USE OF CREATINE PHOSPHATE AND VITAMIN E TO OPTIMIZE CONDITIONS OF CONSERVATION OF THE ISOLATED HEART: EXPERIMENTAL STUDY

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Transplantation of the human heart is now recognized as an effective method of treatment of patients with severe heart diseases when other methods of treatment, both medical and surgical, are impossible. The number of operations of orthotopic allotransplantation of the heart shows a steady increase. By now, more than 2000 transplantations are done annually worldwide [14].

The number of patients needing operations of this kind is much greater than the number which can be performed. One of the main reasons for this relative limitation of the number of operations is the shortage of donors' hearts.

As a rule the donor's heart comes from a distant source, and its transportation to the site of the operation requires high-quality conservation of the isolated organ. Most investigators now accept that the permissible period of conservation before transplantation of the heart does not exceed about 4 h [5, 9, 13, 15]. Prolongation of the safe period of ischemia during conservation naturally would lead to a marked increase in the number of possible operations in clinical practice. Many experimental studies have been undertaken in the attempt to solve this problem, mainly in two directions: conservation by methods with or without perfusion. Perfusion methods, because of technical difficulties, are not used in clinical practice at the present time. Further improvement of nonperfusion methods of conservation by the use of a range of cardioprotective agents to prolong the period of safe cardioplegia has proved to be a more promising approach.

In our own experimental investigations we have used two preparations for this purpose: the high-energy compound creatine phosphate (CP) and the membrane-stabilizing agent α-tocopherol (vitamin E) [1-3, 11, 12].

The results of these investigations are described below.

EXPERIMENTAL METHOD

Experiments were carried out on 60 hearts from noninbred male rats weighing 200-250 g. Under hexobarbital anesthesia (75-100 mg/kg body weight) in the course of 35-60 sec the heart was removed, the aorta cannulated, and perfusion commenced with Krebs–Henseleit solution at 35°C. The heart worked for 5 min without any load on the left ventricle [8]. Perfusion of the coronary system with an ionically balanced cardioplegic solution (CS; Table 1) at 15-16°C then began. The CS was kept in a reservoir at a height of 80 cm above the heart. Perfusion continued for 1 min.

After the end of perfusion the hearts were kept for 6 h in an incubator containing basic CS at 4-6°C. Altogether there were four series of experiments: series I) perfusion of the coronary system with CS; series II) CP was added to the CS in a concentration of 10 mM [11]; series III) perfusion with CS without CP, but after preliminary administration of α-tocopherol to the animal; series IV) CS with the addition of CP after injection of vitamin E.

In series III and IV vitamin E was injected intraperitoneally in a dose of 70 mg/kg body weight 3-4 h before removal of the heart, for injection of this preparation during this time interval has the maximal anti-ischemic effect.

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TABLE 1. Ionically Balanced CS

Nature of ion	Ionic concentration, mM
K^+	26
Na^+	50
Mg^{++}	14
Ca^{++}	1

TABLE 2. Functional State of the Heart before and after Conservation for 6 h in CS, CS + CP, CS + Vitamin E, and CS + CP + Vitamin E

Experimental conditions	Percent recovery of cardiac activity	HR, beats/min	CF	AF	CO	SV, ml/HR/g	dP/dt, mm Hg/sec	External work, mJ/min/g	O_2 consumption, min/g	Efficiency, %
			ml/min/g							
Initial data (n=20)	100	258±8	10,4±0,6	19,0±1,8	29,4±2,0	0,11±0,1	463,8±29,2	301,8±20,5	0,12±0,02	12,6
CS (n=10)	70	60±8*** (23)	3,0±0,6*** (29)	—	—	—	—	—	0,03±0,01***	
CS + CP (n=10)	100	170±12*** (66)	7,3±0,7*** (70)	14,8±2,0 (78)	22,0±2,0** (75)	0,08±0,02 (73)	338,6±10,7 (73)	202,4±23,7** (67,2)	0,09±0,01 (66)	11,2
CS + vitamin E (n=10)	100	214±18* (83)	10,4±0,6 (100)	12,4±1,5** (65)	25,0±1,5 (85)	0,10±0,02 (89)	320±25,7*** (69)	226,6±28,3 (75)	0,10±0,01 (83)	11,1
CS + CP + vitamin E (n=10)	100	243±6 (94)	10,3±0,3 (99)	16,3±1,0 (85,8)	27,2±1,2 (92,5)	0,11±0,03 (100)	425,9±10,8 (92)	253,8±22,5 (84)	0,11±0,01 (92)	11,5

Legend. Asterisks indicate significance of differences between parameters and their initial values: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Values in parentheses.

At the end of 6 h of conservation the hearts were cannulated and perfusion carried out on a Langendorff model for 15 min in order to flush out and rewarm the hearts. The rate of outflow of lactate dehydrogenase (LDH) and calcium from the myocardium was determined in the perfusion fluid flowing from the heart. The heart was then perfused on the Neely model at 35°C for 20 min and the functional state of the working heart after conservation was evaluated [10].

The following parameters of function were recorded: the heart rate (HR), coronary flow (CF), aortic flow (AF), systolic blood pressure (P_s) in the main vessel receiving blood from the left ventricle; the partial pressure of oxygen was measured in AF and CF by a polarographic method on a gas analyzer ("Corning," Denmark). The following parameters were calculated: cardiac output (CO), stroke volume (SV), contractility of the left ventricle and external work of the heart, oxygen consumption, and efficiency of the heart, as the ratio of the external work and oxygen consumption [10]. LDH in the perfusion fluid was determined spectrophotometrically using reagents from "Boehringer" (West Germany); calcium was determined photometrically by the color reaction with glyoxal-*bis*-2-hydroxyanil reagents ("Lachema," Czechoslovakia).

The results of the four series of experiments were compared with initial values obtained on 20 rat hearts on Neely's model before conservation. The results of all the experiments were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Table 2 gives the results of investigations of the functional state of the hearts after conservation, compared with the initial values. It will be clear from Table 2 that when CS alone was used, cardiac activity after 6 h of conservation was restored in 70% of cases (seven of 10 hearts), but virtually no restoration of the pumping function of the heart took place on Neely's model.

Addition of CP to the composition of CS, just as in the experiments in [11], improved the results of conservation of the isolated heart; cardiac activity was restored in 100% of cases, but recovery of the parameters of the pumping function was incomplete (CO, dP/dt during contraction, etc., were reduced by 25-30%). Approximately the same final protective effect was obtained

TABLE 3. Ca and LDH Concentrations in Perfusion Fluid Flowing from Hearts Conserved for 6 h in Hanks' Solution, in CS, in CS with the Addition of CP (10 mM), in CS Preceded by Administration of Vitamin E (70 mg/kg), and in CS with CP, Preceded by Administration of Vitamin E

Chemical substance	Hank's solution (initial data)	Hank's solution (control)	CS	CS + CP	Vitamin E + CS	Vitamin E + CS + CP
LDH units/liter/g	—	1633±76	204±12,3*	398±12,3*	207±26,7*	289±12,3*
Ca, mM/g	2,5±0,12	2,87±0,38**	1,89±0,1**	2,38±0,1	2,4±0,13	2,6±0,2

Legend. *$p < 0.05$ for outflow of LDH into perfusion fluid compared with control; **$p < 0.05$ compared with initial Ca concentration in perfusion fluid.

after preliminary (3-4 h beforehand) administration of vitamin E. With respect to some parameters (HR, CF, and CO) vitamin E was more effective in its action than CP, but according to others (SV, dP/dt contraction, external work, and O_2 consumption) the differences were not significant ($p > 0.05$). Since the action of CP is aimed at maintaining the energy balance of the cardiomyocytes [4] and the action of vitamin E is aimed at stabilizing cell membranes, it was deemed worthwhile to study the combined action of these two preparations. The investigation showed that with such a combination, the contractility and pumping function of the heart after 6 h of conservation at 4-6°C did not differ significantly from the initial state ($p > 0.05$), although with respect to individual parameters there was a tendency for them to fall. If the duration of conservation was increased to 8-9 h, the pumping function of the heart was considerably depressed, and accordingly the conservation time was restricted to 6 h.

Further proof of the better contractility of the cardiomyocytes following low-temperature conservation of the heart in CS with the use of combined pharmacological correction was obtained by reperfusion of the heart on a Langendorff model. The investigation showed (Table 3) that conservation of the heart for 6 h led in all the experiments in which CS was used to a significant decrease in the outflow of LDH compared with control experiments (cold ischemia in Hanks' solution for 6 h at 4-6°C). Some increase in the LDH concentration in the outflowing perfusion fluid after the addition of CP to CS evidently indicates the stronger adaptive action of this preparation on the myocardium, increasing permeability of the cytoplasmic membranes, and also, possibly, facilitating the entry of CP into the cells for that reason [4].

Investigation of the calcium concentration in the outflowing perfusion fluid, aimed at studying the calcium-damaging action of the mitochondria showed that in the control there was a tendency for the outflow of calcium from the myocardium to be increased. After conservation in CS without pharmacological correction of its protective action, a significant decrease in the calcium concentration in the outflowing perfusion fluid was found ($p < 0.05$). When CP was added to the composition of CS, and also if a preliminary injection of vitamin E was given, no significant changes could be found in the calcium concentration in the outflowing perfusion fluid ($p > 0.05$). Experiments with reperfusion of the hearts conserved for 6 h showed that if CS was used, although there was some improvement in the degree of preservation of the cellular structures and membrane permeability was lower than in the control (a decrease in LDH activity), calcium damage to the cardiomyocytes took place (a decrease in Ca in the outflowing solution). On entering the cytosol, calcium then evidently penetrates into the mitochondria, where it disturbs the energy-forming process [6, 7]. This conclusion was confirmed by the virtually complete absence of pumping function of the myocardium after conservation for 6 h in CS (Table 2). Correction of the conserving properties of CS by the addition of CP, preceded by injection of vitamin E, not only led to preservation of the cytoplasmic membranes of the cardiomyocytes, but also maintained calcium homeostasis of the cells.

The study of the functional characteristics showed that calcium homeostasis is evidently best preserved by a combination of pharmacological conservation of the heart with conditions of deep hypothermia, and with the combined use of vitamin E and creatine phosphate.

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REINNERVATION OF A SEGMENT OF THE PANCREAS

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The pancreas is an integral organ which stands at the crossroads of local and general endocrine influences arising from the gland itself and from other organs [3]. However, the mechanisms of the central nervous regulation of this complex internal hormonal and external secretory complex have been inadequately studied. Yet there are grounds for considering that the by no means brilliant late results of pancreatic transplantation in clinical and experimental practice [12], even in cases when the effect of immune conflict is minimal or absent altogether, are not only the result of biological incompatibility, but are also connected with disturbance of regulation of the function of the transplanted organ [16]. The adverse consequences of division of the nervous connections of intestinal and renal transplants, expressed as disturbance of their function at various times after the operation, have been demonstrated by several investigations [4, 6, 7, 10, 11]. It has been shown that the method of transplantation of the kidney and intestine can be used as a model of complete nervous reflex isolation of these organs in order to study the role of nervous reflex influences in the regulation of their functions. In relation to the pancreas, however, this problem has not been studied at all.

The aim of this investigation was to create an experimental model of nervous reflex isolation of the pancreas, without permitting temporary ischemia of the organ. It was necessary to develop an anatomically and physiologically based model of restoration of the innervation of the pancreas after its nervous reflex isolation or transplantation.

EXPERIMENTAL METHOD

Experiments were carried out on 22 dogs, 32 cats, and nine rabbits. To obtain anatomically sound methods of reinnervation, the splenic and hypogastric plexuses of 12 dogs, 10 cats, and five rabbits were studied by dissection and morphometry. The following operations were performed: nervous reflex isolation of the "body—tail" segment of the pancreas of the different animals without disturbance of its blood supply, by dividing all connections between it and the rest of the body, division of the gland

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