 mg as protein. The most significant effect as regards reduction of alcohol consumption was obtained by injection of antibodies to serotonin. For instance, injection of antibodies to serotonin in the highest dose (100 mg) reduced consumption of 15% alcohol solution almost tenfold, whereas the average dose (10 mg) reduced the volume of alcohol solution drunk by the mice by half compared with its initial level. Antibodies to noradrenalin also gave a significant reduction of alcohol consumption by the animals: a dose of 10 mg lowered the alcohol consumption by half, and the highest dose (100 mg) lowered it by two-thirds. Unlike antibodies to serotonin and noradrenalin, those to dopamine had no significant effect on the animals' ethanol consumption. The same effect was obtained by injection of antibodies to BSA and to tetanus toxoid. Meanwhile, gamma-globulin from an unimmunized rabbit, not containing antibodies to neurotransmitters, induced a decrease in alcohol consumption by the animals, although it was significantly smaller than the effect of injection of antibodies to serotonin and

to noradrenalin. The duration of maximal antibody-dependent inhibition of alcohol consumption by animals passively immunized to noradrenalin and to serotonin averaged 3 days, and this was followed by an increase in the volume of ethanol solution consumed.

A return to the original level was recorded on the 10th day after a single injection of antibodies, evidence of the high efficacy of the method of inhibiting alcohol motivation which we used in mice predisposed to alcohol consumption. The specificity of the effect was confirmed by the compensatory increase in water consumption by the mice at the stage of suppression of alcohol motivation. It is a particularly interesting fact that the "psychogenic" action of the antibodies was revealed 1 month after the beginning of alcohol administration to the animals, indicating that the phenomenon of primary addiction to alcohol may be regulated by antibodies to neurotransmitters at the stage of formation of alcohol dependence. Just as with active immunization [4, 8], antibodies to serotonin, when injected intraperitoneally, inhibited alcohol consumption much more intensively than antibodies to noradrenalin. By contrast, antibodies to dopamine had no significant effect on alcohol consumption. Thus the paradoxical fact was noted that the maximal antialcoholic action of antibodies to serotonin and the ineffectiveness of antibodies to dopamine did not correspond to the established notions of the dominant role of the dopaminergic system in the development of alcoholism. Binding of the neurotransmitter with the antibody evidently induces a series of indirect processes in the CNS, as our data published previously indicate [2, 6]. The results demonstrate the good prospects for the use of antibodies to neurotransmitters in the initial stage of development of alcoholism, accompanied by disturbance of permeability of the blood-brain barrier.

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