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PRELIMINARY INJECTION OF PERFLUOROCARBON EMULSION – A NEW METHOD OF ANTIISCHEMIC PROTECTION OF THE MYOCARDIUM

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Preliminary pharmacological preparation of the donor, with the use of β-blockers, calcium antagonists, and antioxidants, is an effective method of protecting the myocardium against ischemic and reperfusion damage [1, 8].

The use of perfluoro compounds (PFCs), widely used because of their physicochemical properties in biology and medicine as gas-carrying [2] and biologically active [3, 13] media, in the context of the present investigation may prove to be promising.

As investigations conducted at the Institute of Biophysics, Academy of Sciences of the USSR, showed the mechanism of biological action is evidently linked with direct interaction between the PFC emulsion and its components and cell membranes [4-7, 9, 10, 12].

The aim of this investigation was to study the effect of preliminary administration of PFC emulsion on the myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits weighing from 2 to 3 kg, divided into seven groups: group 1) receiving no injections (control, $n = 6$); animals of the other groups received preliminary injections as follows, 1, 12, and 24 h before ischemia: salt solution – group 2 (control injection, $n = 15$) and a 4% solution of Proxanol 268 in a salt composition – group 3 ($n = 11$) in doses of 20 ml/kg, and also an emulsion of PFC (perfluorane) with particle diameter of 0.1 μ, stabilized with 4% solution of Proxanol 268 (copolymer unit of polyethylene and polypropylene oxides, mol. wt. 7500, fraction of hydrophobic unit 0.2). The PFC phase in perfluorane was 10 vols. 2. Perfluorane was added in doses of 5 ($n = 11$), 10 ($n = 13$), 20 ($n = 16$), and 30 ($n = 16$) ml/kg – groups 4, 5, 6, and 7 respectively. The crystalloid composition of perfluorane and of the control solution was the same, and they consisted of the following ingredients (in mM): NaCl – 102, KCl – 5, $MgCl_2$ – 1.2, NaH_2PO_4 – 1.2 $NaHCO_3$ – 15, glucose 11. After preliminary injection of perfluorane or the control solutions (without oxygenation) into a rabbit 1, 12, and 24 h before ischemia, the heart was removed from the animal and perfused by Langendorff's method, spontaneously, on recirculation mode for 30 min with Krebs–Henseleit solution (KHS; initial level) under a constant perfusion pressure

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TABLE 1. Some Parameters of Function of the Isolated Rabbit Heart before and after 30 min Ischemia at 37°C

Group	Ischemia	Amplitude of cardiac contractions (mm)		Heart rate, beats/min		Coronary blood flow (ml/min·g)	
		before	after	before	after	before	after
1		22,8±1,6	4,6±0,5	143±11	123±13	8,6±0,7	4,4±0,4
2	1	21,7±2,1	4,5±0,8	149±11	140±9	7,2±0,2	3,8±0,2
	12	22,6±1,8	4,8±0,6	150±12	142±9	8,6±0,2	3,8±0,6
	24	19,1±1,2	4,2±0,9	156±9	143±5	8,9±0,8	4,2±0,3
3	1	21,4±2,5	4,4±0,9	150±12	135±11	—	—
	12	19,2±1,3	4,3±1,0	160±10	155±8	—	—
4	1	21,8±1,3	4,4±0,7	152±8	140±8	—	—
	12	18,0±1,8	4,9±0,7	162±9	155±6	—	—
5	1	18,2±1,6	4,8±0,8	160±11	145±13	—	—
	12	18,3±1,8	7,5±0,8**	158±7	140±12	—	—
6	1	19,7±2,4	7,6±0,9**	158±11	147±8	9,3±0,8	6,2±0,3**
	12	23,4±2,8	13±2,6**	143±9	130±13	8,8±0,8	6,0±0,2**
	24	23,4±2,4	10,5±0,9**	141±12	138±11	7,7±0,3	5,7±0,3**
7	1	23,2±1,7	6,5±0,8	143±8	140±12	—	—
	1*	22,0±2,3	9,1±1,1**	140±13	132±12	—	—
	12	21,7±1,4	9,6±1,3**	145±13	130±11	—	—

Legend. *) Concentration of surfactant in emulsion was 1.5%; **$p < 0.05$ compared with groups 1, 2, 3, and 4.

of 60-70 mm Hg. The perfusion solution (KHS) was oxygenated with carbogen (95% O_2 + 5% CO_2, pO_2 = 250-350 mm Hg). The myocardium was then subjected for 30 min to total ischemia (at 37°C), after which it was reperfused with KHS and restoration of the amplitude of contraction of the isolated heart at the 40th minute of reperfusion was compared with the initial state. The contractile function of the heart was measured by means of a type 6MKhChS mechanotron. The mechanotron was fixed by means of an adaptor to the apex of the heart. The amplifier transformed mechanical oscillations into electrical, which were recorded on an "Élkar" automatic writer and measured in millimeters.

The state of function of the isolated heart before and after ischemia was assessed also with respect to two parameters: coronary blood flow (or velocity of coronary perfusion), determined by direct measurement, and heart rate, recorded on the "Élkar" instrument.

The PFC concentration in the blood (n = 3) was determined 1, 6, 12, and 24 h after injection of the PFC emulsion, by the ^{19}F-NMR method, on a M-400 spectrometer ("Brüel and Kjaer"), and the Proxanol concentration in the blood (n = 3) was determined spectrophotometrically with iodine solution by the method in [11]. Concentrations of PFC were determined in the fraction of sarcoplasmic membranes of cardiomyocytes (n = 3), isolated by the method in [15], on a "Chrom-5" gas–liquid chromatograph.

EXPERIMENTAL RESULTS

The amplitude and frequency of cardiac contractions and the coronary blood flow in the initial state (before ischemia) were identical in all groups ($p > 0.05$; Table 1).

In the group of experiments without preliminary injections partial (to 4.6 ± 0.5 mm) restoration of myocardial contractility after ischemia was observed. Similar changes were found in groups 2, 3, and 4, in which salt solution and 4% Proxanol solution in doses of 20 ml/kg were injected 1 and 24 h before ischemia and the PFC emulsion in a dose of 5 ml/kg 1 h before ischemia. Preliminary injection of the PFC emulsion 1 and 12 h (5 ml/kg) and 1 h (10 ml/kg) before ischemia did not increase the resistance of the myocardium. Prolonging the circulation time of the emulsion in a dose of 10 ml/kg to 12 h significantly increased restoration of the amplitude of contraction after ischemia (to 7.5 ± 0.8 mm). A subsequent increase in the quantity of emulsion injected to 20 ml/kg was no longer dependent on the circulation time of the emulsion in the blood stream, and it significantly enhanced the contractile activity of the heart and the coronary blood flow (Table 1).

The experiments of group 7, in which a preliminary injection of emulsion in a dose of 30 ml/kg was given 1 h before ischemia led to only partial (up to 6.5 ± 0.8 mm) restoration of myocardial contractility ($p > 0.05$). A subsequent decrease in the concentration of Proxanol in the PFC emulsion in this group from 4 to 1.5% caused a significant change in myocardial contractility in the direction of an increase (to 9.1 ± 1.1 mm) in the same time cut (1 h before ischemia). Prolongation of the circulation time (to 12 h) of the same dose of emulsion (30 ml/kg) and with the previous concentration of Proxanol (4%) significantly in-

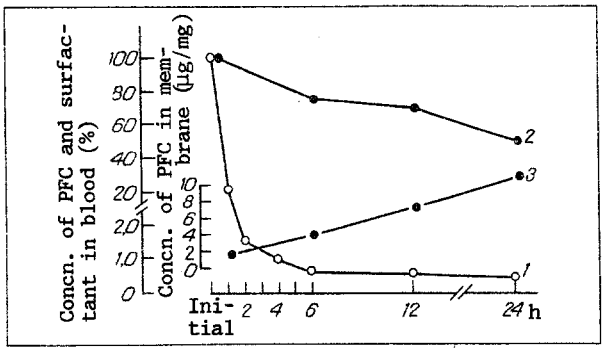

Fig. 1. Concentrations of Proxanol and perfluorocarbon in blood and cardiomyocyte membranes. 1) Proxanol in blood, 2) PFC in blood, 3) PFC in sarcoplasmic membranes (in each time band determinations were made in three (n = 3) independent experiments).

creased (9.6 ± 1.3) the resistance of the myocardium compared with injection of the emulsion 1 h before ischemia. Under these circumstances no significant changes were observed in the heart rate, whether within all the groups or between them (Table 1).

It is clear from the experimental results that removal of the heart from the animal to create 30-min ischemia 1, 12, and 24 h after injection of the emulsion in a dose of 20 ml/kg corresponds to a PFC level of 95, 70, and 50% (2.3, 1.7, and 1.2 g) in the blood stream (Fig. 1, curve 2). The presence of perfluorocarbons was observed in the sarcoplasmic membranes of the cardiomyocytes in a concentration of 2.1, 6, and 10.5 μg/mg membrane protein respectively (Fig. 1, curve 3).

As a result of circulation of PFC in the blood stream, part of the PFC in the membranes gradually dissolves, and this evidently changes their functional properties and increases resistance to ischemia. With a long circulation time (up to 12 h) even a small dose of emulsion (10 ml/kg) can induce an effect, although in short time intervals (1 h before ischemia), and with the same dose, there were no visible changes. A twofold increase in the dose (20 ml/kg), however, with a short circulation time (under 1 h) led to a rapid response of the biosystem (recovery to 7.6 ± 0.9 mm, $p < 0.05$). The hypothesis that an increase in the dose of a preparation administered leads to a linear increase in the resistance of the myocardium to ischemia was not confirmed experimentally. For instance, with an increase in the dose of the PFC emulsion injected (up to 30 ml/kg) the quantity of free Proxanol, unconnected with PFC injected also was increased, for this is present in sufficiently large amounts, in some cases up to two-thirds [14], in PFC emulsion. In high concentrations this could evidently be an adverse effect: as a blocker of slow calcium channels, it could block the working of very important components of cellular self-regulation [10]. Reduction of the Proxanol concentration (to 1.5%), when the emulsion was injected 1 h before ischemia in a dose of 30 ml/kg, caused significantly better recovery of contractile activity than with emulsion stabilized with 4% Proxanol. A significant increase of resistance 12 h after injection of the same dose (30 ml/kg), but with a 4% Proxanol concentration, can evidently be explained on the grounds that Proxanol had almost completely left the blood stream by this time (Fig. 1, curve 1) and it therefore had no effect.

An increase in the resistance of the myocardium to ischemia after preliminary injection of PFC emulsion is associated, in our view, with the presence of PFC in the cardiomyocyte membrane. This process evidently depends on a certain minimal threshold concentration of PFC in the sarcoplasmic membrane, after which an effect may be manifested. For example, 1 h after infusion of the emulsion in a dose of 20 ml/kg, with the minimal recorded PFC concentration in the membrane (2.1 μg/mg), the resistance of the myocardium to ischemia was significantly increased compared with the control injection, but did not differ significantly from infusions given after 12 and 24 h, although the PFC concentration in the membrane continued to rise. This also was confirmed by the fact that an increase in circulation time of small doses of emulsion (10 ml/kg) in the bloodstream from 1 to 12 h increased the resistance of the myocardium to ischemia, just like an increase in the injected doses of emulsion from 10 ml/kg to 20 ml/kg 1 h before ischemia.

Thus the increase in resistance of the myocardium to ischemia which we found is dose- and time-dependent in character.

An increase in permeability of the cell membranes in ischemia is one of the basic, if not decisive, factors aggravating cell damage [1]. It can accordingly be postulated that accumulation of perfluorocarbons in the sarcoplasmic membranes of cardiomyocytes after preliminary injection of a submicron emulsion of PFC modifies the cell membranes and thereby causes a decrease in the severity of ischemic and reperfusion damage to the myocardium. Under these circumstances the protective effect will evidently be due both to the direct influence of PFC on the phospholipid matrix of the membranes and their interaction with

hydrophobic sites on membrane proteins, which may have an effect on restoration of the initial structural and functional homeostasis in a cell that is damaged by ischemia but still viable.

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