Suppression of Alcohol Consumption in C57Bl/6 Mice after Intranasal Administration of Antiserotonin Antibodies

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Experiments on C57Bl/6 mice under conditions of free choice between alcohol and water for 10 months showed that intranasal administration with antiserotonin antibodies in doses of 30 and 15 μg/kg for 2 week decreased alcohol consumption over 1 month after treatment.

Key Words: *antiserotonin antibodies; alcohol motivation*

Antibodies against serotonin (5HT-AB) play a role in the mechanisms of neuroimmune interactions. They can be used in the therapy of neuroimmune disorders, including alcoholism and drug abuse [2-5]. Alcohol dependence and withdrawal syndrome in alcohol-predisposed C57Bl/6 mice are suppressed during active immunization with a serotonin-protein conjugate, passive immunization with 5HT-AB, or intraperitoneal administration of lymphocytes extracorporeally stimulated with 5HT-AB [2, 4,5]. The induction of 5HT-AB production in chronically morphinized animals prevents drug tolerance and withdrawal syndrome [3]. An urgent problem of 5HT-AB immunotherapy of alcoholism and drug abuse is the development of simple effective methods that would allow us to supply antibodies to the brain and regulate central neurotransmitter systems.

Here we studied the immunocorrecting effect of long-term intranasal treatment with 5HT-AB in various doses on alcohol consumption by C57Bl/6 mice in the free choice paradigm for alcohol preference.

MATERIALS AND METHODS

Experiments were performed on 90 male C57Bl/6 mice weighing 20-22 g. The animals were kept in cages (10 mice per cage) and fed a standard diet (dry pelleted food). The animals were given a free choice between 15% alcohol solution and water for 10 months. To avoid the reaction to a specific location of alcohol, water and alcohol bottles were interchanged at least twice a week. We selected the animals that regularly consumed 96% alcohol in a daily dose of not less than 5 g/kg over the past 5 months. These mice were characterized by alcohol dependence. The development of alcohol dependence was estimated by the alcohol deprivation effect (increase in alcohol consumption after short-term alcohol withdrawal). The alcohol deprivation test was performed 4 weeks before the start of the study. The animals were withdrawn from alcohol for 2.5 days. Then the mice were given a free choice between 15% alcohol solution and water. Alcohol consumption was recorded. The increase in alcohol consumption by 1.5-2 times reflected the development of alcohol dependence [8].

5HT-AB were obtained from rabbits routinely immunized with a serotonin-protein conjugate [1,7]. 5HT-AB were purified by means of affine chromatography with cyanogen bromide-activated Sepharose [6].

We performed 2 series of experiments. In series I we selected the effective dose of 5HT-AB for intranasal administration. 5HT-AB were dissolved in 100 μl distilled water and administered in a single dose of 300, 30, 15, or 3 μg/kg once a day

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for 2 weeks. Control animals received intranasally 10 μl distilled water. To exclude possible nonspecific effects of antibodies, in series II we compared the influence of 5HT-AB and γ-globulin on alcohol motivation. Group 1 mice received intranasally 30 μg/kg 5HT-AB in 10 μl distilled water for 2 weeks. Group 2 mice were treated with 30 μg/kg γ-globulin received from intact rabbits and dissolved in 10 μl distilled water. Control animals received intranasally 10 μl distilled water.

The animals were examined over 1.5 months. They had free access to water and 15% alcohol solution. The volume of fluid consumed by mice was measured daily at the same time.

Statistical treatment included parametric one-way analysis of variance. The average variance was compared by means of Student—Newman—Keuls test (Primer software).

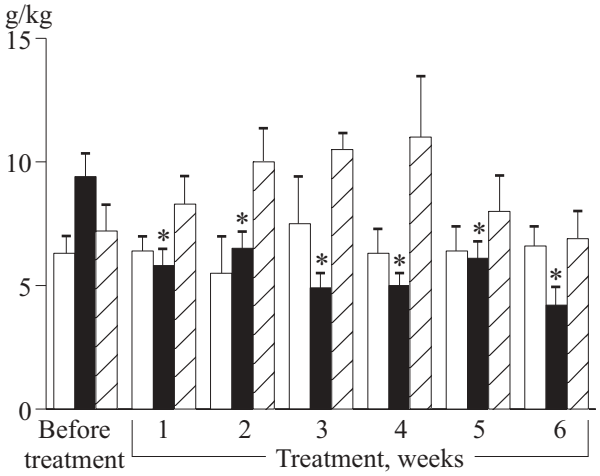

Fig. 1. Effect of 5HT-AB and γ-globulin on consumption of 96% alcohol in C57Bl/6 mice. Light bars, control group; dark bars, 5HT-AB (30 μg/kg); shaded bars, γ-globulin (30 μg/kg). *p<0.05 compared to the baseline level.

RESULTS

Series I was performed on 5 groups of animals. The mice of groups 1, 2, 3, and 4 received intranasally 5HT-AB in doses of 300, 30, 15, and 3 μg/kg, respectively, for 2 weeks. Control animals were treated with the solvent (distilled water). Intranasal administration of 5HT-AB in a dose of 15 μg/kg led to a permanent decrease in alcohol consumption (Table 1). The effect of antibodies developed progressively and was most pronounced by the end of treatment. A 3-fold decrease in the volume of consumed alcohol was revealed over the 1st and 2nd weeks. One and two weeks after the end of antibody treatment, alcohol consumption in these animals decreased by 4.5 times compared to the baseline level. The reduced level of alcohol consumption in mice of this group was observed over 4 weeks after discontinuation of 5HT-AB treatment. Alcohol consumption also decreased during the course and 3 weeks after the end of intranasal treatment with 5HT-AB in a dose of 3 μg/kg. The increase in alcohol consumption not accompanied by the decrease in the volume of consumed water was observed by the 4th week after discontinuation of treatment with 5HT-AB in a dose of 300 μg/kg. No changes in alcohol consumption were found in control animals.

Series II was performed on 3 groups of C57Bl/6 mice. Group 1 mice received intranasally 5HT-AB in a dose of 30 μg/kg for 2 weeks. Group 2 mice were treated with γ-globulin in a dose of 30 μg/kg. Control animals received intranasally distilled water. Experiments with intranasal administration of 5HT-AB confirmed the results of series I (Fig. 1). Treatment with 5HT-AB in a dose of 30 μg/kg was followed by a decrease in alcohol consumption. Treatment with γ-globulin in a dose 30 μg/kg (effective dose of 5HT-AB) had little effect on consumption of 15% alcohol solution in animals with alcohol dependence. No significant changes were revealed in control mice.

Our results show that intranasal administration of 5HT-AB contributes to the decrease in alcohol motivation during alcoholism. Alcohol consump-

TABLE 1. Alcohol Consumption in C57Bl/6 Mice after Intranasal Administration of 5HT-AB ($M\pm m$, g/kg)

Group	Before antibody treatment (baseline level)	Antibody treatment, weeks		Discontinuation of antibody treatment, weeks			
		1	2	1	2	3	4
1 (300 μg/kg), n=10	5.0±2.0	3.0±1.2	2.7±0.6	1.3±0.3	3.7±1.5	9.2±2.3	10.7±1.8*
2 (30 μg/kg), n=10	5.4±1.3	2.2±0.5*	2.3±0.7*	0.9±0.4*	1.3±0.4*	1.5±0.4*	2.4±0.9
3 (15 μg/kg), n=10	5.0±0.5	1.8±0.3*	1.8±0.3*	1.2±0.6*	1.1±0.2*	1.9±0.3*	2.8±0.7*
4 (3 μg/kg), n=10	10.5±4.7	12.1±0.7	12.1±0.7	10.7±0.5	8.8±1.2	4.1±1.2	5.6±1.2
Control, n=20	5.5±0.9	4.9±0.8	4.9±0.8	5.1±0.1	4.8±0.8	5.8±1.0	6.0±1.3

Note. *p<0.05 compared to the baseline level; n, number of animals.

tion by mice decreased only after intranasal administration of 5HT-AB in doses of 15 and 30 μg/kg. 5HT-AB in lower or higher doses were ineffective. The effect of intranasal treatment with 5HT-AB in doses of 15 and 30 μg/kg was persistent and long-lasting. Further clinical studies are required to evaluate whether this simple and effective method of treatment with 5HT-AB may cause a permanent decrease in alcohol motivation.

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