NMR Spectroscopy in Cardiac Surgery

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1 Introduction

Applications of magnetic resonance spectroscopy in medicine have been restricted mostly to the research laboratory. The technique is now entering the field of medical diagnosis and therapy. In the heart, levels of phosphorus metabolites are often correlated with function. Nuclear magnetic resonance spectroscopy has been used to monitor high energy phosphorus metabolite levels in the heart to evaluate the effect of work and ischemic stress. Our applications of magnetic resonance to the practice of cardiac surgical have been in three areas: a) preservation of tissue for transplantation b) optimization of myocardial protective techniques (cardioplegia) and c) monitoring of the heart during the aortic clamping period.

2 Heart Preservation for Transplantation

With increasing demand for a limited number of donor hearts, organ preservation during procurement is critically important. Controversy still exists over issues such as the optimal temperature, optimal solution, and maximum time limit for donor heart preservation. Numerous studies have been, and continue to be, conducted on various animal tissues. Not much data have been obtained in human myocardium primarily because of the difficulty in obtaining adequate quantities of viable tissue for laboratory investigation.

a) Human Atrial Tissue

Portions of human atrial appendages normally discarded during cannulation in the course of surgery requiring cardiopulmonary bypass have been used, with informed patient consent, for studies of heart preservation. We have used ^{31}P and ^{1}H NMR spectroscopy to define the optimal temperature for long-term (up to 24 hours) preservation of high energy metabolite levels and contractile function, and to gain a fundamental understanding of the energy generating pathways in preserved human cardiac tissue [1, 2]. The studies were carried out on a Bruker AM-360 spectrometer. ^{31}P spectra were obtained using a 60° pulse and a 1 s recycle time. ^{1}H spectra were acquired using a spin-echo sequence based on the water-suppressing 1331 pulse sequence. The acquisition of ^{31}P and ^{1}H NMR spectra were interleaved (Figure 1). ^{31}P spectra were used to measure ATP levels on a continuous basis, as an index of net energy preservation. ^{1}H spectra of lactate provided information on generation of ATP through the glycolytic pathway.

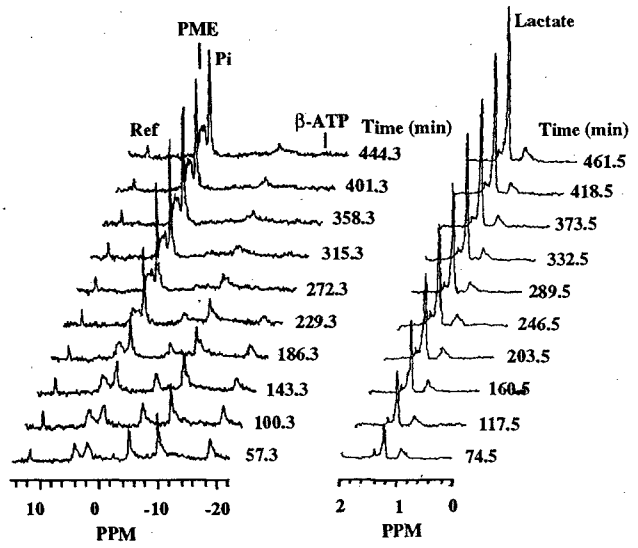

FIGURE 1 ^{31}P (left) and ^{1}H (right) NMR spectra of an atrial appendage (ca. 0.5 g) preserved at 20°C in saline, as a function of time [1] (*ref*, reference capillary; *PME*, phosphomonoester; *Pi*, inorganic phosphate).

Studies of atrial appendages preserved at 1°, 4°, 12° and 20°C in physiological saline (0.9% NaCl) for up to 20 hours demonstrate that preservation of ATP is better at 1° and 4° than at 12° or 20°C. Based on measurements of lactate production, glycolysis is active at all the temperatures, its rate correlating positively with increasing temperature. However, the ATP generated by glycolysis falls short of

demand at all temperatures, but the difference is small at 1° and 4°C (Table 1).

TABLE 1

Energy balance in human cardiac tissue preserved in NaCl 0.9% [1].

Temp. (°C)	ATP*# loss	Lactate* production	ATP*+ generated	ATP*& utilization
1	7	43	65	72
4	8	52	78	86
12	12	106	159	171
20	20	212	318	338

* nmole g^{-1} (wet myocyte mass) min^{-1}.

\# From the rate of change of NMR-visible ATP.

\+ Assuming 1.5 mole of ATP produced per mole of lactate from glycolysis.

& Calculated from (rate of generation of ATP by glycolysis) + (2 x rate of ATP loss). This takes into account the ATP generated by adenylate kinase.

In a separate series of studies, we tested the possibility of improving the maintenance of high energy phosphates at 12°C, one of the higher temperatures currently used in some institutions for the preservation of heart grafts. Our hypothesis was that the poor maintenance of high energy phosphates at 12° and 20°C results from the increased intracellular acidosis that occurs at higher temperatures [1]. Ultimately, acidosis partially inhibits ATP production by glycolysis, the only metabolic pathway for generation of ATP in the anoxic heart. We postulated that the addition of a buffer to the solution used for heart preservation would increase the rate of transport of protons and lactate to the extracellular space, thereby maintaining better intracellular pH homeostasis. Our studies showed that at 12°C, the half-time for loss of ATP increased from 300 minutes in saline to over 900 minutes in a modified Krebs-Henseleit solution containing 100 mM buffer [2, 3]. This observation was confirmed independently using biochemical measurements of high energy phosphates [3]. The beneficial effects of high buffer concentration observed at 12°C did not occur at 4°C (figure 2 [3]), which lead us to postulate that at that temperature, glycolysis was rate limited by the temperature rather than by the acidosis. These studies show the continued need for testing all the conditions to which a graft may be subjected, and the importance of avoiding broad generalizations when dealing with complex multi-enzyme systems.

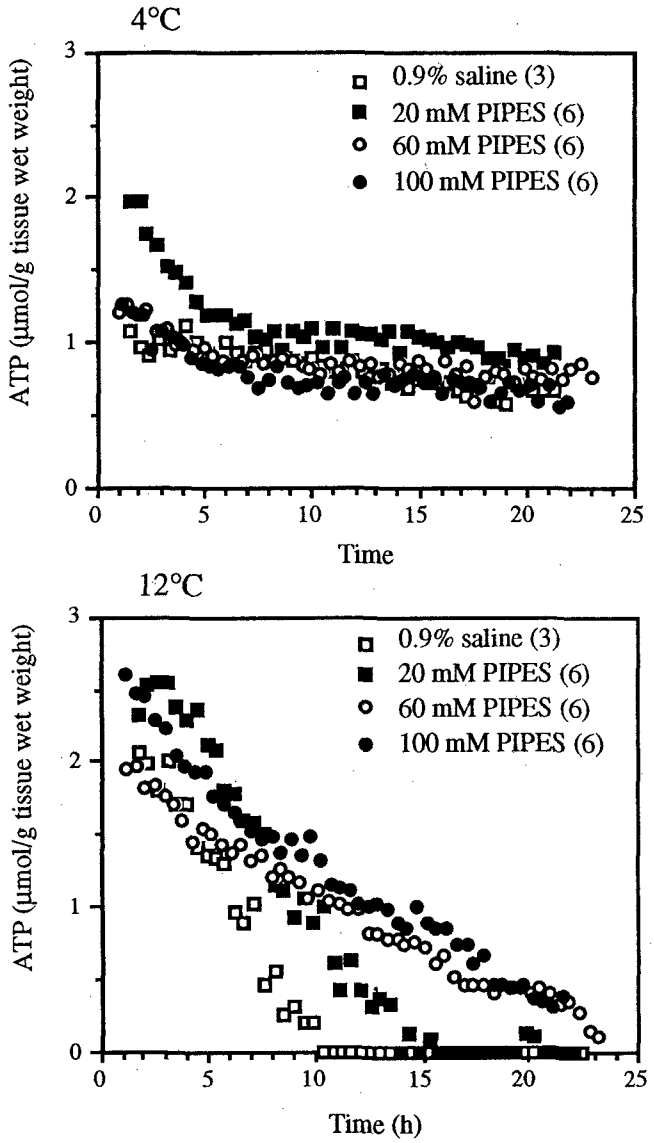

FIGURE 2. Effect of the concentration of PIPES buffer on the preservation of ATP in isolated atrial appendages preserved at 4° or 12 °C in modified Krebs-Henseleit solution [3].

The importance of defining the relationship between high energy phosphate levels and contractile function in human cardiac tissue led to the development of a temperature-controlled NMR microprobe incorporating a perifusion system and a non-magnetic strain gauge (figure 3). The system has been used to study human atrial trabeculae, which are small functional muscle fibers weighing 8 - 25 mg that can be isolated from atrial appendages. The perifusion system provides the tissue with the oxygen and nutrients required for its function. The strain gauge allows for measurement of developed force in

electrically stimulated muscle fibers while NMR simultaneously assesses the high energy phosphate compound levels [4]. In addition, the perifusion system can mimic preservation conditions by allowing muscles to be perifused at low temperature (down to 1°C) during acquisition of NMR data (figure 4).

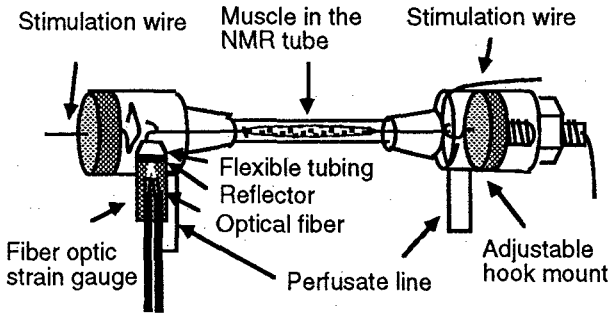

FIGURE 3. Schematic drawing of the microperifusion system used for NMR studies of human atrial trabeculae. The total length of the system is 6.2 cm.

By studying atrial trabeculae in the presence of metabolic inhibitors under conditions simulating preservation, it is possible to assess the contribution of various cellular mechanisms of energy production to the total energy balance of the tissue. Our ^{31}P NMR studies of isolated human atrial trabeculae [5] preserved at 4° and 12°C in oxygenated St-Thomas II solution showed that the high energy phosphates (ATP and phosphocreatine (PCr)) are well maintained during 18 hours of preservation. Contractile studies performed under similar conditions showed high recovery of developed force. Glycolysis, the only pathway available for energy generation under the anoxic conditions existing in large preserved organs, is capable of maintaining ATP levels in hypothermically preserved tissue. Under anoxia, ATP levels are stable for 6 - 10 hours at 12°C, and for a longer period at 4°C. In a resting heart, the major energy source is provide by the lipid catabolism. To test whether this pathway is active at low temperatures, we have measured the ability of the oxydative pathway to maintains ATP levels. At 12°C, when glycolysis is inhibited by iodoacetate, the oxidative pathway can maintained ATP levels, but only if an external source of substrate (10 mM acetate) is present in the perfusate. Thus, the oxidative pathway is functional but depends on both, oxygen and glycolysis.

In tissue preserved ischemically (no flow), metabolic waste products such as lactate cannot be eliminated. This results in considerable extracellular and intracellular acidosis [1] which has profound effects on energy production because glycolysis is inhibited by low pH. Using the atrial trabecula model, we simulated the conditions that exist in the ischemically preserved human heart. Such a large organ (300 - 450 gm) cannot obtain sufficient oxygen to maintain oxidative phosphorylation simply through diffusion from the surrounding medium as in the case with trabeculae. Trabeculae were subjected to acidosis by perifusing the trabeculae with modified St-Thomas preservation solution containing 10 mM lactate at pH 6.0. Anoxia was simultaneously induced with 1 mM cyanide, a potent inhibitor of oxidative phosphorylation, and nitrogen. In the trabeculae, these conditions reproduced those previously observed in the larger atrial appendages where an anoxic core probably existed [1]. Under acidosis and anoxia at 12°C, ATP decreased linearly by 40 to 100% over a 12 h period. At 4°C, ATP decreased less over the same time period.

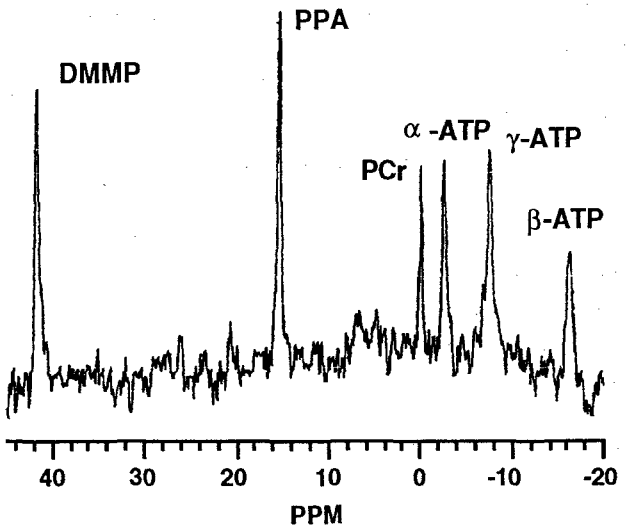

FIGURE 4. ^{31}P NMR spectrum (147 MHz) of a 10.5 mg trabecula perifused at 12°C in modified St-Thomas II solution (60° pulse, 1 sec recycling delay, 2400 scans).

These observations can be reconciled to the following model of energetics: the maintenance of cellular ATP depends on matching of supply and demand. At 4°C, glycolysis appears to be limited by temperature. ATP regeneration cannot be driven at an adequate rate until the feedback drive (ADP + P_i) is increased considerably above the normal level. ATP is then maintained at a low phosphorylation potential. At 12°C glycolysis is not limited by temperature but is limited by low intracellular pH.

Other than gaining a better understanding of energetics in atrial trabeculae, we can ask whether these studies provided the transplant surgeon with any information of practical value? In answer to our initial question on the optimal temperature for preservation of grafts, we have provided evidence that, for preservation times of 5 hours or less, ATP levels are better maintained at 12°C [3]. For longer preservation times, ATP levels are better preserved at the lower temperatures. In addition, increasing the buffer capacity of preservation solutions used at 12°C has a major impact on maintenance of high energy phosphates.

b) Intact Hearts

Most published NMR studies on heart preservation have used rodent hearts, with a particularly large number of studies being performed on the rat heart. We have developed the isolated perfused pig heart for preservation studies [6] because it is architecturally, biochemically, and in size most similar to the human heart. As we discussed above, provision of oxygen and removal of metabolic waste products are critically important for long term heart preservation. Perfusion preservation, which can enhance oxygen delivery and waste removal from the heart, has not yet achieved much clinical application and remains mostly in the realm of the research laboratory. Some of the reasons for this are related to the implementation difficulties in situations in which the heart must be transported over long distances under sterile conditions. In addition, hearts preserved ischemically for less than 5 - 6 hours generally show good recovery of mechanical function after transplantation.

We have consequently focused our efforts on methods of improving long-term ischemic preservation of hearts. The goal is to optimize the conditions currently in use and to extend the safe preservation time between harvest and implantation of the donor organ. This should allow harvest of donor hearts to occur over a wider geographical range and provide for better immunological organ matching.

The studies of isolated, Langendorff perfused pig hearts [6] are performed using a Bruker Biospec instrument equipped with a 4.7 T / 30 cm horizontal bore magnet. The heart is arrested and isolated using techniques similar to those used for human hearts. The isolated heart is then placed in an NMR probe to observe high energy phosphate levels and pH on a continuous basis, with a two minute time resolution, during a hypothermic preservation period that usually lasts 8 hours. Following preservation, the heart is rewarmed to 37°C without removing it from the magnet and NMR spectra are then recorded in the beating heart. A balloon placed in the left ventricle measures the developed pressure and serves as an index of functional recovery of the heart [6]. In this manner, energy levels during and after preservation can be correlated with functional performance of the heart following preservation.

A number of technical difficulties arise in trying to obtain quantitative results from large, isolated, perfused hearts because they change shape when beating and often swell when perfused with solutions other than whole blood. To alleviate the problems caused by the sample moving in a heterogeneous B_1 field and the consequent uncertainty in received signal strength, a high B_1 homogeneity probe was designed [7]. The prototype probe comprised four separate tuned rings on a spherical surface (Figure 5) giving a B_1 field homogeneity of ± 5% over 60% of the radius of a 14 cm sphere (Figure 6).

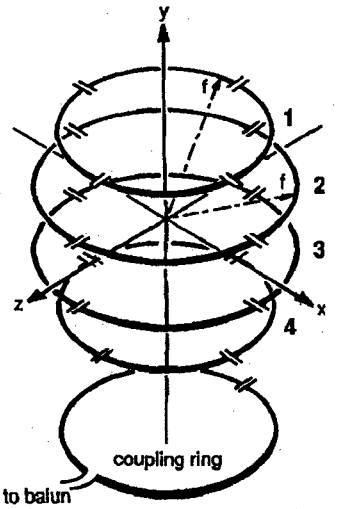

FIGURE 5. Geometry of the 4 coil system [7].

The received signal was rendered less sensitive to the dielectric constant of the sample by distributing the capacitance around the rings. Inter-ring coupling and to a fifth ring used for matching was by induction. The coupling loop was tuned with its own capacitor to Larmor frequency, thereby ensuring that the probe was always on resonance, and rendering the tuning and matching independent. In addition, the use of a low input impedance preamplifier

virtually eliminated the dependence of signal strength on coil loading [8].

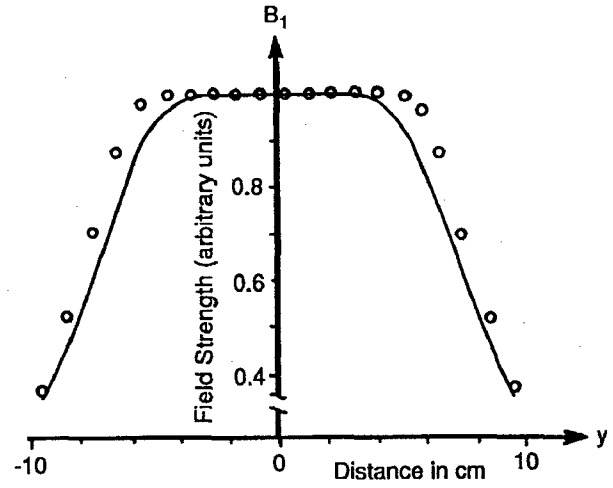

FIGURE 6 Plot down the Y axis of the B_1 field of the probe (open circles, f = 7 cm at 81 MHz) and the form of the plot predicted by the theory.

Strategies for improving hypothermic preservation have ranged from improving the buffer capacity of the preservation solution [6] to the use of secondary cardioplegia [6, 9] to maintain the heart in an arrested state during the entire rewarming phase prior to reperfusion. The purpose of secondary cardioplegia is to allow the energy of the heart to be directed towards the re-establishment of ionic balances that become disrupted by hypothermia and ischemia, rather than to expend energy in mechanical function. We have found that the use of secondary cardioplegia prior to reperfusion does not affect the net energy levels of the heart but rather eliminates ventricular fibrillation that is normally observed upon rewarming of hypothermically preserved hearts [6].

As a result of the increasing requirement for donor organs, a number of organs are frequently harvested from a single donor. This has led to the need for a single preservation solution suitable for all thoracic and abdominal organs. The University of Wisconsin Cold Storage Solution (UW-CSS, DuPont Pharmaceuticals) is currently in use for the preservation of liver, kidney and pancreas. Its utility for heart preservation remains to be determined. We compared the efficacy of UW-CSS to St-Thomas II solution, which is in widespread use for heart preservation [10]. Pig hearts were preserved for 8 hours at 4° or 12°C and then tested functionally after rewarming to 37°C. These temperatures were chosen because they are both in use clinically. At 4°C, UW-CSS and St-Thomas II were equally effective for preservation of heart function. Figure 7 and 8 shows the results obtained with UW-CSS and St-Thomas at 12°C. The lack of functional recovery observed with UW-CSS shows that this solution is unsuitable for use at 12°C. The results also demonstrate the necessity for precise temperature regulation when the solution is used at 4°C. This is not necessary with St-Thomas II solution because recovery is not severely compromised by use at either 4° or 12°C.

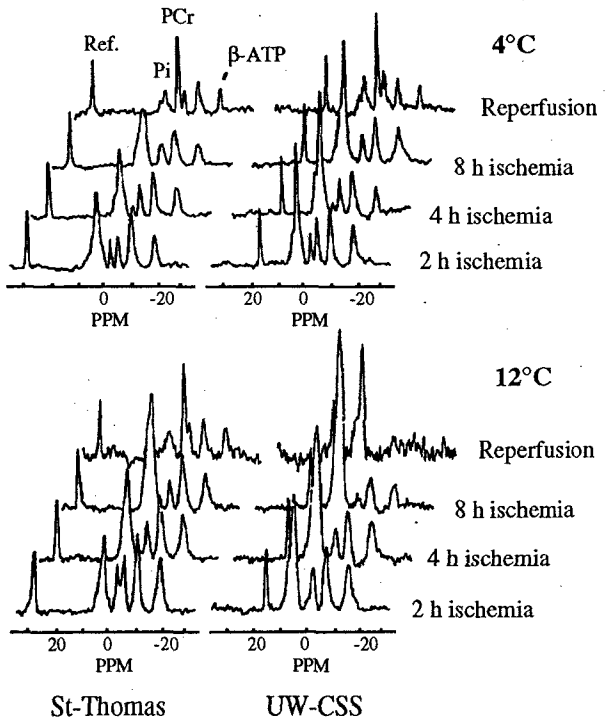

FIGURE 7. Typical time course of the ^{31}P NMR spectra of 4 hearts, two preserved at 4°C (top panels), two preserved at 12°C (bottom panels) [10]. Spectra on the left were obtained from hearts stored with St-Thomas II and spectra on the right were from hearts preserved with UW-CSS. The ATP and PCr disappeared upon reperfusion in the hearts stored with UW-CSS at 12°C.

One of the reasons for the failure of UW-CSS in heart preservation at 12°C could be the calcium paradox. This phenomenon occurs when a heart has been subjected to a calcium-free medium (UW-CSS contains no calcium) and then is reperfused with a solution containing calcium. The calcium paradox results in massive irreversible damage to cell membranes, as calcium from the reperfusion medium overloads the cells. This phenomenon is temperature-dependent and does not occur readily at low temperatures. In order to test the "calcium paradox" hypothesis, we added 0.5 mM calcium (0.08 mM free calcium) to UW-CSS and

repeated our studies at 12°C [11]. Figures 9 and 10 show the improvement observed in the high energy phosphates during reperfusion of a heart preserved with UW-CSS containing calcium.

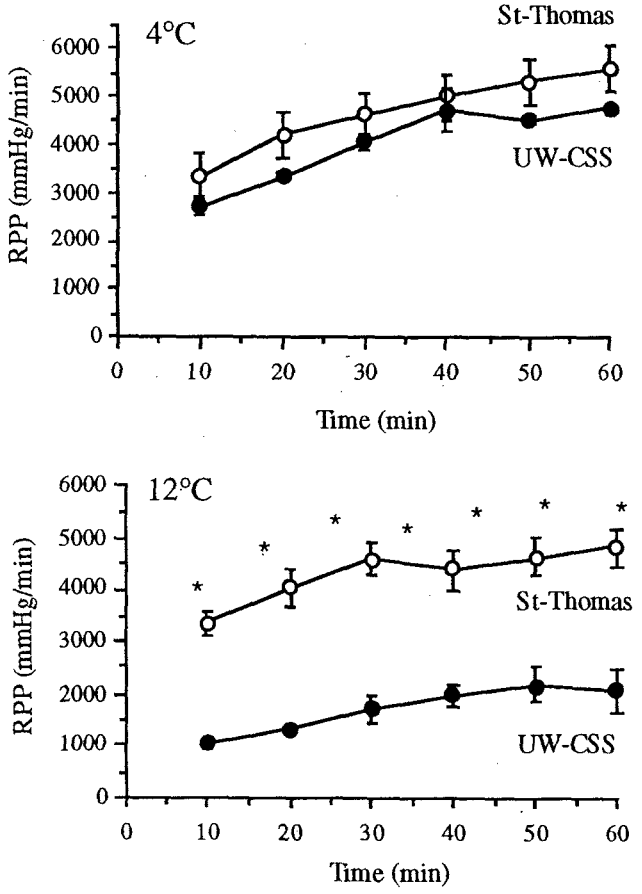

FIGURE 8 Time course of the rate pressure product (RPP: heart rate x developed pressure, a measure of heart function) during reperfusion (n = 7 in each group) [10]. The functional recovery was extremely poor in the hearts stored with UW-CSS at 12°C (* : $p < 0.05$)

These studies show that NMR spectroscopy can be a valuable tool in the design and modification of solutions for protecting the myocardium prior to transplantation. Cardiac surgery is another area in which NMR spectroscopy is being used.

3 Cardiac Surgery

Cardiac surgery is being offered to higher and higher risk patients. This has led to the need for improved methods of myocardial protection during surgery. Traditionally the heart is arrested and kept cold (4°C) with one or more infusions of a crystalloid solution, such as the St-Thomas II solution described above. The hypothermia and ischemia associated with the use of cold crystalloid solutions can impose additional stress on the damaged heart. One of the most recent modifications to cardiac surgical practice has been the use of continuous normothermic blood cardioplegia (CNBC) [12]. The purpose of CNBC is to avoid ischemia. With CNBC, the heart is maintained at 37°C in an arrested state by increasing the potassium concentration of a blood solution that continuously flows through the coronary vessels. Many questions remain unanswered regarding CNBC, such as the route of administration (retrograde and/or antegrade); b) the optimal volume of cardioplegia; c) the flow distribution of cardioplegia, and d) metabolic monitoring of the heart during cardioplegic arrest. For these purposes, a blood-perfused porcine heart model was developed for NMR studies of CNBC. In this model, the heart is continuously perfused with blood while being isolated from the animal. The heart can then be placed in the NMR magnet and its initial function assessed before it is arrested in the magnet. NMR surveillance of the high energy phosphates during CNBC may allow optimization of flow rates and the route of delivery (antegrade and/or retrograde) for maintenance of the energy status of the myocardium. In this context, localized NMR spectroscopy using either spectroscopic imaging [13] or surface gradient coils [14], allows assessment of protective techniques in selected regions of the heart [13, 15] or at various depths across the heart wall [14], respectively.

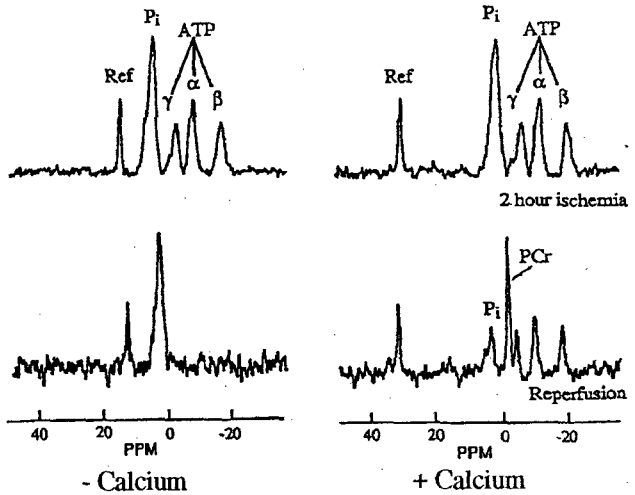

FIGURE 9 Typical ^{31}P NMR spectra of hearts preserved in unmodified UW-CSS (left panel), or with UW-CSS containing Ca^{2+} (right panel) [11]. The peaks of ATP and PCr disappeared upon reperfusion in the heart stored with unmodified UW-CSS.

The difference between the two curves is statistically significant ($p < 0.001$) [11].The NMR technique

may determine whether there is a safe time limit during which blood cardioplegia can be interrupted for surgical visualization. Initially, we evaluated the effect on cardiac energetics and function by interrupting the flow of CNBC, as occurs for instance during aorto-coronary bypass surgery. Our data show that the high energy phosphate profile deteriorates and PCr becomes unobservable within 14 ± 2 minutes when flow is interrupted for 20 minutes in the middle of a 1 hour period of CNBC. This is associated with decreased left ventricular function when the heart is tested after reperfusion, in spite of the fact that both PCr and pH returned to normal within 3 minutes of resuming CNBC [16].

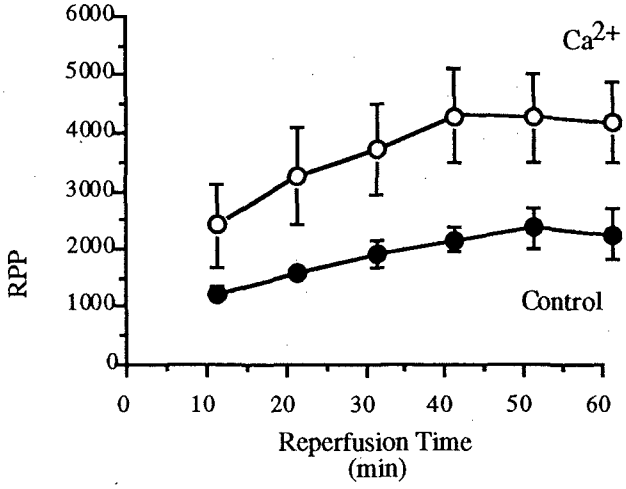

FIGURE 10. Time course of the rate pressure product of hearts reperfused with UW-CSS containing Ca^{2+} (0.5 mM) and without Ca^{2+} (mean ± SD, n=7 per group).

It has been proposed that CNBC could resuscitate the damaged heart during surgery by providing continuous delivery of oxygen and nutrients to the heart. In order to test one aspect of resuscitation, we subjected isolated hearts that had been previously stressed by a 20 minute period of normothermic ischemia to two types of cardioplegia and measured functional recovery following reperfusion [17]. Twenty minutes of normothermic ischemia reduced the ATP levels, measured by ^{31}P NMR, in CONTROL hearts (n=6) to 70 ± 7%. These hearts recovered 86 ± 18 % of their initial function (systolic elastance) when reperfused with normal blood perfusate. The experimental hearts were subjected to either intermittent cold blood cardioplegia (ICBC, n=6) for 5 min at 14°C, every 20 minutes, and repeated 3 times, or CNBC (n=6) for 60 minutes at a flow rate of 0.5 mL min^{-1} g^{-1} heart wet weight. Both ICBC and CNBC prevented exacerbation of the initial ischemic injury. PCr recovered to initial levels following reperfusion in all three groups (CONTROL, ICBC and CNBC) indicating that the mitochondria still possessed sufficient phosphorylating capacity to maintain appropriate physiological activity. ATP levels did not recover to initial levels in any of the groups. This could be related to the loss of nucleotide precursors from the cells during the initial ischemic period. Functional recovery with CNBC was 115 ± 30% compared to ICBC which was 88 ± 9% but there were no significant difference among the three groups by ANOVA ($p>0.05$).

Is there a safe period of normothermic ischemia? From a biochemical perspective, there may be a partial answer. Using ^{31}P NMR spectroscopy, we have seen that during an ischemic episode, PCr decreases before ATP. Theoretical calculations using the enzymatic equilibria of the creatine kinase and adenylate kinase reactions support this observation and have shown that there are two phases of energy depletion [18]: the buffering phase and the depletion phase. During the buffering phase, energy is derived from PCr and the adenine pool is stable. During the depletion phase, energy is primarily derived from adenine nucleotides. As ATP is consumed, AMP is produced which subsequently acts as a substrate for deamination and dephosphorylation reactions, whose products are lost from the cell. Upon reperfusion, although the PCr levels may return to normal, adenine nucleotides may not reach normal levels for a number of days. To avoid imposing a metabolic stress on the myocytes, PCr levels should not be allowed to become depleted to the point where adenine nucleotides will begin to be depleted. Although the role and critical level of ATP necessary for recovery of function in the ischemic heart are controversial, it seems logical to avoid any type of preventable metabolic stress to the heart during surgery. NMR spectroscopy is useful for monitoring the energy depletion and repletion processes in model systems such as the pig heart. This information can subsequently be used to verify the predictions of the theoretical calculations.

NMR can monitor PCr and ATP levels directly and continuously in the isolated perfused heart and *in vivo* [19]. In our studies, PCr levels reflect the balance of energy supply and demand; in the arrested heart they decrease and increase in concert with the availability of oxygen. However NMR techniques are not compatible with direct use in the surgical theatre. Recently developed fiber optic pO_2 probes based on oxygen quenching of fluorescence have been used

to monitor the arrested heart during surgery. We have performed NMR measurements on isolated blood perfused pig hearts and correlated the data with simultaneously measured levels of pO_2 using custom built (Innerspace, Irvine, Calif.) NMR-compatible probes. We have found a good correlation between tissue pO_2 (measured in mmHg) and PCr level in the normal heart. The data are illustrated in Figure 11. Although the data are preliminary, we see that PCr levels drop precipitously when the tissue pO_2 decreases below 30 mmHg. By establishing similar relationships in metabolically damaged or physically abnormal (hypertrophied for instance) hearts it may be possible to provide the surgeon with insight into a) the acceptable limits of oxygen deprivation should delivery of cardioplegia be discontinued during surgery; b) the optimal flow rates of cardioplegia; c) assessment of retrograde versus antegrade delivery; and d) cardioplegic flow distribution across the heart.

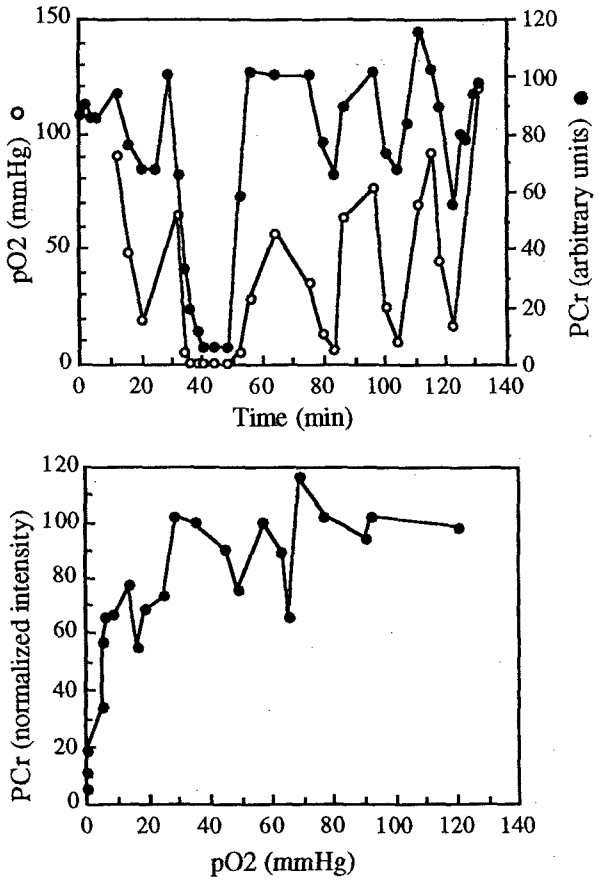

FIGURE 11. Correlation between the pO_2 and PCr levels in the normal heart. Top: pO_2 and PCr in an normal isolated blood perfused pig heart subjected to variations in perfusate flow rate. Bottom: PCr versus pO_2 obtained from the data in the top figure.

4 Conclusions

NMR spectroscopy is becoming an invaluable tool for the development of methods and techniques for heart transplantation and surgery. The advent of very high field (3 - 4 T) wide bore (1 m) magnets and sophisticated localization techniques will continue to increase the relevance of NMR techniques for studies of large isolated organs and intact animal models.

Acknowledgment

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