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ROLE OF CATECHOLAMINES IN THE MECHANISM OF HEART DAMAGE IN RATS WITH ALCOHOL WITHDRAWAL SYNDROME

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UDC 616.127-092.9-02:/615.917:547.
262/.015.156

KEY WORDS: alcohol withdrawal syndrome; isolated rat heart; creatine phosphokinase; lactate dehydrogenase; catecholamines.

It is generally accepted that the development of alcohol cardiomyopathy is due primarily to the toxic action of ethanol. Meanwhile experiments on rats have shown that marked disturbances of the contractile function and carbohydrate metabolism of the heart arise, not at the height of alcohol intoxication, but after the action of ethanol has ended, namely in the period of development of the withdrawal syndrome [7]. Because of activation of the sympathicoadrenal system observed in the alcohol withdrawal syndrome [3, 11], catecholamines (CA) can be regarded as one possible pathogenetic factor of the disturbance of cardiac activity.

The aim of this investigation was to study the rate of disappearance of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) from the perfused heart and the histochemical determination of CA in the rat heart at various times after intensive alcoholization, leading to the development of a withdrawal syndrome.

EXPERIMENTAL METHOD

Experiments were carried out on 82 noninbred male albino rats aged 2-3 months. The alcohol withdrawal syndrome was produced by injecting a 25% solution of ethanol in a dose of 4-5 g/kg body weight into the stomach at intervals of 12 h for 5 days [1]. The animals were killed by decapitation 3-6 h and 1, 2, 3, and 7 days after the last injection of ethanol.

TABLE 1. Rate of Disappearance of Enzymes from the Perfused Rat Heart at Different Times after Alcohol Withdrawal

Time after last injection of ethanol (n =7)	CPK	LDH
Control	0,732±0,053	0,609±0,085
2—6 h	1,086±0,192	0,546±0,085
1 day	1,212±0,205*	0,685±0,109
2 days	1,321±0,154*	0,820±0,103
3 days	1,809±0,081*	1,028±0,068*
7 days	1,728±0,153*	0,797±0,121

Legend. Here and in Table 2: *$p < 0.05$ compared with control. n) Number of animals in each period of observation.

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TABLE 2. Quantitative Estimation of Density of CA-Containing Structures in Rat Heart at Different Times after Alcohol Withdrawal

Time after last injection of ethanol (n = 8)	Character of distribution of CA-containing structures	
	Density of Ca-containing fibers, %	Area of CA-containing structures distributed diffusely (outside fibers, %
Control	86,0±11,4	2,0±0,2
2–6 h	64,0±4,5	19,0±3,4*
1 days	29,0±6,2*	46,0±5,6*
3 days	7,0±0,9*	82,0±4,8*
7 days	38,2±6,5*	4,0±1,2

Legend. Density (area) of CA-containing structures expressed as ratio of number of points of stereometric grid coinciding with fibers containing CA or with zones of fluorescence of CA distributed diffusely outside fibers, to total number of points. Sharp decrease in density of CA-containing fibers on 3rd day after last injection of ethanol could be connected with high intensity of fluorescence of CA located extraneuronally, which masks fluorescence of CA in nerve fibers.

Intact rats served as the control. The rats' hearts were isolated by Langendorff's method and perfused with Krebs–Henseleit solution, saturated with carbogen, at 36.8°C (pH 7.4). The coronary flow rate was 13 ml/min. After 10 min of continuous perfusion of the heart, the change was made to reperfusion, with recirculation of 35 ml of solution, which continued for 30 min. Activity of LDH [4] and CPK [6] was then determined in the recirculating fluid and the heart was dried to constant weight. The rate of disappearance of the enzymes from the heart was calculated in International Units (U) per gram dry weight of heart during reperfusion for 30 min. The histochemical investigation of CA in the heart was carried out on a separate group of animals by the glyoxal method [8]. Sections were studied and photographed by means of a Polyvar luminescence microscope ("Reichert," Austria). The density of CA-containing fibers and the area of diffuse (outside the fibers) distribution of CA were estimated quantitatively separately by a stereometric method [2], the number of points of the stereometric grid which coincided with CA-containing fibers or with zones of diffuse distribution of CA being expressed as percentages. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The animals 3-6 h after the last injection of ethanol were in a state of marked ataxia. On recovery from the state of alcohol intoxication the rats developed signs of an alcohol withdrawal syndrome, which reached their maximum 20-25 h after the last injection of ethanol and disappeared on the 2nd day.

The rate of disappearance of CPK increased after 1 day, reached a maximum on the 3rd day, and remained high on the 7th day after alcohol withdrawal (Table 1). An increase in the rate of disappearance of LDH from the heart was observed on the 2nd day and reached a maximum also on the 3rd day after the last injection of ethanol into the rats. The maximal increase in the rate of disappearance of CPK and LDH compared with the control was 131 and 69% respectively.

Investigation of the character of distribution of CA-containing structures showed (Fig. 1; Table 2) that the density of CA-containing nerve fibers fell sharply after alcohol withdrawal, to reach a minimum on the 3rd day, whereas the change in area of fluorescence of

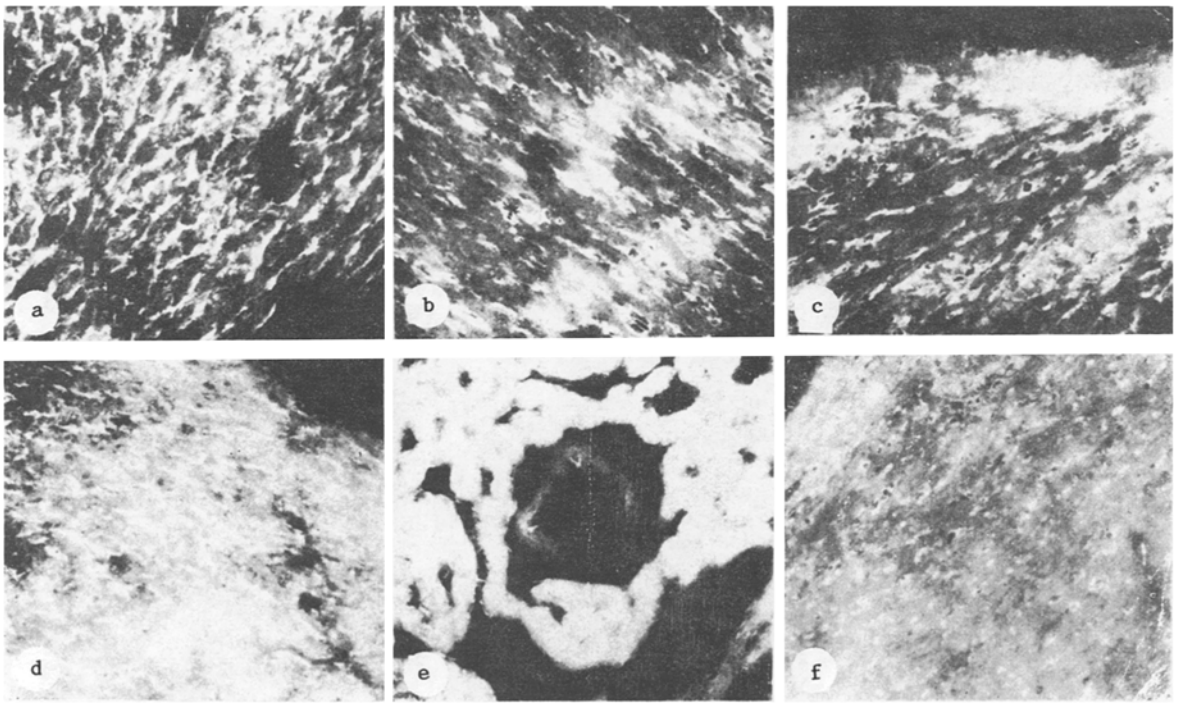

Fig. 1. Distribution of CA-containing structures in rat heart after alcohol withdrawal (reaction with glyoxalic acid). a) CA-containing nerve fibers in intact rat heart (250×); b) small foci of CA diffusion from nerve fibers into cardiomyocytes 2-6 h after alcohol withdrawal (250×); c) multiple foci of extraneuronally located structures containing CA (1st day after alcohol withdrawal, 250×); d) multiple foci of extraneuronally located structures containing CA (3rd day after alcohol withdrawal); e) perivascular cardiomyocytes containing CA (3rd day of withdrawal, 250×); f) appearance of CA-containing neuronal structures (7th day after alcohol withdrawal, 250×).

extraneuronally located CA was directly opposite in character. Small foci of diffusion of CA from nerve fibers into cardiomyocytes were found 2-6 h after the last injection of ethanol (Fig. 1b). The area of extraneuronal fluorescence of CA was significantly increased on the 1st day (Fig. 1c), to reach a maximum on the 3rd day (Fig. 1d, e), and was reduced on the 7th day after alcohol withdrawal (Fig. 1f). The most characteristic feature was accumulation of CA in cardiomyocytes with subendocardial (Fig. 1c) and perivascular locations and in the walls of the large branches of the coronary vessels of the heart (Fig. 1e).

The more rapid disappearance of the enzymes from the heart in the course of perfusion is evidence of disturbance of the integrity of the cell membranes and destruction of myocardial myocytes. The increase in the rate of their disappearance at a time when alcohol had been completely eliminated from the body [1] indicates that the development of heart damage is not attributable to the direct action of ethanol or its metabolites on the myocardium.

The synchronized change in the character of distribution of CA in the heart and the rate of disappearance of the enzymes suggests the high probability of involvement of catecholamines in the genesis of the myocardial damage in the alcohol withdrawal syndrome. The absence of any marked disturbances in the heart at the height of alcohol intoxication is evidently due to the ability of ethanol to protect the myocardium against the necrosis-inducing action of CA [5, 10].

On the basis of these results it is thus possible to assess the cause of the extrasystoles, atrial fibrillation, and raised blood CPK activity in alcoholic patients with an alcohol withdrawal syndrome [9, 12, 13].

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CARBON ISOTOPE FRACTIONATION IN ATHEROSCLEROTIC HUMAN TISSUE

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UDC 616.13-004.6-073.916:546.26.02.
13/-033

KEY WORDS: fractionation of isotopes; carbon; atherosclerosis; prostaglandin E; cAMP.

Biological fractionation of isotopes [4] implies that in living organisms there is a higher proportion of the ^{12}C isotope than in the carbon dioxide of the air. This phenomenon is connected with what is called the normal kinetic isotope effect, in accordance with which the velocity of chemical reactions increases with the participation of lighter isotopes. In biochemical enzymic reactions thermodynamic isotope effects also may arise [1], in connection with the accumulation of heavy isotopes during an increase in energy of the chemical bond. It can be postulated that analysis of isotope distributions in the tissues under normal and pathological conditions may shed some light on the molecular mechanisms of onset of pathological states.

Sensitivity of biological fractionation of carbon isotopes during aging and also in atherosclerosis and senile cataract, was found for the first time in the investigation described below. According to the generally accepted view, the sclerotic process is connected with general slowing of biochemical processes, and we accordingly postulated that during its development a tendency will be observed for the excess of the ^{12}C isotope in sclerotic tissues to be below normal.

EXPERIMENTAL METHOD

Autopsy material was taken from the abdominal aorta, both unchanged areas and also areas showing various stages of atherosclerotic changes in the arterial wall. An adipose strip — a region of the aortic wall with lipoidosis measuring 1 × 2 mm — was excised with all layers of the aortic wall. Fibrous plaques — the aortic wall corresponding to the site of the lesion — also was excised with all its layers. Atheromatous plaques with ulcers — a fragment of the aortic wall — were excised at the center of atheromatous ulceration, with involvement of the aortic wall to different depths. Thus the aortic material for testing could be subdivided in accordance with the generally accepted classification of the atherosclerotic process. A native specimen weighing about 0.1 g was placed in a quartz cuvette and dried in vacuo at room temperature, then introduced into a circulation reactor for oxidation in pure oxygen. The carbon dioxide thus obtained was purified cryogenically to remove impurities and introduced

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 0007-4888/87/0009-1218$12.50