Injury to the Isolated Heart by Adrenalin During Saline Perfusion

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Perfusion of the isolated heart with Krebs' solution containing adrenalin in concentrations of 5 and 20 \( \mu g/ml \) induced micronecroses of the cardiocytes. Perfusion with adrenalin in a concentration of 0.5 \( \mu g/ml \) 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, KH2PO4 1.18, MgSO4 1.18, CaCl2 2.5, NaHCO3 25, Na2-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 CO2 (5%). The acting concentrations of adrenalin were chosen after calculating concentrations activating the neuronal (0.5 \( \mu g/ml \)) and extraneuronal (5 and 20 \( \mu g/ml \)) uptake of catecholamines [11]. The last two concentrations also correspond to those found in the blood of experimental animals after injection of the cardotoxic 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 \( \mu g/ml \) (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 \( \mu \) thick. Lipids were stained with Oil Red 0 in one of the serial sections.
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 \( \chi^2 \) 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 \( \mu \)g/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 ± 0.02%. Perfusion with adrenalin in a dose of 0.5 \( \mu \)g/ml showed the almost complete absence of injuries (0.05 ± 0.03%) and their volume was not significantly different from the control (\( P > 0.05 \)). Adrenalin, in a dose of 5 \( \mu \)g/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 \( \mu \)g/ml. This effect was more