DO CATECHOLAMINES CAUSE DAMAGE TO THE MYOCARDIUM THROUGH HYPERSTIMULATION OF β-RECEPTORS?

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Hypercatecholaminemia is known to lead to the development of irreversible damage to the myocardium. The mechanism of this damage has not yet been explained, although it has been suggested that it is effected through excessive β-adrenergic stimulation [6, 7, 10]. Noradrenalin (NA), adrenalin (A), and isoproterenol (IP) give rise to different β-stimulating effects, and in this series IP is the most powerful β-agonist.

The aim of this investigation was to compare the harmful action of catecholamines differing in their affinity for β-receptors, by the use of two independent quantitative methods of assessing damage (biochemical and morphological) in experiments on a model of the isolated rat heart.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were heparinized, and 20 min later they were anesthetized with thiopental sodium in a dose of 0.2 mg/g intraperitoneally. The thorax of the anesthetized animals was opened and the heart quickly removed and immersed in cold (4°C) perfusion medium. After cardiac activity had ceased the preparation was placed in the chamber of a Langendorff's perfusion system, the aorta was cannulated, and retrograde perfusion with oxygenated (96% O_2 and 4% CO_2) modified Krebs–Henseleit solution in bicarbonate buffer (pH 7.4-7.5) at 37°C was started under a constant perfusion pressure of 80 cm water. L-Noradrenalin bitartrate, L-adrenalin bitartrate, and DL-isoproterenol hydrochloride were used (all reagents were from Sigma, USA).

The following series of experiments were performed series I (control), in which the hearts were perfused with Krebs–Henseleit solution for 160 min of the experiment. In the experiments of series II solutions of catecholamines were used in the following concentrations NA and A 10^{-7} and 10^{-6} M, IP 10^{-7}-10^{-4} M. The concentrations of catecholamines in the perfusion fluid were monitored by Doty's method [3]. The hearts were perfused in accordance with the scheme: 20 min — washing hearts to remove blood, 20 min — control perfusion, 60 min — perfusion with solution containing catecholamine, 60 min — perfusion with Krebs–Henseleit solution.

In all series the perfusion fluid was collected every 20 min and the velocity of the coronary flow was measured. Aliquots of perfusion fluid, collected during this time interval, were taken for analysis of enzyme activity: creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). The rate of relief of the enzyme was calculated and expressed as activity of enzyme liberated into the perfusion fluid during unit time (U/min), and as the total quantity of enzymes released into the perfusion fluid during the whole period of investigation (U). Activity of CPK and LDH was studied by the use of kinetic methods [2, 9] on a reaction velocity analyzer (from LKB, Sweden).

Some hearts in all series of experiments were fixed in buffered 10% formalin solution and embedded in paraffin wax. Sections through the heart 5-7 μ thick, cut in the frontal plane, were stained by Lie's method [5] and with Heidenhain's iron hematoxylin. A general assessment of the changes in the myocardium was made visually. Lesions were counted in specimens stained with Heidenhain's hematoxylin [1].

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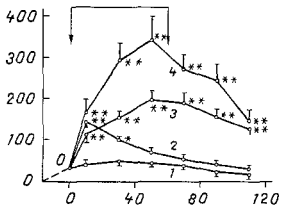

Fig. 1

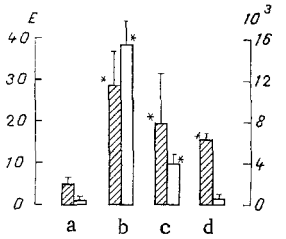

Fig. 2

Fig. 1. Changes in rate of release of CPK into perfusion fluid under the influence of catecholamines. Abscissa, perfusion time (in min); ordinate, CPK activity (in IU/min/g). 1) Control; 2) IP (10^{-4} M); 3) A (10^{-6} M); 4) NA (10^{-6} M). Arrows indicate duration of action of catecholamines. Each point represents $X \pm SE$ (mean ± error of the mean) for 16-18 experiments. *$P < 0.01$, **$P < 0.001$ compared with control.

Fig. 2. Total release of LDH (in U) into perfusion fluid (left) and number of damaged cells ($\times 10^3$) in myocardium (right) under the influence of catecholamines (data shown as $\bar{X} \pm SE$). a) Control; b) NA; c) A; d) IP. Shaded columns represent LDH activity, unshaded — number of damaged cells. *$P < 0.001$ relative to control.

EXPERIMENTAL RESULTS

The experiments showed that the rate of release of CPK and LDH into the coronary blood flow during perfusion with solutions containing NA, A, and IP in a concentration of 10^{-7} was indistinguishable from the control level. Perfusion of the heart with solution containing NA in a concentration of 10^{-6} M led to sharp increase in the rate of release of CPK and LDH into the perfusion fluid (Figs. 1 and 2). In this same concentration adrenalin also caused an increase in the rate of loss of CPK by the myocardium (Fig. 1) and an increase in the total release of LDH into the perfusion fluid (Fig. 2), but by a lesser degree.

It will be clear from Fig. 1 that the character of the change in the release of CPK from the myocardium under the influence of the two catecholamines was identical: the rate of release of CPK into the perfusion fluid began to increase immediately after the beginning of the action of NA and adrenalin, and it fell after perfusion with the catecholamine ceased.

IP in the same (10^{-6} M) concentration and also in a concentration ten times higher (10^{-5} M) caused no increase in the release of CPK and LDH into the coronary blood flow compared with the control. Only when the hearts were perfused with IP in a concentration of 10^{-4} M was this accompanied by an increase in the rate of release of CPK and LDH from the myocardium (Figs. 1 and 2). As calculation of the total release of LDH from the myocardium showed (Fig. 2), this IP-induced loss of LDH by the heart muscle was less than under the influence of NA and A in a concentration of 10^{-6} M.

Morphological investigations also showed (Fig. 2) that NA in a concentration of 10^{-6} M, caused considerable damage to the myocardium and possessed greater damaging capacity than adrenalin. Perfusion of the heart with IP even in a concentration of 10^{-4} M led only to a small increase in the number of injuries of contracture type compared with the control.

The most important result of this investigation was the demonstration of significant differences in the cardiotoxic action of catecholamines, connected with damage to the plasma membranes of myocytes. NA caused the greatest loss of CPK and LDH by the myocardium, exceeding the loss of these enzymes into the perfusion fluid under the influence of the same concentration of adrenalin. This effect of NA also was concerned by a quantitative morphological investigation.

An unexpected fact revealed by these investigations was the comparatively low cardiotoxicity of IP. A concentration of IP ten times greater than the cardiotoxic dose of NA and A did not cause any loss of enzymes from the myocardium. Only in a concentration 100 times higher than that of NA and A did IP cause release of CPK and LDH to a degree significantly

higher than the control level, but nevertheless smaller than under the influence of NA and adrenalin. Similar results also were obtained by counting the number of damaged cells.

Previously it was shown by experiments *in vitro* [10] that loss of enzymes from the myocardium induced by NA is largely inhibited by the β-blocker metoprolol. Propranolol also had an inhibitory effect on leakage of enzymes induced by adrenalin [6]. Correlation was found between the loss of enzymes from the myocardium and the tissue concentration of cAMP, and also the ability of dibutyryl cAMP to induce loss of intracellular enzymes [4]. All this led to the assertion [7] that the cardiotoxic effect of catecholamines is exerted through excessive β-adrenergic stimulation.

IP is known to have a powerful β-stimulating action, and its contact with receptors leads to a sharp rise in the rate of formation of intracellular cAMP [8]. On the basis of investigations cited above [6, 7, 10] and the hypothesis of Opie [7] which generalizes their results, it can be concluded that IP must have a damaging action on the myocardium that is at least comparable with that of NA and adrenalin. The results of the present investigation contradict Opie's hypothesis, and the question of the mechanism of the cardiotoxic effect of catecholamines can evidently not be reduced simply to their influence of β-receptors.

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PHOSPHORYLATION OF RAT LIVER AND ZAJDELA HEPATOMA NUCLEAR MATRIX PROTEINS

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Proteins of the cell nucleus are phosphorylated to a much greater degree than cytoplasmic proteins [1]. The skeletal structure of the cell nucleus or nuclear matrix (NM) consists to the extent of 90-95% of proteins, it preserves the shape of the nucleus, and has high metabolic activity [15]. During phosphorylation of proteins in isolated nuclei of the regenerating rat liver, NM proteins incorporate label twice to three times more actively than total nuclear protein [4].

There is no information in the literature on the characteristics of phosphorylation of NM proteins with tumors. However, a study of protein phosphorylation in cell nuclei of Novikoff's hepatoma and the regenerating rat liver revealed four proteins phosphorylated only in the hepatoma and one only in the liver [14]. Essential differences also were observed in the protein composition of NM of the rat liver, hepatoma 27, and Zajdela's hepatoma [2, 5]. For instance one characteristic feature of tumors is that they contain polypeptides with

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