Dehydrogenase Systems of Different Rat Tissues in Experimental Alcoholism Simulated by Inhalation or Forced Intake of Ethanol

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It is shown that alcoholization of rats during 1.5 months by the inhalation of ethanol vapors with a long-term subsidence into narcotic sleep results in alcohol dependence and marked shifts in the ratio between the activity of malate and lactate dehydrogenases and a change in the isoenzyme spectrum of the latter. This leads to an enhancement of aerobic processes in the brain and skeletal muscle tissues and of anaerobic processes in the liver and myocardium. Semiforced alcoholization of rats during 11 months, with ethanol solution serving as the only source of liquid, moderately lowers the ethanol tolerance and does not affect the dehydrogenase activity in the tissues examined. The effects of ethanol on the activity of functionally associated enzyme systems of malate and lactate dehydrogenases are believed to depend on the method of alcoholization and the type of tissue.

Key Words: *dehydrogenases; ethanol; simulation of alcoholism; carbohydrate metabolism*

The most widespread model of alcoholism is based on semiforced alcoholization of animals [2,5,6]. This is a method whereby animals are given a 7.5-15% ethanol solution as a only source of liquid. Under such experimental conditions the narcotizing effect of ethanol is not obtained, since the animals themselves regulate the frequency of alcohol intake. The mean diurnal dose of alcohol (8-10 g/kg) slightly surpasses the basal level of ethanol metabolism in rats (7.2 g/kg×day) [5]. Semiforced alcoholization under conditions of free choice results in a pronounced increase of the tolerance of alcohol and of its voluntary intake in just 30-40% of alcoholized rats.

In the present study we explored the functional state of the dehydrogenase systems basing ourselves on the notion that systematic intake of high doses of ethanol markedly increases the NADH level in the tissues [3]. We used inhalation of ethanol vapors as an experimental model of alcoholism. The development of alcohol dependence was assessed as a change in the tolerance of ethanol, the level of motivation, and the activity of the enzymes malate dehydrogenase (MDH; EC 1.1.1.37) and lactate dehydrogenase (LDH; EC 1.1.1.27) in different tissues. In addition, the ratio between the activity of MDH and LDH (MDH:LDH) reflects the ratio between the aerobic and anaerobic oxidation of glucose and, therefore, the state of energy metabolism in the tissues [3].

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats initially weighing 180-200 g obtained from the

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TABLE 1. Activity of MDH and LDH in the Tissues of ALcoholized Rats

Tissue	Group of rats	Activity of dehydrogenases, μmol NAD/min per g tissue		MDH:LDH
		MDH	LDH	
Brain	Control I	72±6	27±2	2.7
	Ethanol, 11 months	76±8	29±2	2.6
	Control II	83±7	28±2	3.0
	Inhalation of ethanol	120±6*	34±2	3.5
Liver	Control I	206±21	156±13	1.3
	Ethanol, 11 months	207±19	148±11	1.4
	Control II	245±17	168±9	1.5
	Inhalation of ethanol	202±10*	208±11*	1.0
Heart	Control I	560±32	201±17	2.8
	Ethanol, 11 months	564±27	197±16	2.9
	Control II	593±34	207±18	2.9
	Inhalation of ethanol	535±36	231±19	2.3
Gastrocnemius muscle	Control I	394±28	590±39	0.7
	Ethanol, 11 months	371±32	578±40	0.6
	Control II	485±29	641±36	0.8
	Inhalation of ethanol	468±27	493±22*	0.9

Note. An asterisk denotes $p<0.05$ vs. control group II.

Rappolovo Breeding Center of the Russian Academy of Medical Sciences (St. Petersburg Region). Alcoholization of animals was performed by two methods. The animals of group 1 received a 15% ethanol solution as the only drinking liquid during 11 months, and the control animals received water (control I). Alcoholization of rats of group 2 was performed by ethanol inhalations. The inhalation chamber was a translucent plastic box (120×70×50 cm) with hermetic sealing, to which air saturated with ethanol vapors was delivered by inflow-exhaust ventilation via a system of tubes. Rats grouped in tens were subjected to 19-h inhalations daily (except for days of rest) during 1.5 months. The ethanol expenditures were from 130 mg/liter ethanol vapors at the beginning of the experiment to 260 mg/liter at its end. Ethanol vapors were delivered to the chamber so that 3-3.5 h after the start of inhalation narcotic sleep overcame the rats. The animals were maintained on a standard laboratory diet. Dry chow and water were available throughout the experiment and between inhalations. The control animals received water inhalations (control II). The tolerance of ethanol after the end of alcoholization was determined as the onset and the duration of narcotic sleep caused by intraperitoneal injection of 3.5 g/kg ethanol solution [1] and as the change in the rectal temperature measured with a special thermocouple (accuracy of measurements 0.05°C). For biochemical examinations animals were decapitated immediately after discontinuation of alcoholization. Isolation of the soluble cytoplasmic fraction and measurement of the MDH and LDH activity in the brain, heart, and gastrocnemius muscle, as well as separation of LDH isoenzymes in polyacrylamide gel were performed as described previously [3,4]. Sample size was not less than 10-12 rats in each experimental series. The results were statistically processed using Student's t test.

RESULTS

A study of the tolerance of ethanol after its intraperitoneal injection in a dose of 3.5 g/kg demonstrated that the duration of sleep in the control animals was 25-27 min, whereas semiforced alcoholization lowered this index to 4 min. In the group of rats receiving ethanol inhalations narcotic sleep was not observed at all. After ethanol injection to rats of the control group the rectal temperature dropped 1.8°C on average. Semiforced alcoholization slightly and ethanol inhalation markedly inhibited the drop of the rectal temperature (in these cases the temperature dropped 1.5 and 0.4°C, respectively). It is well known that injection of ethanol in high doses causing narcotic sleep lowers the body temperature [1,7]. How much the temperature drops depends on the depth and duration of narcotic sleep. Hence, the intensity of the narcotic effect of ethanol can be judged from the drop of the rectal temperature. As the ethanol tolerance increases, the change of the rectal temperature caused by ethanol injection diminishes,

TABLE 2. Isoenzyme Spectrum of LDH in the Tissues of Rats Alcoholized by Inhalation of Ethanol Vapors

Tissue	Group of rats	Isoenzymes of LDH, %				
		LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Brain	Control	10.9	14.5	64.3	7.3	3.2
	Inhalation	17.9	17.0	59.7	2.6	2.8
Liver	Control	42.6	37.2	18.7	1.5	0.0
	Inhalation	35.5	33.1	19.4	3.4	8.5
Heart	Control	72.5	12.5	12.0	2.0	0.0
	Inhalation	61.1	10.2	14.7	4.8	9.2
Gastrocnemius muscle	Control	21.3	26.8	24.9	14.9	12.1
	Inhalation	27.0	32.8	22.6	7.2	10.4

i.e., there is a reverse correlation between changes in the rectal temperature and in the tolerance of ethanol. Thus, the increase in ethanol tolerance in both alcoholized groups is clearly pronounced, the degree of the increase being markedly higher for alcoholization by inhalation.

The two control groups differed little with respect to the activity of MDH and LDH. Nevertheless, animals of the control group involved in the experiment during 11 months exhibited a tendency toward a decrease of the MDH and LDH activity in all tissues examined, which is likely to be due to age-specific changes in metabolism (Table 1). Semiforced alcoholization during 11 months did not affect the MDH and LDH activity or the LDH isoenzyme spectrum (Table 2) in the soluble cytoplasmic fraction of the brain, liver, heart, and skeletal muscle. Marked shifts were observed in the total activity of MDH and LDH and in the isoenzyme profile of LDH for alcoholization by inhalation.

An increase in the ratio between the activities (MDH:LDH) in the brain tissue of rats receiving inhalations resulted from a more marked rise of the MDH activity (Table 2). A high MDH:LDH coefficient is known to be indicative of intensive transfer of reduced NADH equivalents from the cytosol to the mitochondria, i.e., of intensive aerobic metabolism [3]. In view of these notions we may speculate that during alcoholization with ethanol vapors the brain cells adapt to enhanced transfer of reduced potentials from cytosol to mitochondria, or, in other words, they adapt to hypoxia [2]. This is also corroborated by the "aerobic shift" of the isoenzyme spectrum of LDH in the brain, namely, a shift toward an increase in the LDH_1 and LDH_2 content.

The MDH:LDH coefficient in the skeletal muscle rose due to a decrease in the LDH activity, and the isoenzyme spectrum of LDH shifted toward an increased percentage of the "aerobic" forms, i.e., the changes of the enzyme profiles which occurred in the skeletal muscle were similar to the changes observed in the brain tissue, which can be regarded as being aimed at adaptive enhancement of the "aerobic" processes. In the case of alcoholization by inhalation an excess amount of NADH, formed in the course of glycolysis in the liver and myocardium, is evidently spent in the lactate dehydrogenase reaction. This is confirmed by the decrease in the MDH activity, the increase in the LDH activity, and the increase of the percentage of the "anaerobic" forms of LDH: LDH_5 and LDH_4.

Thus, the effect of ethanol on the activity of MDH and LDH manifested itself only for injection of alcohol in high doses causing narcotic sleep. Hence, the effects of ethanol on dehydrogenases depend on its dose, on the method of alcoholization, and on the type of tissue.

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