SPLENOPENTIN: A MODULATOR OF IMMUNE AND BEHAVIORAL REACTIONS IN A SECONDARY IMMUNODEFICIENCY STATE INDUCED BY EXPERIMENTAL ALCOHOLISM

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One of the most important tasks in practical immunology is the search for effective methods and means of restoring disturbed functions of the immune system in primary and secondary immunodeficiency states (IDS). Thymus peptides and their analogs are currently attracting attention; one of them is splenopentin, a peptide isolated from the spleen which has the ability to restore the immune response to sheep's red blood cells (SRBC) in irradiated mice [10] and also stimulates differentiation of T and B lymphocytes [9]. Consequently, it can be used as an immunocorrector in secondary IDS. Chronic alcohol intoxication, giving rise to the development of a persistent combined immunodeficiency, characterized by suppression of cellular immunologic reactions, depression of antibody formation following immunization with various antigens, and inhibition of the phagocytic activity of neutrophils and peritoneal macrophages [2, 4, 5, 8], was chosen as the test object. The possible effect of splenopentin on the functional state of the CNS, expressed at the level of alcohol motivation in animals predisposed to the development of experimental alcoholism, appeared to be particularly interesting because of data showing a connection between the immune and nervous systems [6].

The aim of this investigation was to study the effect of splenopentin on immune and behavioral reactions in animals with experimental alcoholism.

EXPERIMENTAL METHOD

Splenopentin was synthesized at the Institute of Biologically Active Substances, Academy of Sciences of the DDR. Experiments were carried out on 120 noninbred mice and 100 C57BL/6 mice initially weighing 18-20 g. In the experiments of series I alcohol intoxication was produced in noninbred mice in the course of 30 days by giving the animals a 15% solution of ethanol instead of water, In experiments to determine antibody-forming cells (AFC) to SRBC six groups of animals were used Mice of the first four groups received alcohol, and those of group 1 were immunized 24 h after the end of alcohol intoxication, i.e., on the 31st day of the experiment, with a 10% suspension of SRBC, injected intraperitoneally in a volume of 0.2 ml; mice of group 2 were immunized 48 h after the end of alcohol intoxication, i.e., on the 2nd day of the abstinence stage. Animals of group 3 received splenopentin in a dose of 250 μg/kg before immunization on the 30th day of alcohol intoxication and they were immunized 24 h later with SRBC; mice of group 4 received splenopentin 24 h after taking ethanol, and they were immunized 48 h later with SRBC; groups 5 and 6 served as controls and consisted of animals not subjected to alcohol intoxication, the mice of group 5 being immunized with SRBC on the 31st day after administration of physiological saline, those of group 6 being immunized after injection of splenopentin. The animals were killed on the 5th day after immunization and the number of AFC in their spleen was counted [11]. Phagocytic activity of the macrophages relative to *Staphylococcus aureus,* strain Zhaev, was determined in cell cultures [7] under identical conditions of

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TABLE 1. Effect of Splenopentin on some Parameters of Immunity in Mice with Chronic Alcohol Intoxication

Group of animals	24 h after withdrawal of ethanol			48 h after withdrawal of ethanol		
	AFC/10^6 lymphocyte	phagocytic number	phagocytic index	AFC/10^6 lymphocyte	phagocytic number	phagocytic index
Alcohol intoxication for 30 days	23,1±2,6*	35,4±0,08*	2,5±0,1*	22,8±1,8*	36,0±0,9*	2,4±0,08*
Alcohol intoxication for 30 days + splenopentin	51,4±2,0	46,3±0,4	3,9±0,09	54,7±2,2	46,8±0,6	3,9±0,09
Splenopentin	54,5±2,2	46,1±0,5	3,6±0,1			
Control	52,6±2,3	45,2±0,3	3,7±0,08			

Legend. Here and in Table 2 *$p < 0.05$.

TABLE 2. Effect of Splenopentin on Consumption of Alcohol and Water by C57B1/6/6 Mice

Group of animals	Consumption of 15% alcohol, g/kg 96°				Consumption of water, in ml/kg			
	.giving splenopentin	1-3 days	4-14 days	15-21 days	Bef.giving splenopentin	1-3 days	4-14 days	15-21 days
Splenopentin 250 µg/kg	9,9±1,2	8,2±2,0	5,8±1,0*	7,3±2,0	3,3±0,8	3,0±0,5	9,8±4,0	2,0±1,0
Splenopentin 500 µ/kg	8,2±1,0	8,2±2,2	4,9±1,1*	6,8±1,8	3,3±1,0	3,0±0,5	14,6±3,6*	3,0±1,2
Control	9,9±1,0	10,0±2,0	9,9±1,2	9,2±1,8	3,3±0,8	4,3±1,0	5,7±4,8	1,2±1,0

alcohol intoxication and administration of splenopentin. The percentage of macrophages involved in phagocytosis (phagocytic number) and the number of bacteria contained in one macrophage (phagocytic index) were determined. In the experiments of series II, aimed at studying the effect of splenopentin on the CNS in C57BL/6 mice, predisposed to alcoholism, the daily level of alcohol consumption was determined for 3 weeks, allowing free choice to the animals between 15% alcohol solution and water, and splenopentin given in doses of 250 and 500 μg/kg. The animals were kept on a standard dry pellet diet. The C57BL/6 mice were kept 10 to a cage, excluding a stress situation. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The results of experiments to study the effect of splenopentin on the immune response to SRBC and on phagocytic activity of the peritoneal macrophages are given in Table 1. They show that alcohol intoxication caused inhibition of AFC production, which continued for 48 h after the end of alcohol consumption. The use of splenopentin under these conditions led to restoration of the normal number of AFC in the spleen, For instance, 24 h after withdrawal of alcohol the number of AFC in the animals' spleen was 23.1 ± 2.6, whereas in animals receiving splenopentin it was 51.4 ± 2.0, and did not differ significantly from the number of AFC in the spleen of intact animals, serving as the control (52.6 ± 2.3). Similar results were obtained with respect to phagocytic activity of the peritoneal macrophages It will be clear from Table 1 that splenopentin restored highly effectively the level of phagocytosis of peritoneal macrophages disturbed by chronic alcohol intoxication.

The results of the study of the effect of splenopentin on C57BL/6 mice, predisposed to the development of experimental alcoholism, during consumption of alcohol are given in Table 2. They show that after administration of splenopentin in doses of 250 and 500 μg/kg the relative alcohol consumption of the animals decreased; a significant decrease, amounting to 50% compared with the control group, in alcohol consumption was recorded, moreover, from the 4th through the 14th day after administration of splenopentin. Alcohol consumption then gradually increased, to regain its initial level by the 21st day after administration of splenopentin. Reduction of the alcohol consumption was observed against the background of an increased uptake of water by the animals.

Thus the investigation showed for the first time that splenopentin can give an effective immunocorrective action on experimental alcohol intoxication in noninbred animals and animals predisposed to alcohol consumption. The mechanisms lying at the basis of these phenomena may be linked, first, with activation of lymphocyte proliferation [9] and inhibition of the function of suppressor T cells, which is activated by alcohol [2], and on the other hand, with its effect on the neurotransmitter systems of the brain, a disturbance of whose function lies at the basis of the pathogenesis of alcoholism [1], and may also be the cause of development of the secondary immunodeficiency state [3].

These results evidently indicate two possible situations for the potential clinical application of splenopentin: to correct disturbances of immunity characteristic of alcoholism [4, 5, 8] and to depress pathological craving for alcohol.

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EXPERIMENTAL INFECTION CAUSED BY THE ISSYK-KUL' ARBOVIRUS

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The Issyk-Kul' virus was first isolated in 1970 from the common noctule (*Nyctalus noctula*), collected in the Issyk-Kul' Depression of the Kirghiz SSR [7]. Later this virus was isolated from other species of bats, ticks, mosquitoes, and birds in Kirghizia and Tadjikistan [1, 2]. Bats are the reservoir of this virus in nature [3, 4]. Issyk-Kul' virus is pathogenic for man; it has been isolated from the blood of a febrile patient who had been collecting bats during the 5 days before his illness (in Issyk-Kul' Depression). On the 20th and 40th day after the beginning of the illness, complement-fixing antibodies were found in the patient's blood in a titer of 1:16 and 1:32 respectively. Virus-neutralizing antibodies were detected on the

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