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INJURY TO THE ISOLATED HEART BY ADRENALIN DURING SALINE PERFUSION

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UDC 615.357.452.015.4:612.172.5

Perfusion of the isolated heart with Krebs' solution containing adrenalin in concentrations of 5 and 20 μg/ml induced micronecroses of the cardiocytes. Perfusion with adrenalin in a concentration of 0.5 μg/ml did not cause micronecroses. Dispersion analysis revealed a statistically significant effect of high concentrations of adrenalin on the intensity of the cardionecrotic effect. The fact that micronecroses develop in the isolated heart when perfused with saline raises doubts about the leading role of the blood factor in the realization of the cardionecrotic effect of catecholamines. The appearance of necroses during exposure to adrenalin in concentrations activating the mechanism of amine uptake by the cardiac muscle cells is evidence of a causal connection between the accumulation of biogenic amines by the myocytes and the development of necrosis in them.

KEY WORDS: isolated heart; adrenalin injury to myocardium; micronecroses of the myocardium

The mechanism of the cardionecrotic action of high concentrations of catecholamines remains unclear. Their damaging effect on the myocardium is exerted against the background of the numerous effects of these agents both in the heart itself and at the level of the whole body [3, 9, 10]. The aim of the present investigation was to study the possible direct harmful action of adrenalin on the myocardium during perfusion of the isolated heart.

EXPERIMENTAL METHOD

Experiments were carried out on 34 noninbred male albino rats weighing 180-200 g. The animals were anesthetized with urethane (1 g/kg, intraperitoneally) with the addition of heparin (5,000 units/kg) and the heart was removed and perfused by Langendorf's method with Krebs' solution in the modification of Neely et al. [13], under a pressure of 70 mm Hg and at 37°C and pH 7.4. The composition of the perfusion solution was as follows (in mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 2.5, $NaHCO_3$ 25, Na_2-EDTA 0.5, and glucose 5.6. Ascorbic acid was added to the solution (20 mg/liter) which was aerated with a mixture of oxygen (95%) and CO_2 (5%). The acting concentrations of adrenalin were chosen after calculating concentrations activating the neuronal (0.5 μg/ml) and extraneuronal (5 and 20 μg/ml) uptake of catecholamines [11]. The last two concentrations also correspond to those found in the blood of experimental animals after injection of the cardiotoxic doses of adrenalin usually used (1-100 mg/kg body weight) [2, 4, 7, 9].

The heart was perfused for 5 min with Krebs' solution and then for 10 min with solutions containing adrenalin hydrochloride in final concentrations of 0.5, 5, and 20 μg/ml (calculated as the base), after which it was again perfused with the original solution for 1.5, 3, or 6 h. To all experiments there was a parallel control in which the heart was perfused without adrenalin. At the end of the experiment the heart was stopped in cold (4°C) Krebs' solution, cut into halves in the frontal plane, and frozen in liquid nitrogen. To detect damage to the myocardium, the reaction with nitro-BT for succinate dehydrogenase activity, after Nachlas et al., was carried out on frozen sections 10 μ thick. Lipids were stained with Oil Red 0 in one of the serial sections.

Department of Pathological Physiology, Patrice Lumumba Peoples' Friendship University, Moscow. Department of Geographic Pathology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 12, pp. 682-684, December, 1977. Original article submitted February 8, 1977.

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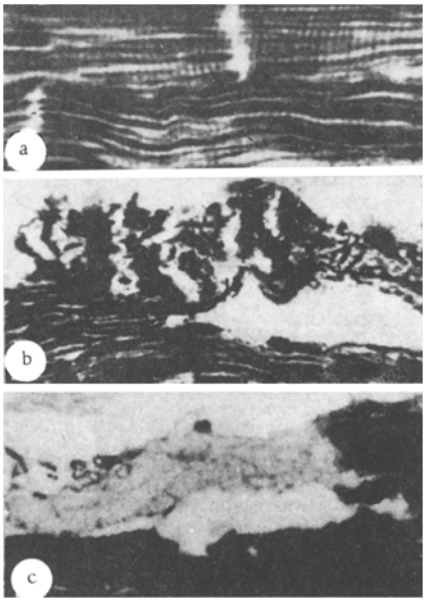

Fig. 1. Succinate dehydrogenase activity in myocytes of isolated heart, 400×. a) Control heart. Perfusion for 3 h without adrenalin. Activity marked mainly by linear deposition of formazan; b) perfusion for 3 h with adrenalin (10 min, 20 μg/ml). Distinctive and irregular deposition of formazan in injured cell; c) perfusion for 12 h with adrenalin (10 min, 20 μg/ml). Negative reaction with nitro-BT in necrotic cell. Section counterstained with eosin.

The degree of injury in the subendocardial and subpericardial layers and at the base and apex of the left ventricle was assessed quantitatively by a stereometric method [1] under a magnification of 200 times, by calculating the relative volume of areas of myocardial damage as a percentage of the total. The numerical indices were subjected to statistical analysis. Significance of differences between mean values was determined by Fisher's criterion using the ψ method [6]. The effect of the perfusion time and dose of adrenalin on the degree of myocardial damage was assessed by dispersion analysis, by determining the index of strength of the effect.

EXPERIMENTAL RESULTS

In all hearts, both control and experimental, the reaction for succinate dehydrogenase revealed high activity of the enzyme, with mostly a linear pattern of formazan deposition characteristic of the intact myocardium (Fig. 1a). After perfusion for 6 h the intensity of the reaction was a very little weaker. In the damaged myocytes changes in the type of deposition of the reaction product were observed, although it was always uniform in character. They consisted of the appearance of single cells or groups of cells in which areas of deep, diffuse staining of the cytoplasm alternated haphazardly with colorless areas, giving the myocardium a "variegated" appearance (Fig. 1b). Such cells were located mainly in the subendocardial layer of the left ventricle and much less frequently in other parts of the heart, especially the right ventricle. The intensity of the reaction with nitro-BT was the same in all damaged myocardiocytes. As several workers have shown [6, 9], the cells described above are in a state of necrobiosis. The irreversibility of the injuries discovered under these experimental conditions was confirmed by an additional experiment in which two hearts, after treatment with adrenalin (20 μg/ml), were perfused for 12 h. Complete disappearance of the reaction for succinate dehydrogenase was observed in these cells under these circumstances (Fig. 1c).

Dispersion analysis of the histostereometric data did not reveal any influence of the perfusion time factor on the severity of injury ($P > 0.05$) and, for that reason, the results showing how the cardionecrotic effect depended on the dose of adrenalin included pooled results obtained at different stages of perfusion. In some of the control hearts (six of 11) solitary "variegated" cells were found. The mean size of the areas of myocardial injury in the rats of the control group was $0.08 \pm 0.02\%$. Perfusion with adrenalin in a dose of 0.5 μg/ml showed the almost complete absence of injuries ($0.05 \pm 0.03\%$) and their volume was not significantly different from the control ($P > 0.05$). Adrenalin, in a dose of 5 μg/ml, caused a sharp and statistically significant ($P < 0.001$) increase in the degree of injury compared with the control and with the dose of 0.5 μg/ml. This effect was more

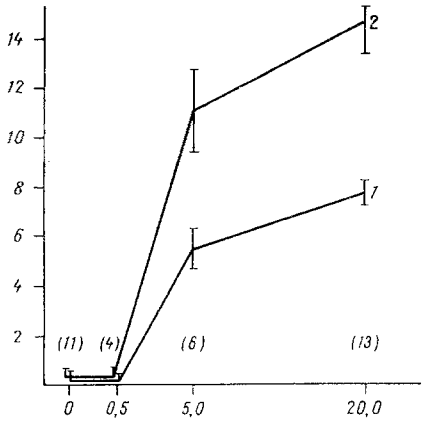

Fig. 2. Cardionecrotic effect as a function of adrenalin concentration in perfusion fluid. Abscissa, adrenalin concentration (in μg/ml); ordinate, ratio (in %) of number of necroses coinciding with points of the stereometric grid to total number of points (P%), with indication of confidence interval (at $P < 0.001$). 1) P% overall in all parts of myocardium of left ventricle; 2) P% in subendocardium. Number of hearts shown in parentheses.

marked still with a dose of 20 μg/ml (Fig. 2). It is worth noting that the intensity of injury to the subendocardial zone correlated with the extent of the damage to the heart as a whole (Fig. 2), so that the damaging effect of adrenalin could be assessed by examination of the subendocardial zone alone. Dispersion analysis revealed the statistically significant effect of high concentrations of adrenalin (5 and 20 μg/ml) on the intensity of the cardionecrotic effect ($P < 0.001$). Staining for lipids in the muscle fibers was always negative.

The "variegated" necrotic cells described above are analogous to those found in the human myocardium after stress [8] and in experimental animals after injection of a cardiotoxic dose of catecholamines [2, 5, 7]. The character of the localization of the micronecroses in the subendocardial zone is typical of irreversible metabolic injuries arising in vivo and can be explained by the functional vulnerability of this part of the myocardium [9]. The very low background level of necroses in some animals in the control series was probably connected with differences in individual sensitivity of rats to the experimental manipulations preceding perfusion.

A special feature of the micronecroses in these experiments was their simultaneous onset, whereas in vivo micronecroses appear at various times up to 48 h after injection of adrenalin [2]. This simultaneous appearance was confirmed by the uniform intensity of the tissue enzymic reaction in the injured cells and by the independence of the size of the lesions of the duration of perfusion. On the basis of this distinguishing feature the effect of the procedure, since it occurred synchronously, could be assessed quantitatively.

Another distinguishing feature of the adrenalin injury arising in the isolated heart was the absence of neutral lipids in the muscle cells detectable histologically, and an invariable accompaniment of the action of adrenalin on the intact organism [2, 5, 7]. This feature can be explained by the absence of lipids, including nonesterified fatty acids, in the perfusion fluid. The role of the plasma lipids in the depression of intracellular oxidative processes [2, 7] and in the onset of the phenomenon of fatty degeneration during hypercatecholaminemia in vivo becomes clearly demonstrable in the light of these findings.

An important role in the mechanism of the micronecrosis-producing effect of the catecholamines is known to be played by circulatory disturbances [10]. The cardiotoxic effect of the catecholamines found during perfusion of a heart–lung preparation with blood containing high concentrations of amines [12] means that the effect of the blood factor on the appearance of injuries to myocardiocytes cannot be ruled out.

The fact that typical micronecroses arise in the isolated heart during its perfusion with saline casts doubt on the role of blood factors in the mechanism of the cardionecrotic effect of the catecholamines. The

appearance of micronecroses under the influence of adrenalin in concentrations activating the mechanism of amine uptake by the heart muscle cells is evidence of a causal connection between the accumulation of catecholamines by the myocytes and the development of necrosis by these cells.

The authors are grateful to Candidate of Medical Sciences L. V. Kakturskii for advice with the statistical analysis of the results.

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