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EFFECT OF DISSEMINATED NECROSIS OF THE MYOCARDIUM ON ATPase ACTIVITY, Ca^{++} TRANSPORT, AND LIPID PEROXIDATION OF MITOCHONDRIAL AND MICROSOMAL MEMBRANES OF THE HEART

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KEY WORDS: myocardial necrosis; microsomes; mitochondria; calcium transport; ATPase; peroxidation of lipids.

The sympathetic nervous system plays an important role in the development of myocardial infarction. Injection of adrenalin or sympathomimetic drugs into animals in doses much higher than normal leads to ischemia followed by the development of foci of necrosis.

One possible mechanism of the action of catecholamines in producing necrosis is an increase in permeability of cell membranes, together with activation of lipases. Changes in these factors may arise as a result of structural changes in protein and lipid components of subcellular membranes. It is particularly important that in the presence of a disturbance of membrane structures of the myocardial cell Ca++ transport is affected and, as a result, an excess of intracellular calcium arises [8]. Together with an excess of fatty acids in the cell, this may be the cause of depression of ATP resynthesis in the mitochondria [5, 6]. An increase in the fatty acid content in the myocardium during hypoxia combined with an increase in the content of promotors of free-radical peroxidation of lipids (PL) [1] also suggests that damage to the cell membranes takes place as a result of PL [3].

Against this background it was decided to study Ca^{++} transport processes through mitochondrial membranes and fragments of the sarcoplasmic reticulum (SR) and the intensity of PL in them, using a model reproducing the development of foci of necrosis by injection of an excess of the β-adrenomimetic isoproterenol.

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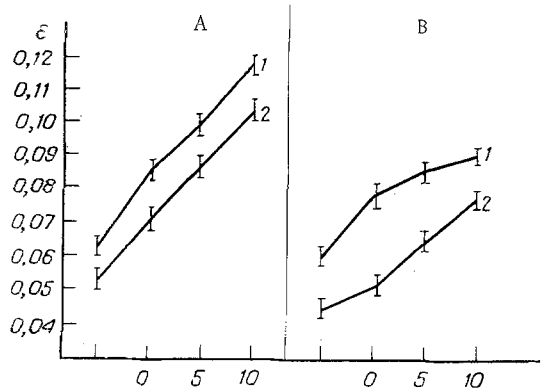

Fig. 1. MDA concentration in microsomes (A) and mitochondria (B) of the rabbit heart affected with disseminated necrosis. Abscissa, time after addition of Fe^{++} (in min); ordinate, optical density (in relative units) (sample contained 1 mg protein). 1) Necrosis, 2) normal.

TABLE 1. ATPase Activity of SR from Rabbit Heart during Ca^{++} Transport after Injection of Isoproterenol (M ± m)

Parameters	Normal	Isoproterenol
V_{ATP}, nmoles P_{in}/mg · min	650±15	324±11*
V_{Ca}, nmoles P_{in}/mg · min	720±12	188±9,8*
Ca/ATP	1,10±0,08	0,55±0,02*

Determination medium: 100 mM KCl; 2 mM ATP; 2 mM $MgCl_2$; 4 mM imidazole; 1.5-6 mM Na oxalate, pH 7.2.
*Difference significant at $P < 0.05$ level.

TABLE 2. Ca^{++} Transport and ATPase Activity of Cardiac Mitochondria after Injection of Isoproterenol

Conditions	Rate of Ca^{2+} accumulation, nmoles Ca^{2+}/mg · min	H^+/Ca^{2+}	Mg-ATPase	2,4-DNP-ATPase
			μmoles P_{in}/mg protein/min	
Normal	57,2±1,3	0,88±0,09	0,18±0,02	0,33±0,05
Expt.	78,5±1,8*	0,74±0,05	0,34±0,03*	0,62±0,04*

* $P<0,02$.

EXPERIMENTAL METHOD

Experiments were carried out on 25 rabbits. Isoproterenol (novodrin) was injected intravenously in a dose of 5 mg/kg body weight. Changes in the heart were monitored histologically and electrocardiographically. The animals were decapitated 24 h after injection of the isoproterenol. Mitochondria and the microsomal fraction, consisting chiefly of fragments of SR, were isolated from the heart by differential centrifugation in 0.25 M sucrose and 0.01 M EDTA

[4]. The rate of accumulation of Ca^{++} and of Ca-ATPase was measured by the use of a pH-meter [7], and mitochondrial Mg (2,4-DNP)-ATPase was determined by two methods [4, 7]. The phosphorus concentration was measured as in [12], protein as in [13], and malonic dialdehyde (MDA) as in [1]. The significance of differences was calculated by Student's test. The ATP-Na_2, albumin, and histidine used were from "Reanal," the Na deoxycholate from "Palfa," and the imidazole and Na oxalate from "Merck."

EXPERIMENTAL RESULTS

The results indicate that disseminated necrosis of the myocardium arising after injection of isoproterenol led to a decrease in the ability of the SR fragments to accumulate Ca^{++}. Meanwhile activity of Ca-ATPase and, correspondingly, the efficiency of the Ca-pump — Ca/ATP — were reduced (Table 1). Similar results have been obtained in other forms of cardiac failure [8, 14].

In mitrochondria isolated from these same hearts the rate of Ca^{++} accumulation was increased by 37%. The H^+/Ca^{++} ratio remained unchanged because of a corresponding rise in the H^+ concentration. Activity of Mg^{++}- and 2,4-DNP-stimulated mitochondrial ATPase was increased approximately twofold (Table 2).

The increase in the rate of calcium accumulation by the mitochondria and, evidently, in the concentration of intramitochondrial Ca^{++} [11], together with changes in the structure of the mitochondrial membranes after injection of isoproterenol [2, 9] are factors inhibiting oxidative phosphorylation and ATP resynthesis [5, 6]. The ATP deficit leads to a decrease in the efficiency of energy-dependent working of the Ca-pump of SR.

The second factor which could change the binding of Ca^{++} by fragments of SR in disseminated myocardial necrosis is an excess of H^+ in the myocardial cell. Such an excess arises on inhibition of the respiratory chain of the mitochondria. These changes as a whole lead to a defect of the contraction—relaxation process. Disturbances of permeability of the myocardial cell membranes to Ca^{++} ions, as other investigations have shown [10, 15], cause structural and functional disturbances of the myocardium.

One possible way whereby cell membranes may be injured, leading to disturbance of their permeability, is through an increase in the intensity of PL. Determination of the end product of PL, namely malonic dialdehyde, in mitochondrial and microsomal membranes showed that under normal conditions the MDA concentration is higher in the microsomal than in the mitochondrial fraction. After injection of isoproterenol, MDA increased in both fractions (Fig. 1).

Disseminated necrosis of the myocardium caused by injection of excessive concentrations of isoproterenol thus affected the permeability of the subcellular membranes to Ca^{++} ions. Under these conditions the efficiency of working of the Ca-pump of the SR fragments was reduced on account of a decrease in the rate of Ca^{++} accumulation and a decrease in Ca-ATPase activity. Under these conditions the rate of Ca^{++} transport into the mitochondria also increased. A definite role in the disturbance of membrane permeability is played by PL, for the concentration of MDA, its end product, increased in the microsomal and mitochondrial fractions.

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CHANGES IN MITOGENETIC RADIATION IN STRUCTURAL-ENERGETIC STATES OF THE LIVER AFTER INJECTION OF GLUCOSE AND AN INCREASE IN VAGAL TONE

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It is shown previously [1] that the unbalanced molecular orderliness which is a feature of the normal liver is intensified during weak nerve stimulation, and it was suggested that this phenomenon is connected with intensification of cell fields [1].

There is no doubt that an increase in molecular orderliness of a substrate facilitates regulation of metabolism. The study of the effect of nervous stimulation on processes connected with the assimilation of glucose by the liver is therefore a natural development of the previous study. We already know that the mouse liver at body temperature emits radiation only (or predominantly) in the visible region [5, 4], but after administration of glucose an ultraviolet component appears temporarily and the visible component is weakened [4]. These data could thus serve as the starting point.

EXPERIMENTAL METHOD

A small (1.5-2 cm^2) area of the surface of the liver was exposed in an unanesthetized rabbit and a small segment of the vagus nerve was exposed in the neck, and electrodes placed beneath it. The duration of the stimulating pulses was 1 msec, their frequency 40 Hz, and their intensity 40-50% of that inducing initial slowing of the heart beat. The liver was moistened with physiological saline at a temperature of 37-38°C. Radiation was recorded on a biological detector with exposures of 10-15 sec [2]. A few minutes before exposure, glucose (2 ml or 6 ml of a 25% solution, i.e., 0.5 or 1.5 g) was injected subcutaneously into the rabbit's thigh.

EXPERIMENTAL RESULTS

The experiments, which included a large series of repeated exposures, separated by 2-min intervals, occupied a considerable time, and for this reason the different variants could not be accommodated on the same animal. However, the following results were sufficiently clearly obtained on 10 rabbits (four control and six experimental).

In the control series with injection of glucose but without nerve stimulation, the radiation was characterized by a curve which was smooth after a small dose of glucose but which fluctuated after a larger dose (Fig. 1a, b).

In the experimental series the radiation emitted by the liver of rabbits in which the vagus nerve was stimulated after administration of glucose was characterized by alternation

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