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EFFECT OF ACUTE ALCOHOLIC INTOXICATION ON ANTIGENIC COMPOSITION OF SOLUBLE RAT-BRAIN PROTEINS

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KEY WORDS: immunoelectrophoresis; brain proteins; antigenic composition; ethanol.

Animals preferring ethanol solution or water when allowed free choice differ in many parameters characterizing the state of their tissue metabolism [4, 7]. Preferential consumption of ethanol by animals, it can be tentatively suggested, is connected with the specific character of their metabolism, including their brain tissue metabolism, whose specificity is largely determined by protein composition. Investigation of the brain protein composition of animals consuming water or ethanol solution when allowed free choice is therefore an important aspect of the study of mechanisms of ethanol preference.

Data in the literature invariably indicate depression of protein synthesis in the brain in acute alcoholic intoxication [6]. However, the effect of a single dose of ethanol on brain protein composition has not been studied.

The aim of this investigation was to study the antigenic composition of soluble brain proteins of rats preferring water or ethanol solution. At the same time, the effect of acute alcohol intoxication was studied on the brain protein composition of rats similar in character of preference (intermediate group).

EXPERIMENTAL METHOD

Male rats weighing 250-300 g were used. The animals were divided into three groups with respect to voluntary consumption of water or 15% ethanol solution by preference. The separation was effected in 10 days. Animals preferring water, those preferring ethanol, and rats of the intermediate group consumed not more than 8%, not less than 50%, and 16-35%, respectively of ethanol solution relative to the total fluid intake. Rats of the intermediate

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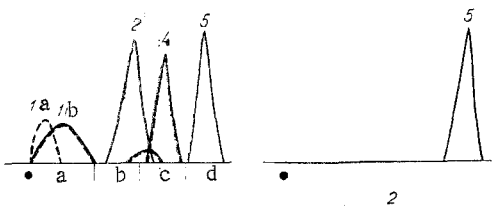

Fig. 1. Immunoelectrophoresis of soluble rat-brain proteins with normal (1) and exhausted (2) antiserum against rat-brain proteins. 1a-5) Numbers of antigens. a-d) Zones of mobility of proteins: a) γ- and β-globulins; b) α-globulins; c) albumins; d) prealbumins. Continuous line represents antigens detected on extraction with veronal buffer; broken line — the same on extraction with physiological saline; continuous and broken lines denote the same with both methods of extraction. Filled circle — point of application of sample.

TABLE 1. Antigenic Composition of Soluble Brain Proteins in Rats of Different Groups

Group of animals	Extraction of brain proteins by	Number of experiment	Antigen content, %					
			1a	1b	2	3	4	5
Preferring water	Veronal buffer	11	0,00	10,6±0,5	29,5±0,5	2,5±0,2	23,8±1,0	33,3±0,7
	Physiological saline	6	16,1±1,7	26,7±1,2	0,00	2,3±0,2	55,0±2,4	0
Intermediate group	Veronal buffer	8	0	11,4±0,7	26,7±1,1 $P<0,05$	3,3±0,3 $P<0,05$	25,3±0,5	31,8±0,9
	Physiological saline	6	14,5±1,1	26,0±2,0	0,00	2,5±0,3	56,2±2,3	0
Preferring ethanol	Veronal buffer	7	0	10,4±0,3	27,6±0,7	3,3±0,3	25,0±0,9	33,5±1,5
	Physiological saline	6	14,5±1,3	24,4±1,8	$P<0,05$* 0	$P<0,05$ 3,5±0,3 $P<0,05$	57,7±2,8	0

Legend. *Relative to animals preferring water.

group were given a single intraperitoneal injection of 25% ethanol solution in a dose of 2.5 g/kg. The animals were used in the experiments 3 h after injection of ethanol. Control animals were injected with distilled water.

The antigenic composition of the soluble proteins was determined in whole rat brain by cross immunoelectrophoresis [9]. The concentration of neurospecific protein S-100 was measured in the cerebellum by rocket immunoelectrophoresis in accordance with the procedure described previously for immunoelectrophoresis of apolipoproteins [2].

Whole rat brain was homogenized in 0.025 M veronal buffer (pH 8.6) or in physiological saline in the ratio 1:4 (w/v). The fraction of extracted proteins was isolated by consecutive centrifugation of the homogenate at 2000g for 20 min and 80,000g for 40 min. The supernatant was used for immunization of rabbits and cross immunoelectrophoresis. Extracts of cerebellar protein for rocket immunoelectrophoresis and liver extracts for exhaustion of the antiserum were prepared in the same way in veronal buffer. Protein S-100 for immunization of the rabbits was purified from bovine brain [11]. The purity of the isolated protein was tested by disc electrophoresis in polyacrylamide gel and by Ouchterlony's immunodiffusion method, using antiserum against protein S-100 provided by S. M. Sviridova (Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk). Rabbit antiserum against brain proteins was obtained by intraperitoneal injection of antigen according to the scheme described previously [2]. Soluble brain proteins and protein S-100 were injected in doses of 3 and 0.5 mg per injection, respectively. Specificity of the rabbit anti-S-100 serum was tested with purified protein S-100 obtained from Dr. Grasso (Institute of Cell Biology, Rome, Italy). Antiserum against soluble rat-brain proteins was exhausted with soluble rat liver and blood antigens by the method in [10].

TABLE 2. Effect of Single Injection of Ethanol on Antigenic Composition of Rat-Brain Proteins

Experimental conditions	Number of experiments	Antigen content, %				
		1b	2	3	4	5
Control	8	11,4±0,7	26,7±1,1	3,3±0,3	25,3±0,5	31,8±0,9
Ethanol	10	14,1±0,2 P=0,05	28,0±1,5	2,1±0,2 P<0,05	23,7±1,3	31,7±1,0

EXPERIMENTAL RESULTS

On immunoelectrophoresis of proteins extracted with veronal buffer and physiological saline, 5 and 4 peaks of antigens amenable to quantitative evaluation were discovered respectively (Table 1). Antigens 1a and 1b were in the zone of mobility of γ- and β-globulins, whereas antigens 2 and 5 were in zones of α-globulins and prealbumins, respectively (Fig. 1).

To study the specificity of these antigens for brain tissue, cross immunoelectrophoresis of blood serum and liver extract of rats with antiserum against soluble rat brain proteins, extracted with veronal buffer, was carried out. Four antigens were found in blood serum and seven in the liver. According to their electrophoretic mobility, brain antigens in the blood serum corresponded to antigen 4, those in the liver to antigens 1 and 3. On the basis of this comparison it could be postulated that antigens 2 and 5 are specific for brain tissue. Subsequent analysis with antiserum against brain proteins, exhausted by liver and blood serum proteins, showed that only antigen 5 is a neurospecific antigen (Fig. 1).

The antigenic composition of brain proteins of animals of the intermediate group was similar to the composition of brain proteins from rats preferring ethanol. Meanwhile, the concentration of antigen 3 in animals preferring water was lower, whereas that of antigen 2 (this antigen was found only on extraction with veronal buffer) was higher than in rats of the remaining two groups. Predominance of antigen 3 in the brain of rats preferring ethanol was observed when brain proteins were extracted by both methods. Consequently, differences in the content of brain antigens which may be associated with the character of ethanol preference relate to nonspecific soluble brain proteins (Table 2).

The concentration of neurospecific protein S-100 in rat brain has been shown to be directly dependent on learning [1, 5, 8]. The concentration of this protein in the cerebellum of rats consuming ethanol or water by preference was 0.30 ± 0.01 and 0.31 ± 0.01 mg/g, i.e., it was virtually identical. This fact is in agreement with data presented above, indicating no correlation between predisposition of rats toward ethanol consumption and the concentration of neurospecific antigen in their brain.

Much evidence has now been obtained to show that the disturbances of metabolism arising in acute and chronic ethanol intoxication are different [3]. It has already been mentioned that a single dose of ethanol depresses protein synthesis in the brain.

Ethanol has a selective action on the content of individual brain proteins (Table 2). The content of antigen 3 in animals receiving ethanol was 1.6 times higher, whereas that of antigen 1b was 1.2 times higher than the corresponding control values. A single injection of ethanol thus causes a significant change in the composition of nonspecific soluble brain antigens.

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EFFECT OF TRANQUILIZERS OF THE BENZODIAZEPINE SERIES ON A FOCUS OF ISCHEMIA AND REDISTRIBUTION OF THE BLOOD FLOW IN THE ISCHEMIC MYOCARDIUM

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Tranquilizers of the benzodiazepine series (diazepam and chlordiazepoxide) are widely used in the combined treatment of ischemic heart disease (IHD). These drugs abolish autonomic components of emotional reactions, including tachycardia and high blood pressure (BP), which are risk factors in IHD, and they strengthen cardiac activity. The aim of this investigation was to study the direct effect of tranquilizers on the functional state of the ischemic myocardium and its blood supply.

EXPERIMENTAL METHOD

A model of acute coronary insufficiency [8] in the authors' modification [6] was used. The essence of the model was creation of an ischemic focus in the myocardium of dogs by simultaneous narrowing of the lumen of the anterior descending branch of the left coronary artery and imposition of a high artificial rhythm on the heart. The degree of ischemia was judged from elevation of the ST segment in the epicardial electrogram. To judge the effect of drugs on the blood supply to the ischemic focus in the myocardium, the method of alternate recording of the retrograde coronary blood flow and retrograde pressure in the distal segment of the anterior descending branch of the left coronary artery, ligated in its middle third, in dogs was used [1, 4]. Redistribution of the blood flow in the heart muscle was judged from the ratio of retrograde coronary blood flow in the territory supplied by the ligated coronary artery and the coronary blood flow in the circumflex branch of the left coronary artery, supplying blood to intact regions of the myocardium (RBF/CBF) [7, 9]. For this purpose, simultaneously with recording the retrograde blood supply to the ischemic focus, the volume velocity of the coronary blood flow in the circumflex branch of the left coronary artery was measured by an ultrasonic method in the coronary arteries [3]. Experiments were carried out on mongrel dogs (15 animals), anesthetized with pentobarbital (40 mg/kg, intravenously), with artificial respiration. Systemic BP (in the carotid artery), the velocity of the coronary blood flow in the circumflex branch of the left coronary artery, and retrograde pressure in the territory of the ligated coronary artery were recorded on the Mingograph-81 apparatus. Drugs were injected intravenously: diazepam in doses of 0.1 and 0.3 mg/kg, chlordiazepoxide in a dose of 1 mg/kg.

EXPERIMENTAL RESULTS

It was shown previously [2, 5] that diazepam and chlordiazepoxide have a marked effect on the blood supply and activity of the intact myocardium. The marked increase in oxyhemoglobin concentration in blood from the coronary sinus of the heart under the influence of the drugs, despite a decrease in volume velocity of the total coronary blood flow, is a noteworthy

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