CATECHOLAMINES IN THE MECHANISM OF THE CARDIONECROTIC ACTION OF ACETALDEHYDE

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UDC 616.127-002.4-02:615.31:547.281.2].015.21:615.357.452-092.9

KEY WORDS: acetaldehyde; catecholamines; micronecroses of the myocardium.

Damage to heart muscles developing in rats with chronic alcohol poisoning is accompanied by the accumulation of catecholamines in the heart [11]. This may be connected with the action of acetaldehyde, which liberates catecholamines and has a stimulating effect on the heart [7, 8, 10]. The possibility cannot be ruled out that the cardiotoxic action of ethanol, which lies at the basis of the development of alcoholic cardiomyopathy, is largely due to the unfavorable influence of acetaldehyde and catecholamines on the myocardium.

The object of this investigation was to study the role of catecholamines in the realization of the cardionecrotic action of acetaldehyde in experiments *in vivo* and on an isolated heart preparation. The latter would rule out effects connected with the possible stimulation of catecholamine release from the chromaffin tissue of the adrenals.

EXPERIMENTAL METHOD

Experiments were carried out on 170 noninbred male rats aged 2-3 months and weighing 200-300 g.

Acetaldehyde was injected intraperitoneally as a 2% solution in physiological saline at 6-8°C, once in a dose of 200 mg/kg or 4 times, at intervals of 15 min, in a total dose of

TABLE 1. Effect of L-dopa, L-α-methyldopa, and α-Methyl-p-tyrosine on Cardionecrotic Effect of Acetaldehyde Inhaled in a Dose of 30 mg/liter

Control groups and number of animals	Percent of necroses	Experimental group and number of animals	Percent of necroses
Intact rats (6)	0,6±0,1	Acetaldehyde (8)	3,6±0,4*
L-dopa (6)	1,8±0,3	Acetaldehyde preceded by L-dopa (7)	7,6±1,3*
L-α-methyldopa (6)	0,9±0,3	Acetaldehyde preceded by L-α-methyldopa (8)	1,3±0,3
α-Methyl-p-tyrosine (6)	1,5±0,2	Acetaldehyde preceded by α-methyl-p-tyrosine (7)	1,4±0,1

Legend. 1) Here and in Table 2, percent of necroses indicates ratio of number of points of stereometric grid coinciding with areas of necrosis to total number of points (in %). 2) *P < 0.05 compared with the control.

Laboratory of Pharmacology of Alcohol, V. P. Serbskii All-Union Research Institute of General and Forensic Psychiatry, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 12, pp. 65-67, December, 1982. Original article submitted June 29, 1982.

 0007-4888/82/9412-1696$07.50

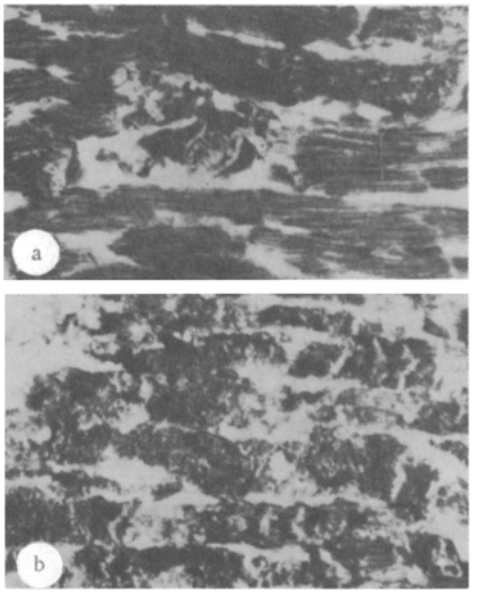

Fig. 1. Micronecroses of the myocardium. Reaction for SDH, 200 ×: a) single injured cells against the background of linear deposits of formazan, characteristic of intact myocardium (inhalation of acetaldehyde in a dose of 30 mg/liter for 1 h); b) focus of injury (administration of adrenalin after intraperitoneal injection of acetaldehyde in a dose of 200 mg/kg). Sections counterstained with eosin.

800 mg/kg, or it was given by inhalation for a period of 60 min, by spraying into the air of the chamber in a dose of 30 mg/liter. Adrenalin hydrotartrate was injected subcutaneously in a dose of 2 mg/kg. L-dopa and L-α-methyldopa were dissovled in 1 N HCl, neutralized with 1 N NaOH to pH 5.0, and injected in a dose of 120 mg/kg body weight; α-methyl-p-tyrosine was suspended in 1% starch gel and injected in a dose of 250 mg/kg. These compounds were given once only, by intraperitoneal injection in a volume of 1 ml/kg body weight, at various times before administration of acetaldehyde. The animals were decapitated 20 h after injection of acetaldehyde or adrenalin.

In experiments on the isolated heart (perfusion with Krebs' solution by Langendorff's method [5]) acetaldehyde was added to the perfusion fluid in concentrations of 1, 3, and 6 mM. The reservoir containing the Krebs' solution to which the acetaldehyde was added was cooled to 8-10°C to prevent evaporation. The duration of perfusion after rinsing the heart for 5 min was 70 min. The temperature of the solution on reaching the heart was 37°C. In some experiments between 10 and 20 min after the beginning of perfusion the heart was perfused with a solution containing, besides acetaldehye, adrenalin in a concentration of 5 or 40 μg/ml.

To discover injuries to the myocardium, the reaction with nitro-BT for succinate dehydrogenase (SDH) activity was carried out on frozen sections 10 μ thick [9]. The degree of injury in the subendocardial layer of the left ventricle and at the base and apex of the heart was estimated quantitatively by a stereometric method [1] under a magnification of 200 times, and the relative volume of the cardiac lesions was calculated in percent. In experiments on the isolated heart the rate of release of lactate dehydrogenase on the heart also was estimated (spectrophotometrically).

EXPERIMENTAL RESULTS AND DISCUSSION

Administration of acetaldehyde to the rats by inhalation led to an increase in the number of damaged cells in the myocardium (3.6 ± 0.4% compared with 0.6 ± 0.1% in the control). These cells were characterized by changes in the appearance of the formazan deposits, which now consisted of alternation of regions of intense, diffuse staining of the cytoplasm with colorless regions, giving them a "speckled" appearance (Fig. 1). In some myocytes the reaction for SDH had almost completely disappeared. Changes of this sort usually are found in

TABLE 2. Effect of Acetaldehyde and Cardionecrotic Effect of Adrenalin during Perfusion of the Isolated Heart

Experimental conditions	Number of observations	Percent of necroses (M ± m)	Rate of release of LDH from the heart, Units/70 min perfusion (M ± m)
Control perfusion	8	1,2±0,2	0,469±0,063
Adrenalin 5 μg/ml	8	—	1,049±0,074
The same, preceded by acetaldehyde in concn. of:			
3 mM	7	—	1,012±0,091
6 mM	8	—	0,902±0,107
Adrenalin 40 μg/ml	8	4,5±0,7	1,517±0,116
1 mM	7	4,5±0,6	1,462±0,103
3 mM	7	4,3±0,7	1,404±0,127
6 mM	8	4,4±0,5	1,417±0,114

stress or as a result of the action of toxic doses of adrenalin, they are irreversible, and they reflect a state of necrobiosis [2, 6]. The damaged cells were located mainly at the base or apex of the heart and in the subendocardium of the left ventricle. Similar lesions also were found after four injections of acetaldehyde in a total dose of 800 mg/kg (3.7 ± 0.9% compared with 1.3 ± 0.1% in the control). Acetaldehyde, injected as a single dose of 200 mg/kg, had no necrosis-inducing action (1.7 ± 0.3% compared with 1.4 ± 0.2% in the control).

Injection of adrenalin in a toxic dose, such as is usually used to model catecholamine lesions of the heart, led to a massive focus of necrosis in the myocardium (12.0 ± 1.7%). Preliminary (5 min beforehand) intraperitoneal injection of acetaldehyde in a dose of 200 mg/kg potentiated the necrosis-inducing action of adrenalin (18.6 ± 2.0%).

In the experiments with inhalation of acetaldehyde (Table 1) preliminary injection of L-dopa 30 min before the experiment was found to potentiate the cardionecrotic action of acetaldehyde. Injection of L-α-methyldopa, on the other hand, in the same dose prevented the development of myocardial injuries induced by acetaldehyde. A similar effect was found when α-methyl-p-tyrosine was injected 6 h before inhalation.

The character of spontaneous (also observed in the control) injuries to the myocardium in the isolated heart preparation was the same as in the experiments *in vivo*. Acetaldehyde, even in a concentration of 6 mM, known to be definitely higher than the blood concentration in experiments *in vivo* (1.68 ± 0.28 mM), had no necrosis-inducing action. Perfusion with solutions containing adrenalin in concentrations of 5 and 40 μg/ml caused a lesion of the heart whose volume depended on the adrenalin concentration (Table 2). Simultaneous perfusion with solutions containing adrenalin and acetaldehyde in concentrations of 1, 3, and 6 mM did not change the volume of the myocardial lesions induced by adrenalin alone.

The necrosis-inducing action of acetaldehyde was potentiated by injection of the catecholamine precursor L-dopa and, conversely, it was prevented by injection of L-α-methyldopa and α-methyl-p-tyrosine, which have a sympatholytic action. Injection of acetaldehyde in a dose not causing the development of necrosis significantly enhanced the cardionecrotic effect of adrenalin. Meanwhile, as shown by the experiments on the isolated heart, the volume of myocardial lesions induced by adrenalin was unchanged in the presence of acetaldehyde. Acetaldehyde itself, under these same conditions, likewise did not induce necrosis. Acetaldehyde thus does not directly affect realization of the pathological action of adrenalin. Its toxic effect is probably realized by stimulation of liberation of biogenic amines from neurons and chromaffin tissue. The phenomenon of activation of the sympathico-adrenal system in alcohol poisoning, accompanied by elevation of the blood catecholamine level and by accumulation of catecholamines in the heart muscle, is well known [3, 4, 11]. The presence of acetaldehyde under conditions of chronic alcohol poisoning may evidently facilitate the accumulation of high concentrations of catecholamines in the myocardium, causing the development of micronecroses, and may thereby contribute to the formation of cardiosclerosis, hypertrophy of the myocardium, and other manifestations of alcoholic cardiomyopathy.

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