EFFECT OF MODE OF ALCOHOL LOADING AND WITHDRAWAL ON CONTRACTILITY, GLUCOSE UPTAKE, AND LACTATE RELEASE IN ISOLATED PERFUSED RAT HEARTS

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UDC 612.172+612.173.1:547.455.623].014.46:547.262

KEY WORDS: ethanol; withdrawal response; heart, carbohydrate metabolism; necrosis of myocardium.

Alcoholic myocardiopathy (AMC) is a frequent complication of alcohol abuse. To create a model of AMC in rats, continuous long-term administration of ethanol is usually used. Meanwhile human alcohol intake is not continuous but, as a rule, alternates with a state of hangover or abstinence. The role of the postintoxication syndrome in the pathogenesis of AMC has not been investigated.

The aim of this investigation was to study contractility and carbohydrate metabolism in the myocardium of rats under different conditions of alcohol loading: inducing and not inducing withdrawal reactions.

EXPERIMENTAL METHODS

Animals of group 1 received a single intraventricular injection of 25% ethanol solution in a dose of 8 g/kg. For 3 months the animals of group 2 were allowed free access to 10% ethanol solution and to water. Saccharine (0.125%) and sodium chloride (1%) were added to the ethanol solution to stimulate its consumption [14]. The mean daily ethanol intake in this group was 4.8 ± 0.3 g/kg. Rats of group 3 were given intraventricular injections of 25% ethanol solution twice a day in a dose of 8-9 g/kg daily [1]. The rats were anesthetized with urethane 3-4 h and 1, 3, and 6 days after the last injection of ethanol or after access to it was discontinued, and the heart was removed and perfused by Langendorff's method under a hydrostatic pressure of 100 mm Hg. The perfusion fluid consisted of Krebs–Henseleit solution to which a 1% solution of gelatinol, which significantly increased the stability of working of the heart, was added to improve transcapillary fluid exchange. Considering that gelatinol contains Ca^{++} and Na^{+} ions, appropriate corrections were made to the Krebs–Henseleit solution; the pH of the buffer solution, on saturation with carbogen (95% O_2 and 5% CO_2) and at 37°C, was 7.4. After 10 min of continuous perfusion of the heart a change was made to reperfusion with recirculating solution in a volume of 50 ml. The total duration of reperfusion was 50 min. The peak systolic pressure (PSP) in the left ventricle, the heart rate (HR) and the coronary flow (CF) were recorded. The PSP was measured by means of an electromanometer (Statham), connected to a latex balloon, introduced through the auricle of the left atrium into the left ventricle. The tension time index (TTI) of the myocardium was calculated by the formula (PSP × HR × T)/1000, where T is the tension time of the left ventricle [12].

Considering that activation of glycolysis is one of the earliest signs of AMC [7, 10] the glucose uptake and lactate release by the hear were studied. In the period from the 10th to the 30th minute of reperfusion, 2-ml samples of perfusion fluid and of glycogen in the rats' heart were determined by enzymic methods [2, 4, 5]. The blood ethanol level was determined by gas chromatography [6]. For histological investigation, the hearts of 10 rats of group 3, 8 days after the last injection of ethanol, were fixed in formalin, and frontal sections were embedded in paraffin wax. The dewaxed sections were stained with hematoxylin and eosin.

Laboratory of Pharmacology and Toxicology of Alcohol, V. P. Serbskii All-Union Scientific-Research Institute of General and Forensic Psychiatry, Moscow. Department of Pathological Physiology, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Morozov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 5, pp. 575-578, May, 1986. Original article submitted April 4, 1985.

 0007-4888/86/0005-0630$12.50

TABLE 1. PSP, HR, TTI, and CF of Isolated Rat Heart at Different Times after a Single Injection of Ethanol (group 1), and Chronic (3 months, group 2) and Forced (5 days, group 3) Alcohol Loading

Group of animals	Experimental conditions	Number of animals	Duration of reperfusion, min							
			30				50			
			PSP, mm Hg	HR, beats/min	TTI	CF, ml/min	PSP, mm Hg	HR, beats/min	TTI	CF, ml/min
1.	Control	8	132±5	229±7	1,55±0,05	17,6±0,9	126±7	234±11	1,51±0,11	16,7±1,2
	after injection of ethanol:									
	3—4 h	8	136±3	244±9	1,55±0,06	22,5±1,2*	139±4	244±11	1,61±0,10	21,8±1,5*
	1 day	6	138±5	257±9*	1,72±0,09	22,8±1,4*	140±5	265±6*	1,73±0,09	21,2±0,8*
	3 days	8	130±4	232±9	1,50±0,07	19,1±1,2	126±5	236±12	1,45±0,07	18,4±1,1
2.	Control	8	133±8	225±10	1,50±0,14	23,8±1,2	124±7	229±8	1,40±0,15	22,1±1,6
	after stopping access to ethanol									
	3—4 h	8	124±7	238±9	1,40±0,11	21,8±0,9	114±6	236±6	1,30±0,09	20,9±1,5
	1 day	8	122±6	229±11	1,38±0,12	18,7±1,5*	111±7	216±6	1,16±0,06	15,7±1,7*
	3 days	8	125±3	214±10	1,33±0,07	24,0±1,5	125±3	229±8	1,39±0,06	21,9±1,6
3.	Control	11	132±5	229±7	1,56±0,05	17,6±0,9	126±7	234±11	1,51±0,11	16,7±1,2
	after last injection of ethanol									
	3—4 h	8	124±5	225±8	1,41±0,08	17,6±0,8	124±6	229±11	1,45±0,10	17,9±1,0
	1 day	8	112±6*	231±6	1,13±0,05*	16,4±1,6	104±7*	236±7	1,09±0,06*	15,0±1,1
	3 days	8	126±6	191±17*	1,06±0,13*	14,2±0,8	124±5	208±11	1,06±0,07*	14,8±0,8
	6 days	8	127±7	255±12	1,58±0,11	19,9±1,3	122±5	259±12	1,54±0,10	18,1±0,9

Note. Here and in Table 2: *) $p < 0.05$ compared with corresponding control. TTI expressed in mm Hg·sec/min·1000.

TABLE 2. Glucose Intake and Lactate Release of Isolated Heart and Glycogen Concentration in Heart of Rats at Different Times After Single Injection of Ethanol (group 1) and Chronic (3 months, group 2) and Forced (5 days, group 3) Alcohol Loading

Group of animals	Experimental conditions	Number of animals	Glucose uptake, μ moles/g dry weight of tissue	Lactate excretion	Glycogen, μmoles glucose/g dry weight of tissue
1.	Control	8	84±12	17±3	—
	After injection of ethanol				
	3—4 h	7	74±6	17±4	—
	1 day	6	75±7	16±5	—
	3 days	8	80±15	17±2	—
2.	Control	8	92±8	28±6	54±4
	After stopping access to ethanol:				
	3—4 h	8	130±8*	23±3	59±6
	1 day	8	114±9	32±3	66±7
	3 days	8	101±10	38±5	67±7
3.	Control	8	84±12	17±3	51±6
	After last injection of ethanol:				
	3—4 h	8	102±18	24±3	16±2*
	1 day	8	176±24*	36±3*	63±7
	3 days	8	121±14	24±3	34±4*
	6 days	8	133±15*	22±5	88±6*

Note. Glucose uptake and lactate excretion estimated during period from 10th to 30th minutes of perfusion; glycogen in heart tissue was determined on animals of a separate group.

RESULTS

No withdrawal reaction was observed in the rats of group 1. Some rats of group 2 showed isolated signs of a weak withdrawal reaction. Clear signs of a withdrawal reaction [1] were observed in the animals of group 3 and they reached a peak of severity 18-24 h after the last injection of ethanol. The ethanol concentration in the blood 3-4 h after a single injection (group 1) and the last injection (group 3) was 113 ± 10 and 101 ± 12 mM, falling after 24 h to under 2 mM. In the rats of group 2, during alcohol loading the blood ethanol concentration at different times of day (9 a.m. and 5 p.m.) did not exceed 5 mM. In the rats of groups 1 and 2, disturbances of contractility of the heart were not discovered at any time

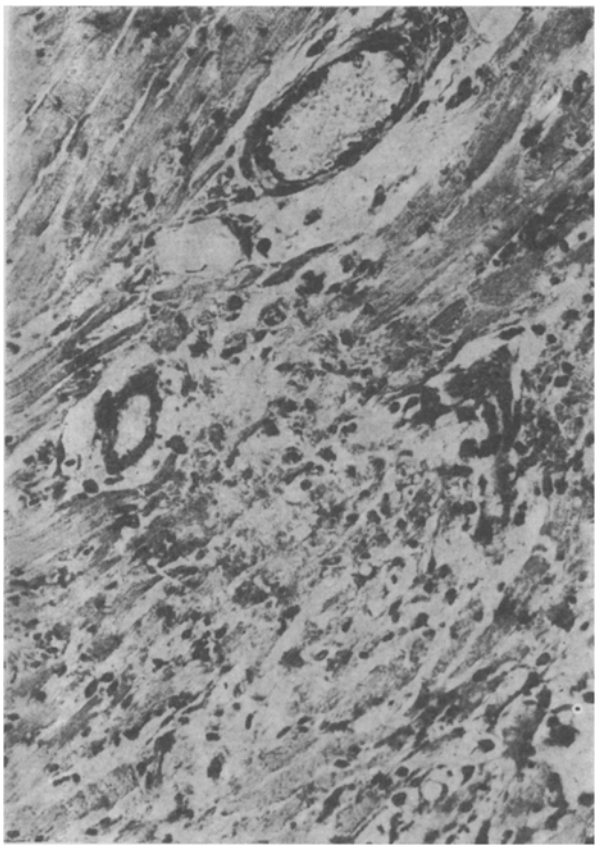

Fig. 1. Perivascular area of necrosis. Hematoxylin-eosin, 500×. Explanation in text.

of the investigation (Table 1). In the rats of group 3, 24 h after the last injection of ethanol, at the time of greatest severity of the withdrawal reaction, PSP and TTI were reduced by 15 and 28% respectively. On the 3rd day after the last injection of ethanol, TTI remained low due to slowing of the heart. By the 6th day no disturbance of contractility or rhythm of the heart could be found.

The glucose uptake and lactate release by the heart in rats of group 1 were unchanged at all times after injection of ethanol (Table 2). In the animals of group 2, the glucose uptake, 3-4 h after access to ethanol had stopped, was raised by 41%, and it fell to the control level 24 h later. The rate of lactate release was unchanged. No significant changes in the glycogen level in the heart were observed under these circumstances.

The glucose uptake and lactate release by the heart of rats of group 3, 3-4 h after the last injection of ethanol were the same as in intact rats. After 1 day the glucose uptake was doubled, after 3 days it had fallen to the control level, and after 6 days it showed a further rise. The increased glucose uptake 1 day after the last injection of ethanol was accompanied by increased lactate release. The glycogen concentration in the myocardium of the rats of group 3, used in the experiments 3-4 h after the last injection of ethanol, was reduced by 70%, it returned to normal after 1 day, fell again on the 3rd day, and rose sharply on the 6th day of the investigation (Table 2).

Histologically, single and multiple confluent necrotic areas, perivascular in distribution, were discovered in the hearts of the rats of group 3 (Fig. 1). Thus acute ethanol intoxication is not reflected in the state of contractility or carbohydrate metabolism of the isolated rat heart. Long-term ethanol consumption by rats under free choice conditions likewise does not lead to the development of disturbances of cardiac contractility. The increase in glucose uptake by the heart of these animals, in the absence of changes in lactate release, is evidence of activation of glycolysis without any significant disturbances of biological oxidation, in agreement with data obtained by other workers [7, 10]. The ethanol withdrawal reaction, which develops after intensive alcohol loading of short duration, leads to a disturbance of the contractile function of the heart and of its rhythm.

Changes arising in carbohydrate metabolism under these circumstances are similar to those found when the energy metabolism of the myocardium is disturbed [13]. A fact of fundamental importance is that disturbances of cardiac activity develop in the postintoxication period, when the ethanol concentration in the body had fallen to its background level. The morphological substrate for these disturbances consists of disseminated necrotic foci in the myocardium. After a single injection of ethanol in a sublethal dose, and after chronic alcohol loading of rats, no necrotic lesions can be found in the heart [3, 8].

These results are evidence of the important role of the postintoxication syndrome in the pathogenesis of AMC. It seems probable that the unsuccessful attempts to produce experimental models of AMC [8, 9] are due to the use of continuous alcohol loading of the animals and the absence of periodic withdrawal reactions.

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