OXIDATIVE MYOCARDIAL DAMAGE: PROTECTIVE ACTION OF EXOGENOUS PHOSPHOCREATINE

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An important role in heart damage during ischemia and, in particular, reperfusion is played by active forms of oxygen [7, 8]. Catecholamine-induced myocardial damage also is largely determined by generation of oxygen radicals [14]. Postischemic and catecholamine-induced myocardial damage exhibits considerable similarity in its morphological and biochemical features with disturbances of the structure and metabolism of the heart arising during incubation with hydroperoxides [5, 10]. Peroxidation of cell components can thus be regarded as a mechanism of irreversible myocardial damage. Investigations [1, 3, 6] have shown that exogenous phosphocreatine (PC) has a protective action during both ischemia and reperfusion of the isolated rat heart and during administration of isoproterenol. However, the mechanism of the cardioprotective effect of PC is not sufficiently clear.

The aim of this investigation was to determine whether the protective action of PC in catecholamine-induced and ischemic heart damage may be linked with an increase in the resistance of the myocardium to oxidation.

EXPERIMENTAL METHOD

Experiments were carried out on isolated hearts of male Wistar rats, using hydrogen peroxide as oxidizing agent. Retrograde perfusion was conducted with a perfusion pump ("Cole-Palmer," USA) with Krebs-Henseleit (K-H) solution; the rate of flow of the perfusion fluid was 12-14 ml/min/g wet weight of tissue. The K-H solution was saturated with carbogen (95% O_2 and 5% CO_2) and its pH was adjusted to 7.40 at 37°C. H_2O_2 (90 μM) was added to the K-H solution immediately before perfusion. When the disodium salt of PC (10 mM) was added to the perfusion fluid, appropriate corrections were made to Na^+ (by reducing the NaCl concentration by 20 mM) and Ca^{2+} concentrations (increasing the $CaCl_2$ concentration to 20%). To study contractility of the heart a catheter with latex balloon, connected to a Statham P23Db pressure transducer ("Gould," USA) was introduced into the chamber of the left ventricle. After perfusion with H_2O_2 material from the myocardium was taken and fixed in 3.5% glutaraldehyde ("Merck," West Germany), postfixed in 1% osmic acid, dehydrated in alcohols of increasing concentrations, and embedded in Epon-Araldite. To demonstrate early disturbances of the integrity of the sarcolemma, ionic lanthanum was used [2]. Ultrathin sections were cut on an OM-IZ ultramicrotome ("Reichert," Austria). The material was studied under the JEM-100B electron microscope (Japan).

EXPERIMENTAL RESULTS

During H_2O_2-induced perfusion of the isolated rat heart, in control experiments a gradual rise of the diastolic pressure was observed starting with the 20th minute of perfusion, and was found to be irreversible, for when H_2O_2 was removed from the perfusion fluid the degree of contracture was virtually not reduced (Fig. 1a). The development of irreversible contracture, evi

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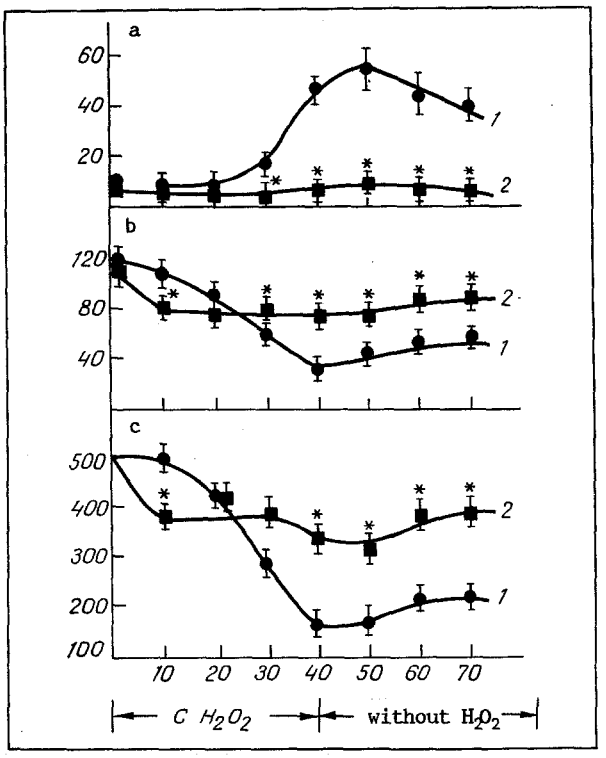

Fig. 1. Effect of PC on contractility of isolated rat heart during H_2O_2-perfusion ($M \pm m$). Abscissa, time of perfusion (in min); ordinate: a) diastolic pressure, b) developed pressure, c) work of the heart (developed pressure × number of contractions per second, in mm Hg/sec). 1) Control, 2) after injection of PC. *$p < 0.05$ compared with control.

dence of Ca^{2+}-overloading of the cardiomyocytes, also was observed in [4, 9] during perfusion of the isolated rat heart with hydroperoxides. Addition of PC (10 mM) to the perfusion fluid completely prevented the rise of diastolic pressure in the left ventricle during perfusion with H_2O_2 for 40 min.

The developed pressure (Fig. 1b) and also the contractility of the left ventricle and work of the heart during H_2O_2-induced perfusion were immediately reduced in the experiments with PC, whereas in the control experiments the developed pressure, its derivative, and the work of the heart remained at their initial level for the first 10 min (Fig. 1b, c). Since exogenous PC does not penetrate inside the myocardial cells [6], and since the sarcolemma plays a decisive role in the binding and transport of Ca^{2+} ions, which participate in contraction, it can be postulated that PC, during perfusion with H_2O_2, limits the trans-sarcolemmal inflow of Ca^{2+} Rossi and co-workers with H_2O_2, limits the trans-sarcolemmal inflow of Ca^{2+}. Rossi and co-workers [13] also showed that PC increases myocardial resistance to a sudden increase in the extracellular Ca^{2+} concentration. Starting with the 10th minute, contractility of the isolated rat heart was gradually reduced in the control experiments, so that by the 20th minute the developed pressure, its first derivative, and the work of the heart were actually identical in the two experimental groups, after which myocardial contractility in the control fell sharply, and this was accompanied by a simultaneous rise of the diastolic pressure (Fig. 1). Inhibition of myocardial contractility during incubation with hydroperoxides, as was demonstrated in [4, 9, 15], is probably due to irreversible damage to a considerable cardiomyocyte population, accompanied by loss of mass of functioning tissue. Removal of H_2O_2 from the perfusion fluid in the control experiments did not restore contractility of the heart, whereas in experiments with injection of PC the velocity of contraction and relaxation of the left ventricle at the 30th minute of perfusion with K-H solution did not differ from its initial values, and the developed pressure and work of the heart came close to them (Fig. 1b, c).

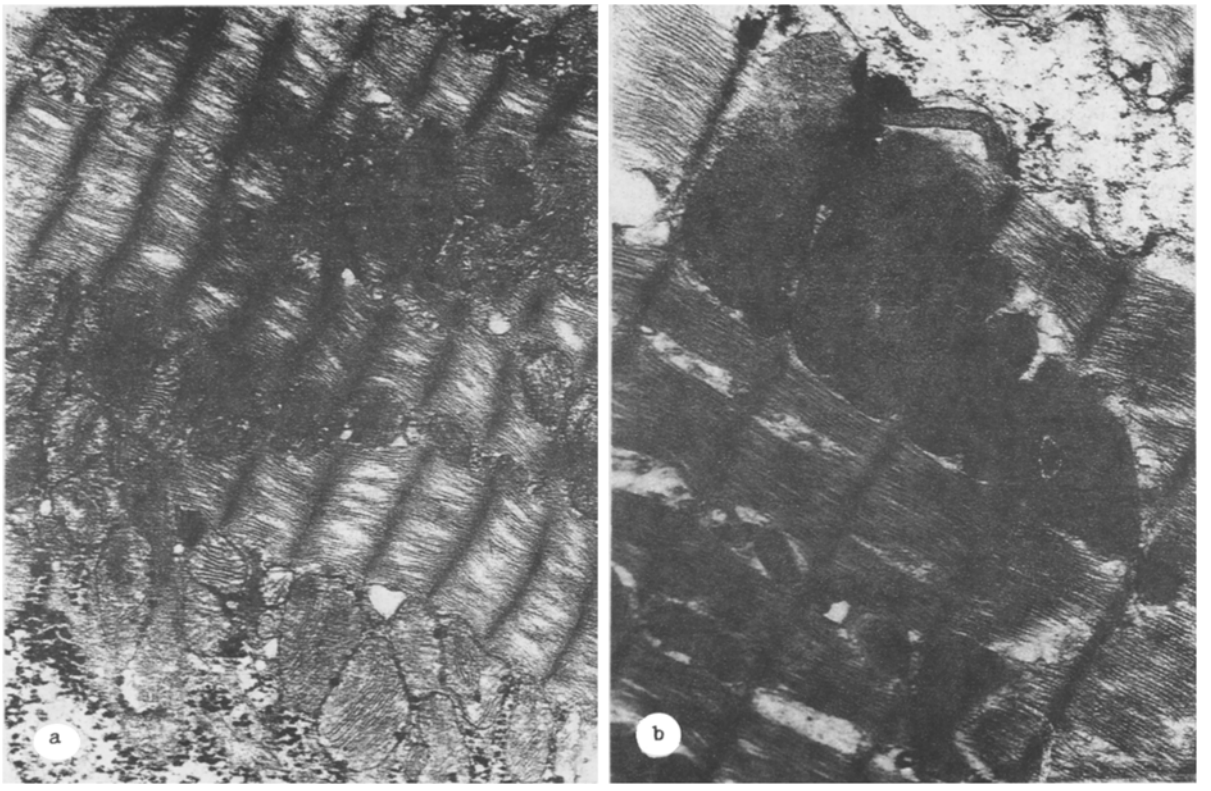

Fig. 2. Effect of PC on ultrastructure of contractile cardiomyocytes during perfusion of isolated rat heart with H_2O_2. a) Myocardium during H_2O_2 perfusion (control). Granules of tracer penetrate sarcoplasm (arrow), sarcomeres are overcontracted. M) Mitochondria, Mf) myofibrils. Unstained preparation. Magnification 5500. b) Myocardium (during H_2O_2 perfusion) with PC. Particles of ionic lanthanum can be seen (arrow) in lumen of T-tubules. Unstained preparation. Magnification 9500.

Since Barsacchi and co-workers [4] found a high degree of correlation between inhibition of myocardial contractility and accumulation of peroxides of phospholipids in cell membranes during H_2O_2 perfusion of the isolated rat heart, we studied the structural integrity of the cardiomyocyte membrane systems with the aid of ionic lanthanum, a transmembrane tracer. Ultrastructural study of the control myocardium revealed that H_2O_2-perfusion caused marked damage to mitochondrial membranes, as is shown by penetration of ionic lanthanum particles into the mitochondrial matrix (Fig. 2a). Metabolism of peroxides is accompanied by exhaustion of reduced glutathione (GSH) together with accompanying oxidation of NADH and damage to mitochondria [11]. However, another investigation [15] showed that perfusion of the isolated rat heart with peroxides in concentrations below 150 μM did not cause significant oxidation of pyridine nucleotides in mitochondria. However, even high concentrations of peroxides did not lead to irreversible damage to isolated mitochondria, unless they were preloaded with Ca^{2+} ions [11]. Irreversible mitochondrial damage in the whole cell is evidently the result of the combined action of hydroperoxides and Ca^{2+} overloading. In the control experiment, signs of overloading of the cells with Ca^{2+} ions were in fact observed: cardiomyocytes were in a contracted state, some cells were overcontracted, and the sarcoplasmic reticulum was vesicular. A sharp increase in the intracellular Ca^{2+} ion concentration was largely responsible for uncontrolled inflow of Ca^{2+} known by penetration of granules of the tracer into the sarcoplasmic matrix (Fig. 2a).

In the experiments with PC, on the other hand, the sarcolemma remained impermeable for particles of the tracer, evidence of the preservation of its structural integrity during perfusion with H_2O_2. There were no signs of Ca^{2+} overloading, the myofibrils were in a relaxed state, and the structure of the sarcoplasmic reticulum was normal. Meanwhile, virtually all the mitochondria were swollen and reduction of the cristae and washing out of the mitochondrial matrix were observed (Fig. 2b). Consequently, H_2O_2 metabolism, just as in the control experiments, leads to mitochondrial damage on account of exhaustion of GSH. In the absence of Ca^{2+} overloading, however, mitochondrial damage does not become irreversible in character, as is shown by the almost complete restoration of contractility of the isolated heart on removal of H_2O_2 from the perfusion fluid.

Thus exogenous PC has a protective effect during H_2O_2 perfusion of the isolated rat heart, as shown by improvement of its contractile function, prevention of irreversible contracture, and preservation of the structural integrity of the sarcolemma of the cardiomyocytes. The protective factor is evidently connected with stabilization of the phospholipid structure of the membranes and inhibition of sarcolemmal 5'-nucleotidase [12].

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