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^{31}P-NMR study of cardiac preservation: St. Thomas' Hospital cardioplegic solution versus UW preservation solution

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Abstract. Ex vivo cardiac preservation was evaluated by measuring the catabolism of high-energy phosphate (ATP and creatine phosphate, CrP) using ^{31}P-NMR spectroscopy. After cardioplegic arrest St. Thomas' Hospital cardioplegic solution (group A), and University of Wisconsin (UW) preservation solution (group B) were tested. The hearts were mounted in the 4.7 T horizontal bore magnet of the NMR spectrometer and were continuously perfused with the test solution under 25 cm H_2O pressure for 6 h at 10 °C. Peak heights of the β-phosphate of ATP and CrP were measured and expressed as percentages of the initial value. For both group A and group B, ATP declined less rapidly during preservation than CrP. In group A, ATP remained constant for 60 min while CrP decreased from the onset of preservation. After 6 h of preservation 28.3% of ATP and 24.5% of CrP remained (group A). On the other hand, in group B, levels of both ATP and CrP remained much more stable: CrP did not decrease during the first 3 h of preservation, while ATP started to decrease after 5 h. At the end of preservation 76.1% of ATP and 71.5% of CrP were still present. We conclude that UW solution is superior to St. Thomas' Hospital solution for the preservation of high-energy phosphates during 6 h cardiac preservation with continuous hypothermic low-flow perfusion.

Key words: Heart preservation, in rat – UW, heart preservation – St. Thomas' Hospital solution, heart preservation – NMR study, in heart preservation

The main objective of organ preservation is an optimal functional recovery after transplantation. Functional recovery may be correlated with maintenance of an energized state of the organ during preservation. Although some controversy about this issue remains, we believe that at least 70% of ATP needs to be preserved to assure a functional recovery [12].

Preliminary studies have shown that the best preservation method is a continuous hypothermic low-flow coronary perfusion [12, 24]. However, different solutions to perfuse the heart can be used. The UW solution was developed by Belzer et al. [5] for kidney preservation and used recently as a multi-organ preservation solution [3]. Preliminary results have revealed that the UW solution resulted in prolonged preservation periods without excessive loss of function upon normothermic reperfusion [28, 32, 35]. Kidney and pancreas can be preserved for up to 72 h and liver for up to 48 h. Very few studies report on the preservation of hearts using the UW solution. Cold storage preservation of canine hearts for 12 h with UW solution did not seem to dramatically decrease function upon reperfusion [33], neither did a 4 h hypothermic storage period of rat hearts [22]. Successful functional recovery after up to 24-h periods of heart storage have been reported recently [37].

This is in contrast to the results obtained with the more widespread cardioplegic solutions like the St. Thomas' Hospital or the Bretschneider-HTK solution. These

Table 1. Composition of preservation solutions. Units are m*M* unless otherwise mentioned, except for viscosities which are in relation to water at 10 °C

	St. Thomas No. 1	St. Thomas No. 2	UW
NaCl	144.0	91.5	–
KCl	20.0	14.8	–
$MgCl_2$	16.0	15.0	–
$CaCl_2$	2.4	1.2	–
$NaHCO_3$	–	25.0	–
KH_2PO_4	–	1.2	25.0
$MgSO_4$	–	1.3	5.0
Procaine HCl	1.0	–	–
Raffinose	–	–	30.0
Glutathione	–	–	3.0
Allopurinol	–	–	1.0
Adenosine	–	–	5.0
K-lactobionate	–	–	100.0
Hydroxyethyl starch	–	–	5%
	–		
Viscosity at 10 °C	–	1.024	3.283

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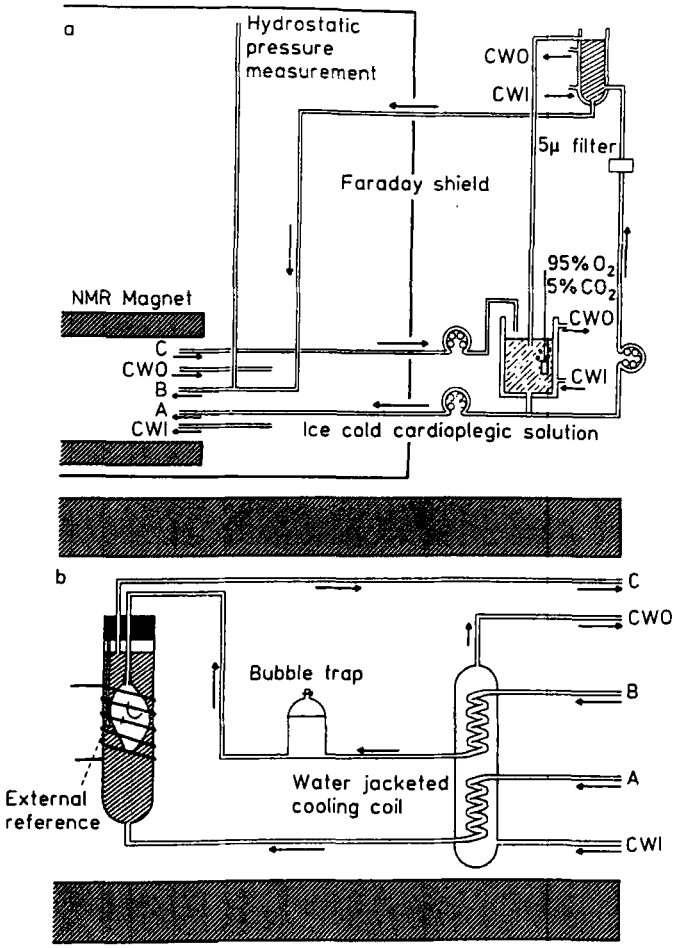

Fig. 1. **a** Experimental set-up of a preserved rat heart in the NMR apparatus. An upper reservoir (200 ml) is connected with the aorta via a bubble trap and a water-jacketed cooling coil. The fluid in this upper reservoir is pumped through a 5 μm filter and the level is adjusted to perfuse the heart under a constant pressure. The hydrostatic pressure measurement system allows the perfusion pressure in the aortic line to be continuously monitored close to the heart *(B)*. The system ends in a graduated cylinder. Cooling of the heart is achieved by perfusion with cold solution *(B)* and by infusing cold solution through the sample tube *(A)*. A suction line *(C)* prevents the sample tube from overflowing and returns the excess fluid back to the main reservoir outside the NMR cage. The perfusion fluid in this main reservoir was continuously gassed with a mixture of 95% O_2 and 5% CO_2. Cooling of the whole system is assured by using water-jacketed fluid reservoirs through which ice-cold water is circulated (*CWI*, cold water inflow; *CWO*, cold water outflow). The external NMR reference is phosphonitrilic chloride trimer. **b** Enlarged model of NMR magnet

are routinely used in Europe for short-term open-heart surgery. Long-term ex vivo preservation of the heart using these solutions is still in an experimental stage. Some of these experiments have demonstrated clearly that hearts can be preserved for 12–48 h and subsequently regain their normal function upon reperfusion [16, 38]. In some studies, the superiority of the UW solution as compared with St. Thomas' Hospital or Bretschneider-HTK cardioplegic solution has been demonstrated [22, 33].

It has been shown previously that hypothermia protects the heart against ischemia [17]. The high-energy phosphates (HEPs), ATP and CrP, seem to be best preserved when the temperature is around 0 °C, while catabolism is more pronounced at higher temperatures, e.g. at 12 or 18 °C. A controversy still exists concerning the best preservation temperature to assure optimal functional recovery. Indeed, some results indicate that higher preservation temperatures are better than the 0 °C condition [11, 26]. Continuous hypothermic perfusion of the heart also results in better preservation than intermittent perfusion which, in turn, seems to be better than simple cold storage [1, 12, 19]. Different species have been used as experimental animals by different investigators: rat, rabbit, guinea pig, dog, pig, baboon and humans. Species differences, with respect to cardiac preservation, have been reported. The rat heart, in particular, seems to be very sensitive to ischemia [13, 25].

Our methodology was adapted to these findings. A continuous low-flow hypothermic (10 °C) perfusion was performed in rat hearts to study HEP catabolism during long-term ex vivo preservation. The catabolism was followed using ^{31}P-NMR spectroscopy to enable continuous monitoring rather than using intermittent standard tissue sampling and subsequent biochemical analysis. The latter procedure delivers only 'snap-shots' of the breakdown process, and requires a large number of experimental animals because of the nature of tissue sampling and the smallness of the rat heart. Moreover, there exists a significant amount of evidence that tissue levels of ATP during organ preservation may be relevant for subsequent viability upon normothermic reperfusion [20, 36]. Hence, quick detection of ATP levels may have clinical value for organ transplantation. We studied the catabolism of HEPs during 6 h of cardiac preservation using either St. Thomas' Hospital or UW solution as continuous perfusion solution of the hearts.

Methods

Anesthesia and cardioplegic arrest

Male Wistar rats (300–400 g) were heparinized (100 U/kg IP) and anesthetized with Na-pentobarbital (65 mg/kg IP). The trachea was exposed and cannulated. Using a Bird respirator, artificial ventilation was initiated with a 50% mixture of oxygen and room air at 80 cycles/min and positive end-expiratory pressure of 15 cm H_2O.

The heart was excised and trimmed of large vessels. During this manipulation the heart remained immersed in cardioplegic solution at room temperature. The ascending aorta was freed and cannulated with a piece of PE-240 tubing (Intramedic, non-radiopaque polyethylene tubing). A small flange on this tubing prevented the heart from slipping off the cannula. This cannula was connected to a reservoir positioned 120 cm above the heart level and which was filled with either St. Thomas' Hospital cardioplegic solution or UW preservation solution (for composition see Table 1). The solutions were made up freshly before each experiment, gassed with a 95%/5% O_2/CO_2 mixture and filtered through a 5 μm Millipore filter.

Immediately after cannulation of the aorta an antegrade flush of the coronary arteries was initiated with 5 ml of solution at room temperature and with 20 ml of the same test solution at 4 °C. This method has been shown to increase coronary flow during the subsequent preservation period [7, 8]. This perfusion caused the myocardial temperature to decrease gradually from room temperature to 10–15 °C when using the St. Thomas' Hospital solution. Pilot

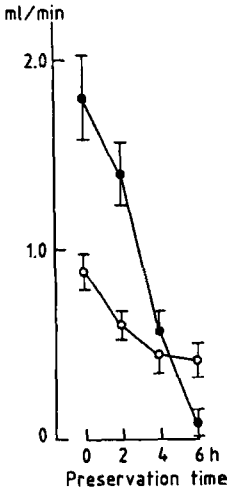

Fig. 2. Mean values (± SEM) of the coronary flow measured during hypothermic preservation. The coronary flow in group A was significantly higher during the first 2 h of preservation ($P < 0.001$). In both experimental groups, coronary flow decreased. This decrease was significant from the 2-h point onwards ($P < 0.01$). ● St. Thomas' Hospital solution; ○ UW solution

experiments had shown that this method did not induce the desired temperature decrease when using the UW solution due to its high viscosity, resulting in low coronary flow and in a too-slow myocardial cooling. Therefore, we immersed the UW-hearts in UW solution at about 10 °C from the start of the cardioplegic flush. It was assumed that the myocardial temperature equilibrated immediately with the temperature of the immersion fluid. At the end of the flush, coronary flow was measured.

Hypothermic preservation in the NMR magnet

Immediately after the cardioplegic flush the heart was mounted in the horizontal magnet of a NMR spectrometer. The experimental set-up is shown in Fig. 1. The heart was directly connected to an upper reservoir (200 ml) positioned outside the NMR cage at a distance of about 2 m from the core of the magnet. The heart was perfused under a constant hydrostatic pressure of 20 cm H_2O. The suspended heart was thermostatically controlled at 10–12 °C by flushing the sample tube continuously with cold preservation solution at 100 ml/min. The coronary effluent and the perfusate, directly pumped into the tube for cooling, were removed by suction from the top of the tube, and were recirculated. To avoid condensation on the solenoid surrounding the sample tube, the core of the magnet was ventilated with cool dry air. Coronary flow was measured intermittently. The hydrostatic perfusion pressure was measured using a side-line on the aortic cannula, which ended in a small graduated cylinder. When the flow in the main aortic line (line B, Fig. 1) was interrupted, the rate of drainage of the small graduated cylinder was used to measure coronary perfusion flow.

Using this set-up, six rat hearts were preserved using St. Thomas' Hospital cardioplegic solution (group A) and six hearts using UW preservation solution as perfusion media (group B). Preservation time was 6 h or until β-ATP became undetectable.

^{31}P-NMR spectroscopy

^{31}P-NMR measurements were performed at 81.1 MHz in the 4.7 T horizontal superconducting magnet with a 30-cm-wide bore (BRUKER Biospec, Karlsruhe, FRG). A six-turn solenoid surrounded the 20 mm sample tube containing the perfused rat heart. Signals were acquired every 20 s using a 60 ° excitation pulse of 5 µs. The interpulse delay of 20 s ensured that each of the peaks in the spectrum had relaxed back to its equilibrium value before the application of the next pulse. This was verified by increasing the delay up to 40 s, for which value no further increase in peak heights was noted. This condition ensured that metabolite concentrations could be compared on the basis of their spectral peak heights. In order to obtain a sufficient signal-to-noise ratio, 64 successive signals were accumulated, yielding a NMR spectrum every 20 min. The accumulated time-domain signals were weighted with an exponential decay (corresponding to a line broadening of 10 Hz) in

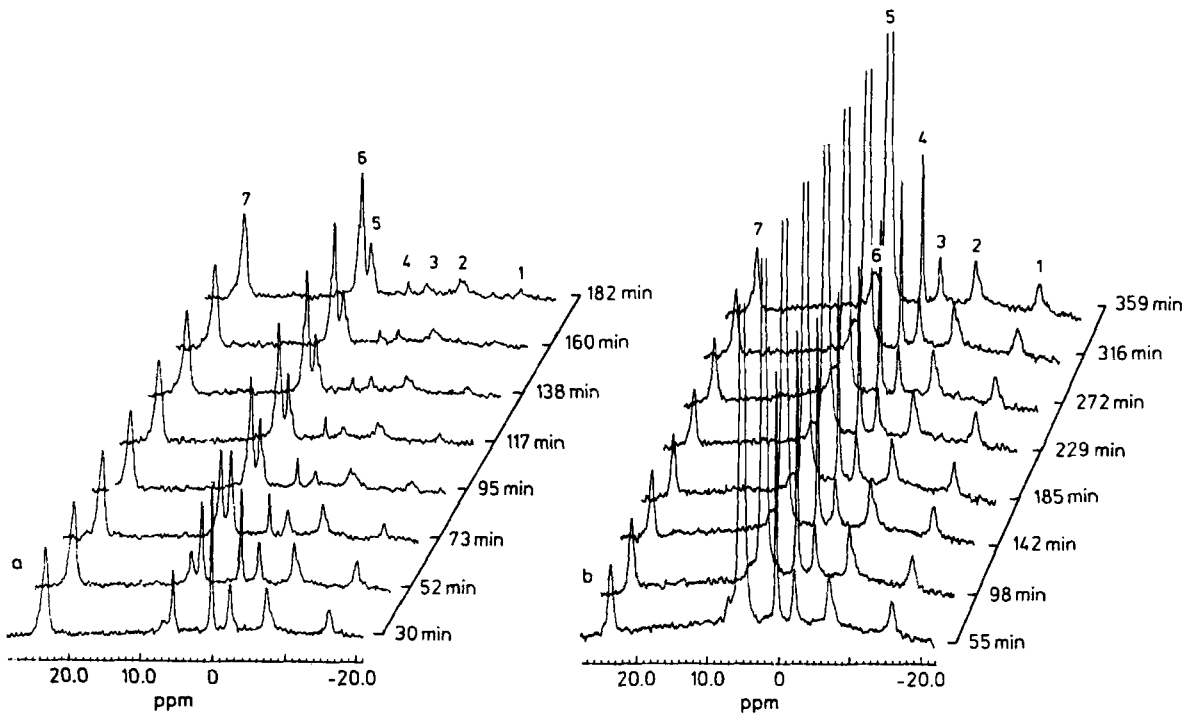

Fig. 3a, b. ^{31}P-NMR stacked spectra obtained during hypothermic preservation. St. Thomas' Hospital cardioplegic solution; UW preservation solution. Chemical shifts are expressed in parts per million ppm relative to the position of the CrP signal *(4)*, which is assigned the value 0 ppm. Signals are assigned (from right to left) to the beta- *(1)*, alpha- *(2)* and gamma-phosphates *(3)* of ATP, CrP *(4)*, inorganic phosphate *(5)*, phosphomonodiesters *(6)* and the external standard *(7)*. Each spectrum is the accumulation of 64 acquisitions with a time delay of 20 s

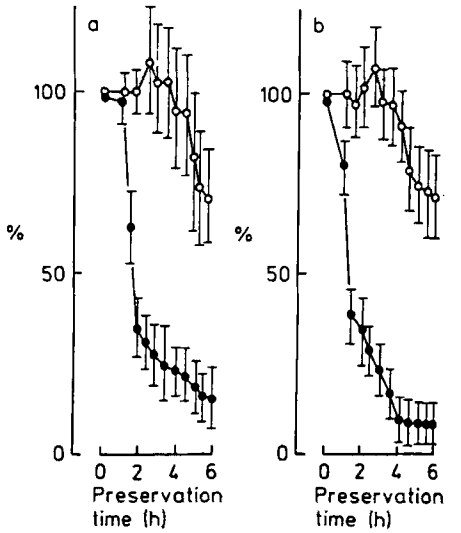

Fig. 4. Normalized values of (a) ATP and (b) CrP peak heights (control value (the first spectrum obtained) is 100%; mean values ± SEM) obtained in group A (St. Thomas' Hospital cardioplegic solution) and in group B (UW solution). In group B the number of observations each time was six. In group A, however, the number decreased gradually from the 2-h time point onwards as a result of the progressive reduction in detectability of the ATP and CrP peaks. ($n = 5$ at 2 h, $n = 3$ at 3 h, $n = 2$ at 4 h and $n = 2$ at 6 h). Between 2 and 5 h of preservation the ATP and CrP peaks were significantly higher in group B than in group A ($P < 0.001$). ● St. Thomas' Hospital solution; ○ UW solution

order to improve the signal-to-noise ratio of the spectra after Fourier transformation. Phosphonitrilic chloride trimer with the addition of Ca^{3+} to reduce the relaxation time (15 μEq ^{31}P) [15] was used as an external standard to correct for possible instrumental drift during the 6 h of measurement. The position of the CrP peak was taken as the reference for the frequency scale (0 ppm). Relative changes in metabolite concentrations were evaluated from the changes in spectral heights.

Due to the different time delays needed to shim the NMR magnet at the onset of each experiment (5–10 min) the occurrence of the first spectrum obtained was slightly offset in the various experiments. Peak heights of ATP and of CrP were averaged in 30-min intervals throughout each experiment, and the mean values (± SEM) obtained in these time intervals are represented in the figures.

Results

At the end of the initial cardioplegic flush, prior to mounting the heart in the NMR magnet, the coronary flow was measured under a hydrostatic pressure of 120 cm H_2O. For group A the coronary flow was 15.3 ml/min ± 2.9 SD, and for group B it was reduced to 7.0 ml/min ± 1.0 SD ($P < 0.001$). These differences may be due to different viscosities. At time 0 the average coronary flow of the heart positioned in the magnet was 1.94 ml/min ± 0.29 SD for group A and 0.93 ml/min ± 0.15 SD for group B ($P < 0.001$). During preservation, coronary flow decreased gradually in both groups and after 5–6 h it had dropped to about 10% of the starting value in group A and to about 30% in group B (Fig. 2).

Stacked plots of the NMR spectra from a typical experiment in group A and in group B are shown in Fig. 3. Spectra were obtained every 20 min. Results of peak heights of ATP and CrP were averaged in 30 min time intervals during the preservation period. Peak heights measured in the subsequent time intervals were expressed as a percentage of the first spectrum obtained which served as control. The high phosphate concentration of the UW solution (25 m*M*) caused the huge peak at about 5 ppm. Peak heights (and hence also concentrations) of CrP and ATP were comparable in both experimental series at the start of the preservation. However, they decreased during the subsequent hours of hypothermic preservation. For group A, it can be seen that the ATP peaks attenuate more quickly and nearly disappear completely at the 4-h time point (Fig. 4).

The changes in CrP peaks are even more dramatic than those for ATP. The peaks, increasing in height between +3 and +7 ppm originate from accumulating phosphodiesters, inorganic phosphate and phosphomonoesters. For group B, the accumulation of phosphate esters was undetectable mainly due to the large 25 m*M* peak of inorganic phosphate, and the decline of the ATP and CrP peaks is clearly much slower than for group A. Figure 4 shows the relative decrease of the ATP and CrP peaks for both groups. For group A, a fast decay of CrP and of ATP was observed: after 2 h, 30–45% of the initial values remained. ATP remained constant only during the first hour. At the end of preservation the residual level was about 10–20% for both HEPs. ATP and CrP levels were much more stable in group B: CrP remained fairly constant for 3 h and ATP for 4.5 h. CrP levels decreased slowly between 3 and 6 h of preservation and, after 6 h, 70% of the initial level was still preserved. ATP decreased only during the last 1.5 h to reach about 75% of the initial value at the 6 h time point.

Discussion

Best preservation of HEPs in rat hearts with continuous low-flow hypothermic perfusion was obtained using the UW solution.

In a recent study HEP catabolism in rabbit hearts preserved for 12 h was investigated by NMR [10]. The preservation method used was very similar to ours, but the hearts were perfused continuously at 4 °C using a pressure of 60 mm Hg. English et al. compared the St. Thomas' Hospital No. 1 solution with a regular Krebs-Henseleit solution, the Bretschneider's cardioplegic solution and a modified St. Thomas' Hospital No. 1 solution, called CP5 which contained 30 m*M* glucose and 0.1 m*M* calcium. In this study, CrP became undetectable within 2.5 h when using the St. Thomas' Hospital No. 1 solution, and, when using their best CP5 solution, CrP was broken down completely within 4–6 h. Using St. Thomas' Hospital solution, ATP became undetectable within 3 h in some experiments. ATP levels decayed more slowly with CP5 than with either of the other solutions.

The present study produced very similar results for the St. Thomas' Hospital solution. A very fast breakdown of CrP occurred and a somewhat slower breakdown of ATP. However, some evidence exists that the rate of breakdown found in this study is slower than that re-

ported by English et al. [10]. This was definitely the case for CrP: in English's study peak detection became impossible after 2.5 h, while in our study at least about 20% of the initial CrP peak remained during the whole preservation period. This might be either the result of the different species used [25], or simply because different perfusion pressures were used [34]. Another possibility might be that English's group used the St. Thomas' Hospital No. 1 cardioplegic solution, while we used the No. 2 solution (no procaine, reduced calcium content; see Table 1). Indeed, Ledingham et al. [21] showed that the No. 2 cardioplegic solution results in a better myocardial preservation than the No. 1 solution.

Our methodology also allowed a continuous perfusion of the heart during the complete course of the experiment, whereas in English's study the hearts were periodically placed in the NMR magnet to acquire a spectrum, but remaining most of the time in a refrigerator. During transport no perfusion occurred, and this might have altered the rate of catabolism of ATP and CrP. English's best result, with respect to HEP preservation, was obtained using CP5, but in our study the best result was obtained with the UW solution. As mentioned by Ledingham et al. [21], and later confirmed by English et al. [10], a decrease in the calcium concentration might have very beneficial effects, which might be one of the reasons for the observed improved properties of CP5 compared to the St. Thomas' Hospital solution. Calcium-free solutions are, however, not very effective for heart preservation, but a low calcium concentration is essential [14]. A low calcium concentration is also necessary to prevent the calcium paradox (reperfusion cell damage after a period of calcium-free perfusion) [29].

Another explanation for the improved results of CP5 and UW solution might be the increased concentration of oncotic agents. Evidence for the beneficial effects of glucose during ischemia can be found in clinical and experimental studies [6, 30]. Also, ribose prevents cell swelling [27]. Raffinose, with a high molecular weight (MW, 594), and the impermeant lactobionate (MW, 358) are used in the UW solution to prevent cell swelling and to create a high osmolarity [2, 4, 35]. To prevent expansion of the extracellular space hydroxyethyl starch is added (50 g/l) and the presence of precursors for ATP resynthesis (adenosine) might have had additional benefits in maintaining the ATP level during preservation. Indeed, it has been shown recently that the presence of increased amounts of adenosine in the perfusate (15–30 μmol/l) during normothermic ischemic conditions, results in increased tissue ATP levels [18]. Additional benefit may be expected from albumin, since this component also reduces the permeability of myocardial capillaries to hydrophilic solutes of varying molecular sizes [9, 23, 31].

Conclusion

The present study clearly shows that the preservation of HEPs in a stored donor heart strongly depends on the cardioplegic or preservation solution used for hypothermic perfusion of the coronary arteries. The UW solution preserved ATP and CrP better than the St. Thomas' Hospital cardioplegic solution in an ex vivo hypothermic perfused rat heart.

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