The results are thus evidence that the basic action of allapinine is inhibition of sodium conductance of membranes of excitable objects. Allapinine has an inhibitory action on the sodium current, without changing the threshold of its activation, in experiments both on cardiomyocytes and on single trigeminal ganglionic neurons.

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SERUM CREATINE KINASE ISOZYME SPECTRUM OF RATS DURING AGING AND ACUTE ALCOHOL INTOXICATION

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KEY WORDS: creatine kinase isozymes, age, ethanol, blood, brain

Ethanol has a many-sided action on all systems of the body [1-3]. Many workers have shown that ethanol penetrates freely through the blood brain barrier, and thus disturbs its protective function [1]. The direct action of alcohol and acetaldehyde on cells may lead to increased "flowability" of the cell membranes [2], thereby changing their permeability. An early response of brain tissue to ethanol poisoning is disturbance of oxidative processes and acidification of the intracellular medium, and disturbance of tissue respiration [1]. Similar changes in energy metabolism may also lead to increased cellular permeability for several metabolites and proteins, one of which is creatine kinase.

Creatine kinase (CK) is a cytoplasmic enzyme involved in energy metabolism. A significant increase in CK activity in the blood of rats has been demonstrated after a single injection of a large dose of ethanol [7]. There is also information on the increase in CK activity in the blood serum of alcoholics [4].

We know that CK exists in three molecular forms, each characterized by marked organ-specificity. Skeletal muscle contains MM-CK, MB-CK is a cardiospecific isozyme, and BB-CK is found mainly in the brain, smooth muscle, and gonads [5].

The question accordingly arises: on account of which isozymes does the increase in total CK activity in the blood serum take place in acute alcohol intoxication, and from which tissues does the enzyme leak into the blood stream. Other interesting questions are age changes in the activity of this enzyme. The investigation described below was carried out to study these problems.

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TABLE 1. Total CK Activity in Blood Serum of Rats of Different Ages and Rats with Acute Alcohol Intoxication (M ± m)

Group of animals	Blood serum CK activity, IU/liter
Intact rats aged 4 months (n = 12)	2000±90
Intact rats aged 24 months (n = 8)	750±130*
Intact rats aged 4 months + physiological saline (n = 12)	2100±110
Administration of ethanol	
in dose of 3 g/kg (n = 12)	3300±120*
in dose of 5 g/kg (n = 12)	5400±170*

Legend. *p < 0.05 compared with intact group (4 months).

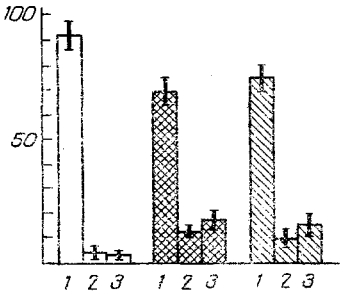

Fig. 1. CK isozyme spectrum in blood serum of rats of different ages and rats with acute alcohol intoxication. Abscissa, isozyme profiles of different groups of rats. Unshaded columns — intact rats aged 4 months, cross-hatched columns — intact rats aged 24 months, obliquely shaded — intact rats aged 4 months + acute alcohol intoxication. 1) MM isozyme of CK, 2) MB isozyme, 3) BB isozyme. Ordinate, content of CK isozymes in blood (in per cent).

EXPERIMENTAL METHOD

Experiments were carried out on 56 male rats weighing 180-200 and 350-400 g. To reveal age changes in serum CK activity intact animals aged 4 and 24 months, whose blood was shown to contain activity of CK and its isozymes, were used.

The study of the effect of acute alcohol intoxication on activity of CK and its isozymes in the tissues and biological fluids of rats, two groups of animals aged 4 months were used. Each animal was given an intraperitoneal injection of 25% ethanol solution in a dose of 3 and 5 g/kg 3 h before the beginning of the experiment. The control consisted of rats receiving an injection of a corresponding volume of physiological saline. Before decapitation, cerebrospinal fluid was taken from these animals under superficial ether anesthesia. Activity of CK and its isozyme profile were determined in brain extract obtained from the cerebral cortex, CSF, and blood serum. Total activity of the enzyme was determined colorimetrically [6]. The isozymes were separated by chromatography on a column with Sephadex DEAE A50 [7]. The protein content was determined by the microbiuret method. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Analysis of the data on total CK activity in the blood serum of rats with acute alcohol intoxication, and also in intact rats of different ages, revealed that the intact animals aged 4-5 months differed strongly in activity of CK and its isozyme spectrum from intact rats aged 24 months (Table 1). This observed age-related decrease in total blood CK activity is perhaps the result of a change in the level of general metabolism, slowing of tissue renewal processes, diminution of motor activity, and so on. The causes of age changes in the isozyme profile of CK, which shifts with age toward an increase in the contribution of MB- and BB-forms, are apparently more complex (Fig. 1).

TABLE 2. CK Activity in Brain, CSF, and Blood Serum of Rats with Acute Alcohol Intoxication (M ± m, n = 12)

Experimental conditions	Brain, IU/mg protein	CSF, IU/liter	Blood serum, IU/liter
Control (physiological saline)	9,20±0,12	25±4	2100±110
Administration of ethanol			
in dose of 3 g/kg	8,01±0,10*	430±58*	3300±120*
in dose of 5 g/kg	6,90±0,15*	1015±120*	5310±172*

Legend. *$p < 0.05$ compared with corresponding control.

The next series of experiments to study the effect of acute alcohol intoxication on the serum CK activity of rats was conducted on animals aged 4 months. As Table 1 shows, after administration of ethanol in a dose of 3 g/kg total activity in the blood rose sharply due to an increase in activity of all three forms of the enzyme. At the same time there was an abrupt change in the relative percentages of the isozymes (Fig. 1). These data are evidence that ethanol causes structural damage to cell membranes at the whole body level, but the brain and myocardium are most severely affected, for the absolute increase in activity for the MB- and BB-isozymes was greatest (by 4 and 10 times respectively), whereas MM-CK activity was increased only by 1.4 times. We suggested that the source of serum BB-CK activity is brain tissue. To test this hypothesis, we studied the time course of CK activity in the brain—CSF—blood system (Table 2). Clearly, CK activity following administration of ethanol to the rats decreased in the brain, but increased in the CSF and blood; these changes, moreover, were dose-dependent in character. This suggests that under the influence of acute alcohol intoxication the permeability of the blood-brain barrier and of cells of the cerebral cortex is disturbed, as a result of which leakage of the globular CK protein can take place from the cell cytoplasm into the CSF, and thence into the blood, which may lead to changes in the blood levels of the BB isozyme.

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