Mechanisms of Ischemic Myocardial Cell Damage Assessed by Phosphorus-31 Nuclear Magnetic Resonance

JOHN T. FLAHERTY, M.D., MYRON L. WEISFELDT, M.D., BERNADEINE H. BULKLEY, M.D., TIMOTHY J. GARDNER, M.D., VINCENT L. GOTT, M.D., AND WILLIAM E. JACOBUS, PH.D.

SUMMARY  Phosphorus-31 nuclear magnetic resonance (31P NMR) can estimate tissue intracellular pH as well as the content of high-energy phosphate metabolites in isolated perfused hearts. We used 31P NMR to examine mechanisms associated with the recovery of ventricular function in hearts subjected to global ischemia and reperfusion, with special emphasis on intracellular pH, a previously unreported variable. Single-dose and multiple-dose administration of a hyperkalemic cardioplegic solution were compared with hypothermia alone in 18 isolated perfused rabbit hearts. Hearts in group 1 were subjected to 24°C hypothermia during 60 minutes of global ischemia; group 2 hearts received a single injection of 37-mM KCl cardioplegic solution at 10°C at the onset of ischemia; and group 3 hearts received a similar initial cardioplegic injection followed by two subsequent 24°C injections at 20-minute intervals during the ischemic period. Using an intraventricular balloon, maximal dP/dt provided a quantitative index of left ventricular performance before and after ischemia. Return of ventricular function expressed as a percentage of control was 54 ± 11% for group 1, 84 ± 6% for group 2, and 101 ± 18% for group 3. Differences in the rate of development of intracellular acidosis were noted during the 60-minute ischemic period. Intracellular pH fell to 6.09 ± 0.12 in group 1, 6.31 ± 0.09 in group 2, and 6.79 ± 0.03 in group 3. In all three groups intracellular pH returned to control (pH 7.20) within 10 minutes of reflow.

The metabolic correlates of functional recovery appeared to be the tissue content of ATP at the end of ischemia and after reflow. ATP content at the end of ischemia was 22 ± 2% of control in group 1 hearts, 31 ± 4% in group 2 and 64 ± 2% in group 3. After 45 minutes of reperfusion, ATP levels recovered to 33 ± 9% of control in group 1, to 71 ± 9% in group 2 and to 86 ± 6% in group 3. Although there were no differences between groups in the content of creatine phosphate after 60 minutes of ischemia, the rates of creatine phosphate decline were dissimilar. Further, during the early reflow period, a marked overshoot in tissue creatine phosphate was detected, especially in groups 1 and 2. Histologic damage assessed by light microscopy correlated with the metabolic data, confirming that multidose cardioplegia provided the best preservation of cellular morphology.

These results demonstrate that the magnitude of intracellular acidosis and the associated increase in inorganic phosphate correlate inversely with recovery of postischemic ventricular structure and function. ATP, but not creatine phosphate, content correlates with return of contractile performance after reperfusion. The overshoot in creatine phosphate during early reperfusion might impede optimal restoration of ATP content and, as a result, optimal recovery of cell functions.

THE ADMINISTRATION of single and multiple doses of a hyperkalemic cardioplegic solution during prolonged global ischemia improves myocardial preservation.1-4 However, the mechanisms of improved protection are not clear. In previous studies, mass spectrometry was used to quantitatively index myocardial metabolism during global ischemia. The magnitude of the rise in myocardial carbon dioxide tension (Pmco2) during 1 hour of ischemia correlated inversely with subsequent recovery of ventricular function.5-7 With respect to the cellular content of adenosine triphosphate (ATP) and creatine phosphate (PCr), Hearse et al.8 and others9-11 demonstrated a correlation between functional recovery and the tissue content of these metabolites. Although there has been much speculation concerning the role of intracellular acidosis as a causative agent in ischemic damage, the relationship of the time course of intracellular pH (pHi) during ischemia and the resultant recovery of myocardial function has not been documented.

Tissue pHi can be determined from the chemical shift of the inorganic phosphate (Pi) peak in phosphorus-31 nuclear magnetic resonance (31P NMR) spectra. This method of measuring pHi was first described by Moon and Richards12 for use in erythrocytes, but was later applied to the skeletal muscle2-16 and to the intact heart.14,16,17 Intracellular pH is considered to have an important, if not critical, role in the pathogenesis of ischemic injury.16-18 Therefore, we used 31P NMR to serially and noninvasively monitor pHi, as well as the ATP and PCr content, in studies of myocardial preservation.

The present study was designed to test the following hypotheses: (1) that a single dose of potassium cardioplegic solution, shown to retard the rate of rise in

From the Departments of Medicine, Physiological Chemistry, and Surgery, The Johns Hopkins Medical Institutions, Baltimore, Maryland.

Supported by USPHS grants 1 RO 1 HL-19414 and 5 RO 1 HL-22060 and by SCOR in Ischemic Heart Disease P 50 HL-17655, NHLBI.

Dr. Flaherty was supported by NHLBI Research Career Development Award KO 4 HL-00019.

Dr. Jacobus is an Established Investigator of the American Heart Association.

Address for correspondence: John T. Flaherty, M.D., Division of Cardiology, The Johns Hopkins Medical Institutions, 601 North Wolfe Street, Baltimore, Maryland 21205.


561
Pm\textsubscript{CO}_2 during global ischemia,\textsuperscript{4} retards the rate of development of intracellular acidosis as well as the rate of use of ATP and PCr; (2) that multiple doses of cardioplegic solution during ischemia further slow the rate of fall in pH\textsubscript{1}, as well as the decline of ATP and PCr; and (3) that the degree of recovery of ventricular function and the degree of morphologic protection after ischemia and reperfusion are related directly to preservation of high-energy phosphate compounds and inversely to the severity of intracellular acidosis during ischemia.

**Materials and Methods**

**Perfusion Methods**

Female New Zealand white rabbits that weighed 1.2–2.0 kg were heparinized and anesthetized; their hearts were removed and quickly placed into iced saline (150 mM NaCl). The perfusion apparatus was a modified Langendorff preparation with the ascending aorta terminally cannulated and perfused from a reservoir maintained at 37°C (fig. 1). The perfusate, a modified Krebs-Ringers bicarbonate buffer solution, contained 117 mM sodium chloride, 6 mM potassium chloride, 3.0 mM calcium chloride, 1.0 mM magnesium sulphate, 0.6 mM EDTA and 16.7 mM glucose. The final pH was adjusted to 7.40 by adding 24 mM sodium bicarbonate. The perfusate was bubbled continuously with a mixture of 95% oxygen and 5% carbon dioxide. The perfusate contained no Pi. Therefore, any Pi signal in the NMR spectra must be derived from tissue Pi. In control experiments there was no difference in long-term functional or metabolic stability when rabbit hearts were perfused for 2 hours with either phosphate-containing or phosphate-free perfusate. The perfusion pressure was 110 cm of water. Perfusate flow was removed from the NMR sample tube by vacuum aspiration.

The heart rate was maintained at 150–170 beats/min by right ventricular pacing with a wick soaked in saturated potassium chloride, encased in polyethylene tubing, and connected to a Grass SD-9 stimulator. A latex balloon tied to the end of a 100-cm length of PE 190 tubing was carefully purged of air bubbles and connected by a three-way stopcock to a Statham P23Db transducer. Isovolumic left ventricular pressure was recorded during preischemic control and postsischemic reflow periods with a Brush two-channel direct-writing recorder. The first derivative of ventricular pressure was simultaneously recorded with an analog differentiator. The balloon was initially inflated by syringe with enough fluid to produce a left ventricular end-diastolic pressure of 10 mm Hg; subsequent measurements of left ventricular developed pressure and maximal dP/dt were made at the end-diastolic volume that resulted. All hearts were subjected to 1 hour of global ischemia at 24 ± 1°C. Aortic inflow was totally interrupted by cross clamping the perfusion line. Cold cardioplegic solution (10°C) was injected immediately thereafter into the aortic root through a catheter attached to a sidearm just above the heart. The cardioplegic solution contained 34 mM potassium chloride, 68 mM sodium chloride and 98 mM glucose. Sodium bicarbonate was added to titrate the pH of the solution to 7.40, which required approximately 24 mM sodium bicarbonate, yielding a final osmolality of approximately 330 mosmol/l. Forty-five minutes of normothermic reperfusion at 35 ± 2°C was used after the ischemic period. During reperfusion, functional recovery was assessed. Postsischemic recovery was calculated as a percentage of the preischemic control function. The balloon was deflated and pacing was discontinued for the 60-minute ischemic period. Pacing was re instituted if the spontaneous heart rate was less than 150 beats/min, and the balloon was reinflated 15 minutes after reflow was started, just before the measurement of ventricular function.

**Nuclear Magnetic Resonance Methods**

Phosphorus-31 NMR spectra were obtained on a Bruker WH-180 spectrometer at a 4.2-tesla field in a wide-bore superconducting magnet. At this field, phosphorus resonates at a frequency of 72.89 MHz; the diameter of the phosphorus probe was 25 mm. This instrument was operated in the pulsed Fourier transform mode and was interfaced to a Bruker 1080
computer; the data were collected on low-density magnetic disks. Because of the field stability of the superconducting magnet, a field/frequency lock was not required. Proton-decoupled spectra were collected during the relaxation period after 45° pulses delivered at two-second intervals. The relaxation rates expressed as T1 under these conditions were 1.64 seconds for Pi, 3.33 seconds for glycerol phosphoryl choline (GPC), 2.72 seconds for PCr and 0.89 for β ATP. The slowest relaxing signal (GPC) should fully recover in 1.25 seconds or less. Thus, the 2-second delay between pulses results in the acquisition of minimally saturated spectra. The data were accumulated with a 4K table at a spectral width of 5000 Hz. Typical spectra (150–300 pulses) for control, ischemia and reflow are shown in figure 2. The 60-minute multidose KCl spectrum shows the marked decline in PCr and the stoichiometric increase in the Pi peak. The calibration bar above the Pi peak also indicates the chemical shift of the Pi peak under control conditions. In the second spectrum (ischemic), upfield migration of the Pi peak is present. The magnitude of this shift is used to estimate the decrease in tissue pH1.

Estimation of Tissue Intracellular pH

Intracellular pH was measured from the chemical shift (δo) of the Pi peak by the following equation:

\[
pH_i = pK - \log \frac{\delta_o - \delta_B}{\delta_A - \delta_o}
\]

To minimize tissue inhomogeneity effects, chemical shifts were measured relative to the resonance of PCr, which is relatively independent of pH over the range of pH encountered in these studies (pKₐ = 4.6). Under these conditions, the constants used in this equation are pK = 6.90, δₐ = 3.299 ppm, and δₐ = 5.805 ppm, as previously reported.¹⁷

The validity of this equation is illustrated in figure 3. In this figure, the solid line was mathematically derived by the insertion of appropriate values of δo to calculate the expected pH1. The actual data points were derived from NMR experiments as follows. A 10-mM K₂HPO₄ solution was titrated through the pH range from 9.0–5.0. At each pH value, a 200-pulse NMR spectrum was obtained, and the chemical shift of the Pi peak was measured relative to a vertically aligned sample of PCr (pH 7.2). In control experiments, the chemical shift of the Pi peak was not affected by altering the concentration of monovalent and divalent cations within physiologic ranges; nor were the NMR phosphate titration data altered when phosphate was titrated in a supernatant solution from a heart homogenate. The close agreement between the theoretical curve and the NMR data under all conditions suggests that the method may be appropriate for measuring pH1 in intact tissue.

Quantitation of Metabolites

The estimation of tissue levels of PCr, ATP and Pi were obtained by planimetric measurement of the areas under the individual peaks using the appropriate normalization constants (fig. 2). A Hewlett Packard digitizer was used to perform the area integrations. Quantitative data thus derived for PCr, ATP and Pi content are expressed as a percentage of the preischemic control levels. Data are presented as mean ± SEM. Statistical analyses were performed using the unpaired t test or the repeated-measures analysis of variance where appropriate.

Morphologic Methods

Transmural biopsies obtained for light microscopic examination were fixed in 10% buffered formalin. Ad-
Additional samples were fixed in cold 3% gluteraldehyde in 0.1 M phosphate buffer, washed with several changes of 0.1 M phosphate buffer (pH 7.4), postfixed for 90 minutes with osmium tetroxide in sucrose-phosphate buffer, dehydrated in a graded series of alcohols and acetone, embedded in epoxy resin and cut into semithin (1 μ thick) sections. These sections were stained with toluidine blue and examined with the light microscope. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with the electron microscope (AEI). Specimens for light microscopy were embedded in paraffin, sectioned and stained with hematoxylin and eosin and phosphotungstic acid hematoxylin.

Ischemic damage was estimated by the presence and extent of contraction band injury across the entire transmural section of the ventricle at the level of the papillary muscles. The amount of injury was assessed quantitatively by grading the slides at 400 × magnification with an overlying 6 × 6 grid. Foci of contraction band injury within a box in the grid were counted in each of 10 randomly selected fields. The grading was done on two occasions without knowledge of the identity of the histologic section. For each slide the score for 10 fields were summed, and a mean score was determined for each group. Electron micrographs were evaluated qualitatively using the presence and severity of mitochondrial swelling, disruption and mineralization, the extent of contraction and disorganization of sarcomeres, and the clumping of nuclear chromatin as signs of ischemic injury. The electron micrographs (six to 10 per heart) were read blindly. The ischemic injury was graded as mild, intermediate or severe.

**Experimental Design**

Eighteen hearts were studied using three experimental protocols. Five hearts received no cardioplegia, but were maintained under hypothermic conditions (23–25°C) for the entire 60 minutes of total ischemia (group 1). Five hearts received a single 10-ml bolus of cold (10°C) cardioplegic solution immediately after the perfusion line was clamped, and remained hypothermic throughout the ischemic period (group 2). In eight hearts, the initial bolus of cold cardioplegic solution was introduced at the onset of ischemia, followed by two additional boluses of solution at 23–25°C after 20 and 40 minutes of ischemia (group 3).

A control 31P NMR spectrum was obtained before the ischemic period using 300 pulses (10 minutes) (fig. 2). After the onset of ischemia, three 150-pulse spectra (5 minutes) were sequentially obtained during the time intervals 0–5, 5–10 and 10–15 minutes. Three subsequent 300-pulse spectra were then collected at 20–30, 35–45 and 50–60 minutes of ischemia. During the normothermic (37°C) reflow period, 150-pulse spectra were collected at 5–10 and 10–15 minutes, and 300-pulse spectra were collected 20–30 and 35–45 minutes after the cross clamp was released.

Ventricular function was recorded during the preischemic control period and then after 15, 30 and 45 minutes of reperfusion. Hearts that did not receive cardioplegia (group 1) were defibrillated by briefly removing the heart from the magnet 5 minutes after reflow began and applying a single direct-current countershock of 1–2 W-sec. All hearts that received cardioplegic solution began beating spontaneously immediately after reperfusion and did not require electrical cardioversion.

**Results**

**Intracellular pH**

In the hearts treated with hypothermia alone (group 1), pH, decreased progressively from a control value of 7.20 ± 0.01 to 6.09 ± 0.12 at the end of the 60-minute ischemic period (p < 0.001 vs control) (fig. 4). After reperfusion was started, pH, underwent a slight overshoot and then rapidly returned to control values within the first 5 minutes and remained stable and in the normal range during the remaining 40 minutes of reperfusion. The administration of a single dose of potassium cardioplegia significantly decreased the rate of fall in pH, in group 2 hearts. During ischemia, pH, was significantly higher in hearts treated with cardioplegia than those in the untreated group (p = 0.0019 vs group 1). After 60 minutes of global...
ischemia, pH$_1$ had decreased to 6.31 ± 0.09 in the hearts given cardioplegia (group 2), compared with 6.09 ± 0.12 in the group 1 hearts. After reperfusion, pH$_1$ in group 2 hearts also returned to normal with 5 minutes of reflow, without undergoing overshoot ($p = 0.016$ vs group 1). Hearts that received multiple doses of potassium cardioplegia (group 3) demonstrated a further slowing in the rate of decrease in pH$_1$ throughout the ischemic period: pH$_1$ decreased to 6.79 ± 0.03. Throughout the ischemic period, pH$_1$ was significantly less acidic than after single-dose cardioplegia ($p < 0.001$ vs group 2). In group 3, pH$_1$ returned to normal within 10 minutes after reperfusion was begun and remained there for the remainder of the 45-minute reflow period.

**Creatine Phosphate**

After the onset of global ischemia, PCr levels decreased rapidly in all three groups (fig. 5). The early rate of decrease in PCr content differed in the three groups, but after the ischemic period, hearts in all groups were equally depleted of PCr. After 60 minutes of ischemia, PCr reached 3 ± 2% of control in group 1, 5 ± 1% of control in group 2 and 6 ± 2% of control in group 3 (NS). After initiation of reflow, PCr levels increased rapidly in all three groups. Hearts in groups 1 and 2 exceeded control content during the first 10 minutes of reperfusion, peaking at 160–180% before decreasing to 110–130% after 45 minutes of reperfusion. In contrast, hearts that received multiple doses of cardioplegia had a smaller overshoot, reaching only 115% of control during the first 10 minutes of reflow and then stabilizing at 104 ± 5% of control by the end of the reflow period. After 45 minutes of reflow, the PCr content did not differ significantly between the three groups, despite marked differences in ventricular function.

**Adenosine Triphosphate**

In hearts kept hypothermic without cardioplegia during ischemia (group 1), ATP fell progressively to 22 ± 2% of control, a content significantly lower than the 31 ± 4% in hearts that received single-dose cardioplegia (group 2) ($p < 0.05$ vs group 1) (fig. 6). The ATP content in hearts that received multiple-dose cardioplegia (group 3) was even higher (64 ± 2% of control) after 60 minutes of ischemia ($p < 0.05$ vs group 2). After reperfusion, the ATP content of group 1 increased to 43 ± 6% of control within 10 minutes and declined to 33 ± 9% of control after 45 minutes of reflow. In group 2 hearts, ATP returned to 80 ± 11% of control after 10 minutes of reflow and to 71 ± 9%...
after 45 minutes of reflow ($p < 0.05$ vs group 1). In group 3 hearts, ATP returned to even higher levels (94 ± 12% of control) after 10 minutes and was 86 ± 8% after 45 minutes of reflow.

**Inorganic Phosphate**

Pi increased rapidly during the first 15 minutes of ischemia in all groups of hearts and subsequently the rate of rise declined. Peak amounts were reached at the end of the 60-minute ischemic period. Group 1 hearts reached 1580 ± 720% of control, group 2 hearts 789 ± 153% and group 3 hearts 836 ± 160%. As with PCr, there were no significant differences between these values. During reperfusion, Pi returned rapidly toward, but did not reach, control levels in all groups of hearts. After reperfusion, Pi was 208 ± 41% of control in group 1, 257 ± 62% in group 2 and 181 ± 36% in group 3 (all NS).

**Left Ventricular Function**

Before the onset of ischemia, control developed pressure was 101 ± 17, 122 ± 5 and 106 ± 8 mm Hg in groups 1, 2 and 3, respectively (NS). After 60 minutes of ischemia and 45 minutes of reperfusion, maximal positive dP/dt returned to 54 ± 11% of control in group 1 hearts, 84 ± 6% in group 2 ($p < 0.05$ vs group 1) (fig. 7) and 101 ± 18% in group 3. A similar pattern of preservation of ventricular function could be seen by comparing recovery of peak left ventricular developed pressure, also an index of postischemic ventricular function. Peak left ventricular developed pressure returned to 67 ± 5% of control in group 1, 98 ± 12% in group 2 ($p < 0.05$ vs group 1) and 102 ± 5% in group 3.

**Light and Electron Microscopic Analysis of Morphologic Damage**

Histologic evaluation by light microscopy showed significant differences in the mean injury score; the multidose cardioplegia group showed the least injury. A high injury score reflects severe morphologic damage. A typical low-power field for group 1 hearts is shown in figure 8. Group 1 hearts (hypothermia alone) had an injury score of 82.4 ± 13.5, group 2 hearts (single-dose cardioplegia) had a score of 52.8 ± 3.1, and group 3 hearts (multidose cardioplegia) had a
The magnitude of intracellular acidosis and the associated increase in Pi content varied inversely with the degree of recovery of postischemic left ventricular function. Serial measurement of myocardial ATP and PCr during ischemia and reperfusion revealed that only ATP content correlated with preservation of ventricular performance. Postischemic ventricular function appeared to be independent of PCr content.

Melrose et al. first proposed chemical cardioplegia to preserve ischemic myocardium during elective cardiac surgical procedures. Gott et al. and Greenberg et al. showed that topical myocardial hypothermia also provides protection, as evidenced by improved preservation of high-energy phosphate metabolites and better postischemic ventricular function. However, little is known about the basic mechanisms at the molecular level by which ischemic cell damage can be prevented. The biophysical technique of 31P NMR appears to be a useful method for probing the metabolic changes associated with global ischemia and reperfusion. With the NMR we can obtain information such as pH, which is difficult if not impossible to acquire by previous techniques.

Many attempts have been made to correlate ischemic metabolic events with postischemic ventricular performance. The content of ATP and PCr in myocardial biopsies or in freeze-clamped hearts has been used to index ischemic metabolism. Studies from our laboratory have shown the usefulness of mass spectrometry in measuring myocardial gas tensions and quantitatively indexing the severity of global myocardial ischemia. The role of intracellular acidosis during the early phases of ischemia has been described, but no correlation has been demonstrated between the severity of acidosis during global ischemia and the recovery of contractility after reperfusion. Except for the microelectrode technique, which can induce cellular damage, NMR is the only method currently available for assessing tissue pH under conditions of no flow. The use of weak acids or weak bases to calculate a transmembrane pH gradient requires perfusion of the tissue as well as equilibration across membranes, whose permeability properties may be altered by ischemia. Because flow is required, these latter methods cannot be applied during total ischemia. Thus, 31P NMR appears to be a unique method by which to serially measure pH under conditions comparable to those encountered during cardiac surgery.

Although the NMR values for pH are informative, they may be subject to error in absolute calibration. Salhany et al. reported a pH of 7.0 ± 0.1 in Langendorff-perfused, nonworking guinea pig hearts. In the in vivo rat heart, pH was reported to be 7.11 ± 0.01. These variations could be related to differences in species or perfusion models or to NMR variables. For example, magnetic field homogeneity could be influenced by the irregular geometry of the beating heart, which is in addition a nonspinning sample. However, calculation of the chemical shift of phosphate relative to another intracellular metabolite such as PCr should minimize these effects. The Oxford...
group reported Pi chemical shift in normal hearts to be 4.92 ppm from PCr. Using this value they estimate pH, at 7.11. However, when this number was inserted into our titration equation, we calculated a pH of 7.17, which is within the range of experimental error for reported normal values of 7.18–7.20. This suggests that the problem with NMR pH data may lie with the titration curve calibration and its effect on the calculation of the data. Such calibration errors would affect principally the calculation of absolute pH and should have only small effects on the estimation of changes in pH in a given heart. Therefore, our data on the magnitude of intracellular acidosis during ischemia are both valid and important.

Additional important features of NMR are its time resolution and its ability to acquire pH, PCr and Pi simultaneously in an isolated perfused heart. When serial measurements of ATP and PCr content during ischemia are made by standard analytic techniques, they are by necessity made on hearts which were different from those in which ventricular function was assessed. The NMR method, in contrast, allows pH, and phosphate metabolites to be measured in the hearts in which functional recovery is being assessed and also permits serial data to be obtained frequently during the ischemic and reflow periods.

Our data and the data obtained by Reibel and Rovetto suggest that there may be little need for concern about preservation of PCr or creatine during global ischemia. We observed complete and, in fact, supranormal recovery of PCr in hearts that exhibited marked abnormalities of postischemic ventricular structure and function. Further, the discovery of a PCr overshoot during reperfusion raises several important issues. Compared with other tissues, both heart and skeletal muscle contain considerably less Pi than the number of acceptor sites for phosphorylation, e.g., creatine and ADP. That is, because the quantities of Pi are limited, heart and skeletal muscle can never fully convert all their ADP and creatine into ATP and PCr. Liver, kidney and brain tissue contain more Pi than available acceptor sites. In the normal heart, therefore, Pi incorporation into ATP and PCr appears to be balanced. However, during reflow of postischemic hearts that had excess Pi available, phosphate appears to be preferentially incorporated into PCr presumably by the coupling of mitochondrial creatine kinase to the reactions of electron transport. Thus, preferential incorporation of Pi into PCr could critically reduce the amount of Pi available for ATP resynthesis during recovery from an ischemic insult and thereby limit the amount of ATP available for vital bioenergetic activities.

It is also possible that excess transfer of phosphate into PCr during early reflow could directly inhibit myocardial metabolism. In 1968, Atkinson proposed that the adenylate energy charge was a key factor in the regulation of cellular metabolism. The energy charge is defined as the ratio of the high-energy adenylates over the total adenylate pool: [ATP + 1/2

![Figure 9. Electron micrographs from a heart in group 1 showing evidence of ischemic injury. There is marked chromatin clumping of the nucleus (nuc); the sarcomeres (s) are in disarray and are contracted; some are showing contraction band (cb) formation; and the mitochondria (m) are swollen and contain electron dense granules (g), a probable sign of irreversible injury (original magnification left × 8000; right × 20,000).](image-url)
ADP]/[ATP + ADP + AMP]. The validity of this factor in regulating metabolism in heart muscle, which also contains PCr and creatine, has recently been challenged. Seraydarian\textsuperscript{7} suggested that in the heart, mitochondrial respiration may instead be controlled by the PCr mole fraction: [PCr]/[PCr + creatine]. Therefore, a supranormal concentration of PCr could act as an inhibitory signal to aerobic as well as glycolytic pathways.\textsuperscript{38}

Our results further suggest that preservation of ATP content is the better metabolic correlate of functional recovery. Other reports describe excellent preservation of myocardial function in the presence of reduced levels of ATP. A threshold content of ATP for posts ischemic recovery has been proposed by Bretschneider\textsuperscript{38} and a critical pool of ATP necessary for preservation of membrane integrity by several other groups.\textsuperscript{8, 40, 41} While our data appear compatible with these concepts, further direct experimental validation is required because no specific ATP-requiring protective reaction has been identified.

Maintenance of adenine nucleotides in the form of ATP is an effective mechanism for minimizing another potentially harmful event — nucleotide base degradation to adenosine. It is important to prevent degradation to adenosine because resynthesis of ATP requires the action of adenosine kinase (E.C. 2.7.1.20) to initially form AMP, then myokinase, and finally oxidative phosphorylation or glycolysis to form ATP. However, it also requires ATP to make ATP because the first two steps, catalyzed by adenosine kinase and myokinase, use ATP as substrate. Thus, hearts in which ATP content has been severely reduced by ischemia have limited ability to regenerate ATP because of the reduced concentration of priming substrate. An additional obstacle to the full regeneration of ATP by posts ischemic myocardium is the very low content of adenylate kinase in cardiac muscle. Even in the presence of adequate ATP, the maximal rate of ATP regeneration starting with adenosine is severely restricted. Support for the beneficial effects of preventing nucleotide degradation comes from a study in which EHNA (erythro 9 hydroxy-3-nonyl adenine), an inhibitor of adenosine deaminase, was used.\textsuperscript{42} The resultant accumulation of adenosine would act by mass action to retard the further degradation of AMP to adenosine by 5'-nucleotidase. Combined administration of additional adenosine would be expected to provide further product inhibition.

Global ischemia results in the net hydrolysis of ATP, which generates both an inorganic phosphate and a hydrogen ion\textsuperscript{42}: ATP + H$_2$O $\rightarrow$ ADP + Pi + H$^+$. Differences in the rate of accumulation of excess protons under different conditions of myocardial preservation are dramatically apparent when hydrogen ion concentration instead of pH is plotted against duration of ischemia (fig. 10). Comparison of the data in figure 10 with the ATP data in figure 6 reveals that hearts with the slowest rate of hydrogen ion accumulation had the best preservation of ATP. However, mechanisms of ischemic cell damage might include the decline in ATP content, the degree of intracellular acidosis and the rise in Pi content. The magnitude and duration of acidosis during ischemia could contribute to irreversible cellular damage by inducing acid activation of lysosomal hydrolases.\textsuperscript{44} The rise in Pi content during ischemia could itself interfere with myocardial metabolism by irreversibly altering the localization and thus the activity of mitochondrial creatine kinase.\textsuperscript{45}

The mechanism by which potassium cardioplegia improves myocardial preservation appears to be related to the induction of complete electrical and mechanical standstill. This would result in a decrease in the rate of use of both substrate and high-energy phosphate metabolites as well as the production of excess protons. If we assume that ATP turnover is similar in the cases of both single-dose and multidose administration of cardioplegia, then the better preservation of ATP with multidose must result from more effective anaerobic metabolism. Multidose administration of the cardioplegic solution could not only wash out hydrogen ions but also provide additional buffering capacity in the form of bicarbonate and additional substrate in the form of glucose. The importance of reducing the severity of acidosis for ischemic myocardial preservation was suggested by the results of a study from our laboratory that compared BES buffer (pK$_A$ 7.17) with TRIS (pK$_A$ 8.16) and bicarbonate (pK$_A$ 6.18) buffering of a cardioplegic solution administered in a multidose fashion.\textsuperscript{46} Maintenance of pH$_I$ and functional recovery were better when BES buffer or bicarbonate was used than when Tris buffer was used.

In conclusion, $^{31}$P NMR is a valuable new tool for studying interventions designed to reduce the severity of ischemic and reperfusion injury due to prolonged global ischemia. The degree of decline in pH$_I$ and the associated increase in Pi correlate inversely with recovery of postischemic ventricular structure and
function. Further, ATP, and not PCr, content correlates with the return of contractile performance. Finally, the PCr content appears to overshoot inappropriately during the early reflow period, potentially impeding optimal restoration of myocardial ATP content, which may be critical for recovery of vital cell functions.

References

33. Vary TC, Angelakos ET, Schaffer SW: Relationship between adenine nucleotide metabolism and irreversible ischemic tissue damage in isolatedperfused rat heart. Circ Res 45: 218, 1979
Comparative Value of the Cold-pressor Test and Supine Bicycle Exercise to Detect Subjects with Coronary Artery Disease Using Radionuclide Ventriculography

DANTE E. MANYARI, M.D., ANDRE J. NOLEWAJKA, M.D., M.C.L.S.C., PAUL PURVES, RTNM, ALLAN DONNER, PH.D., AND WILLIAM J. KOSTUK, M.D.

SUMMARY Left ventricular ejection fraction (EF) and wall motion studies were performed using blood pool cardiac scintigraphy before and during the cold-pressor test (CPT) and bicycle exercise. Twenty normal subjects responded to the CPT with no change or a significant increase (7% or more) of the EF and no new wall motion abnormalities. Mean EF increased significantly ($p < 0.01$). Two subjects responded abnormally to the CPT, one with a significant decrease (7% or more) in EF and another with the development of new wall motion abnormalities. During exercise, EF increased significantly in all but one subject ($p < 0.001$). No new wall motion abnormality was seen.

In 20 patients with coronary artery disease (CAD) and normal resting left ventricular function, mean EF decreased ($p < 0.001$) during the CPT, but only 11 subjects could be identified individually by a drop in EF of 7% or more. During exercise, 18 of the 20 patients responded abnormally (failure to increase EF by 7% or more). Twelve patients showed new wall motion abnormalities during CPT and 15 during exercise. Three patients during the CPT and one during exercise had normal EF response while developing new wall motion abnormalities.

Thus, the sensitivity of radionuclide EF changes during the CPT to detect subjects with CAD was 55%. It increased to 70% when wall motion analysis and EF changes were considered. The specificity was then 90% and the predictive accuracy was 88%. The sensitivity of radionuclide studies during exercise, considering EF changes and wall motion analysis under otherwise similar conditions, was 95%. Specificity and predictive accuracy were also 95%. We conclude that the CPT is not as sensitive as exercise for detecting subjects with CAD by radionuclide cardiac angiography. The CPT may be a useful intervention in subjects in whom adequate exercise cannot be accomplished.

SINCE Hines and Brown described the cold-pressor test (CPT) in 1932, it has been used to identify subjects with hypertension and atherosclerosis. However, its acceptance in the clinical setting was limited, mainly because the initial hypothesis that the vascular reactivity in subjects with these conditions is different from that in normal subjects has not been verified. More recently, it has been reported that the CPT may induce local and global left ventricular dys-function in many patients with coronary artery disease (CAD). Furthermore, because assessment of left ventricular function during exercise may be cumbersome and time-consuming, the CPT may be a preferred method of stress to detect subjects with CAD. This alternative however, has not been adequately tested.

We undertook this study to determine the comparative values of isotonic leg exercise and the CPT for identifying subjects with CAD and normal left ventricular function, by noninvasively assessing left ventricular function changes during these forms of stress.

Methods

Patients

The control group included 20 normal subjects, ages 24–66 years (mean 39 years). Fifteen were males and

From the Cardiac Investigation Unit, Department of Medicine, University Hospital, University of Western Ontario, London, Canada.

Supported by a grant from the Ontario Heart Foundation.

Address for correspondence: Dr. W.J. Kostuk, Cardiac Investigation Unit, University Hospital, Box 5339, Terminal A, London, Ontario, Canada N6A 5A5.

Received March 20, 1981, revision accepted June 8, 1981.

Mechanisms of ischemic myocardial cell damage assessed by phosphorus-31 nuclear magnetic resonance.
J T Flaherty, M L Weisfeldt, B H Bulkley, T J Gardner, V L Gott and W E Jacobus

Circulation. 1982;65:561-570
doi: 10.1161/01.CIR.65.3.561
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/65/3/561

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/