Cellular Protection during Myocardial Ischemia

The Development and Characterization of a Procedure for the Induction of Reversible Ischemic Arrest

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SUMMARY An isolated perfused working rat heart model was used to investigate the extent to which various protective agents, used either singly or in combination, were able to increase the resistance of the heart to periods of transient ischemia. The aim of the studies was to develop a solution which, if infused into the coronary vessels just prior to the onset of ischemia, would rapidly induce arrest and would also counteract several of the deleterious cellular changes known to occur during myocardial ischemia. Agents which induce cardiac arrest, modify cellular ion loss, affect substrate utilization, energy production and energy stores, affect coronary vessel diameter and cell swelling, prevent dysrhythmias, and affect metabolic rate were investigated. The additive effects of these agents were evaluated. An aqueous solution was formulated which contained high concentrations of potassium and magnesium, in combination with adenosine triphosphate, creatine phosphate and procaine. This solution increased the recovery of the ischemic (37°C for 30 min) rat heart from 0% to 93%. The safe period of ischemia could be further increased by the use of hypothermia.

OPEN HEART SURGERY requires ideally a still and relaxed heart. Cardiac arrest (cardioplegia) in diastole can be induced by several procedures10 which may or may not involve coronary perfusion. While few workers would question the metabolic and morphological advantages of maintaining coronary perfusion throughout the period of arrest, the simplicity and practical advantages of nonperfusion methods has resulted in the widespread use and ad- 
vocation* of ischemic arrest. However, the use of ischemic arrest has been criticized11-13 because associated with its prolonged use is the onset of irreversible metabolic and ultrastructural damage. Two important questions have therefore arisen. First, what is the maximum duration of ischemia that can be tolerated by the myocardium before the onset of major irreversible damage? Second, is there any way in which this period can be extended or the onset of irreversible damage be reduced or delayed?


Immediatedly following the onset of ischemia a number of functional, metabolic, and morphological changes occur.14 These changes are initially of a reversible nature and if blood flow is restored to the ischemic tissue during this phase of reversible damage there is a complete resumption of normal metabolism and function. If ischemia is maintained for longer periods of time, irreversible damage occurs, the restoration of blood flow no longer consistently reverses injury, and a permanent impairment of functional capacity occurs. The time taken for the onset of irreversible damage is determined by a number of factors such as the severity of ischemia, the nutritional and hormonal status of the tissue, the availability of energy supplies such as glycogen, adenosine triphosphate (ATP), and creatine phosphate (CP), the metabolic capacity for anaerobic energy production, the contractile state of the tissue, the age and temperature of the tissue, and the composition of the coronary blood in the tissue at the onset of ischemia.

The temperature of the myocardium and the composition of the extracellular fluid during ischemia provide an effective means of modifying the rate at which ischemic tissue deteriorates. The use of topical hypothermia and the consequent reduction of metabolic rate affords considerable protection to the ischemic myocardium.15-18 Similarly,
the pioneering work of Bretschneider\textsuperscript{19} and Kirsch\textsuperscript{20, 21} on the infusion of various cardioplegic and protective solutions into the coronary circulation just prior to ischemia has emphasized the value of this approach.

The objective of the studies reported in this paper was to use the isolated rat heart to search for and individually assess the potential protective value of a variety of agents which could be combined on a rational basis to form a solution which, if infused into the coronary bed prior to ischemia, could combat the deleterious changes induced by ischemia and thereby afford protection to the myocardium. The requirements of such a solution were that it should arrest the heart rapidly in diastole, be nontoxic, and also be freely and rapidly reversible on reperfusion. In addition to its cardioplegic action it was hoped that such a solution would also minimize ischemic changes such as acidosis, cell swelling, depletion of energy reserves, loss of ions, metabolic disruption, and the occurrence of dysrhythmias.

Methods

Two hundred and eighty-eight male rats (280–320 g) of the Sprague-Dawley strain maintained on a standard diet were used in these experiments.

Perfusion Techniques

The perfusion techniques have been described previously.\textsuperscript{2} Following excision of the heart, the aorta and the left atrium were cannulated. The perfusion circuit was designed so that it could be used for two modes of perfusion each of which could be readily converted to the other: 1) \textit{Non-working Langendorff Preparation}. Hearts were perfused via the aorta as described by Langendorff\textsuperscript{22} with a perfusion pressure of 65 cm H\textsubscript{2}O. This mode of perfusion was used for an initial washout period and also for periods of coronary infusion prior to the onset of ischemia. 2) \textit{Working System}. Hearts were perfused via the left atrium as described by Neely et al.\textsuperscript{23} at an atrial perfusion pressure of 20 cm H\textsubscript{2}O. The left ventricle spontaneously ejected 40–50 ml perfusate/min against a hydrostatic pressure of 100 cm H\textsubscript{2}O. The aortic flow and the coronary flow could be pooled and recirculated. This preparation was used for the control working period prior to the ischemic period and for the recovery period following ischemia.

With the exception of any periods of hypothermia or ischemia, the hearts were maintained in the normothermic state by perfusion at 37°C. Accurate temperature control was essential in all studies, and the temperature of the perfusate was continuously monitored using a thermistor probe and a telethermometer. In periodic control checks, a microthermistor was introduced into the aortic cannula and also into the heart chamber to confirm adequate temperature control. At all times the heart was kept in a water-jacketed chamber that was maintained at the same temperature as the perfusion fluid and through which was passed the same gas mixture that was used to aerate the perfusate. Prior to its introduction into the heart chamber, the gas was bubbled through water that was maintained at the same temperature as the perfusate and the heart chamber. In experiments involving tissue ischemia (either normothermic or hypothermic) the hearts were maintained in a sealed, temperature-controlled heart chamber, and coronary flow was halted by clamping both the atrial and aortic cannulae.

Perfusion Medium

Krebs-Henseleit bicarbonate buffer,\textsuperscript{24} pH 7.4 (normal buffer), was the standard perfusion fluid. During working control and recovery periods glucose (11 mM) was included in the buffer. In studies involving the alteration of the ionic composition of the perfusate, the elevation of the concentration of any ion, e.g., potassium, was compensated for by the appropriate reduction of another ion (e.g., sodium). The perfusion fluid was equilibrated with 95\% O\textsubscript{2} + 5\% CO\textsubscript{2} (aortic O\textsubscript{2} partial pressure was over 600 mm Hg). Precautions\textsuperscript{25} were taken to prevent the precipitation of calcium. Before it was used, the perfusion fluid was filtered through a cellulose acetate filter with 5.0 μm pores.

Perfusion Time Sequence

To allow an estimate of recovery and to eliminate errors resulting from variation between individual hearts, a control working perfusion period preceded all experimental periods. Immediately after mounting, the heart was perfused for a 5 min washout period by a non-recirculating Langendorff type of perfusion. The preparation was then converted to a working heart system for a 15 min control period. During this time, the stability of the preparation could be confirmed, and the control values for peak systolic pressure, heart rate (derived electronically from the pressure recordings), aortic flow rate (measured with a flowthrough electromagnetic flowmeter in-line with the aorta), and coronary flow rate of the heart were established. As an index of external work, aortic flow rate against a hydrostatic pressure of 100 cm H\textsubscript{2}O was monitored. At the end of the control period, the hearts were subjected to a 2 min period of coronary infusion (Langendorff perfusion via the side arm of the aorta) during which time the normal glucose containing perfusate could be washed out of the heart and the coronary bed be filled with a new solution. The hearts were then made ischemic by clamping the aortic and atrial cannulae and in this way the infused solution was trapped in the heart. The hearts were then subjected to a 30 minute ischemic period. At the end of the ischemic period, the hearts were converted back to atrially perfused working preparations (with an initial 2 min non-recirculating phase to wash out and eliminate any of the infusate). The recovery of aortic flow, heart rate, and peak systolic pressure was recorded over a 15 min period, and recovery values were expressed as percent of the control values.

Expression of Data

Results are expressed in two forms, graphically and as tables. In the graphical representation the recovery of each heart is calculated as a percentage of its own control value (obtained before the ischemic period). These individualized percentage values are then statistically Meaning for the various times during the recovery period and are graphically represented. In the tables of results certain key times are selected (t = 18 min for the control value, i.e., 18 min after the start of the experiment, and t = 5, 10, and 15 min after the end of the ischemic period for the recovery values) and the absolute values are statistically Meaning for those times. It should be noted that accurate percentage recovery values cannot be obtained directly from the tables since the averaging of the control and recovery values prior to the calcula-
tion of percentage recovery negates the ability to utilize individualized controls. The graphical representation is therefore of particular value since it eliminates the element of individual-to-individual variation.

Results

Ischemic Controls

To provide a comparative baseline for these studies the effect of simple normothermic ischemia was investigated. At the end of the working control period, hearts (N = 6) were subjected to a 2 min infusion period with normal buffer (buffer A). This procedure ensured that there was no residual glucose trapped in the coronary bed. The aortic and left atrial cannulae were then clamped and the heart was subjected to a 30 min period of total ischemia at 37°C. At the end of this period, all hearts failed to recover (fig. 1, table 1).

Modification of Anionic and Cationic Balance

In an attempt to improve the recovery from ischemia we modified the ionic composition of the extracellular fluid trapped in the heart during ischemia. There were three reasons for this decision. Firstly, we have previously stressed the importance of the rapid induction of arrest during ischemia and have suggested that the prevention of ischemic beating and hence the conservation of limited energy supplies will afford considerable protection to the myocardium. Secondly, it is possible that the elevation of the extracellular concentration of various ions such as potassium may reduce the potentially damaging intracellular ion loss which is known to occur during ischemia. Thirdly it has been suggested that certain ions, e.g., aspartate can exert a marked protective effect upon the ischemic myocardium.

Hearts were therefore subjected to a 2 min pre-ischemic period of coronary infusion with a variety of solutions containing elevated concentrations of one or more of the following: potassium chloride, potassium citrate, potassium aspartate, and magnesium chloride.

Trapping 16 mM potassium chloride (buffer B) in the coronary tree caused a very rapid induction of cardiac arrest and improved post-ischemic recovery such that hearts (N = 6) subjected to 30 min ischemia recovered to 29.9 ± 8.0% of their control aortic flow rate. In additional experiments (N = 6 for each group) the 16 mM potassium chloride was replaced by 16 mM potassium aspartate (buffer C) or 16 mM potassium citrate (buffer D). The recoveries were 28.1 ± 7.6% and 11.1 ± 5.4% respectively. In a further series of experiments (N = 6 hearts for each group) hearts were subjected to pre-ischemic perfusion with solutions containing 16 mM potassium chloride plus 16 mM magnesium chloride (buffer E) or 16 mM potassium chloride plus 16 mM magnesium aspartate (buffer F). The hearts were sub-

<table>
<thead>
<tr>
<th>Buffer code</th>
<th>Composition of pre-ischemic infusate</th>
<th>Aortic flow rate (ml/min)</th>
<th>Peak systolic pressure (cm H2O)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
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</tr>
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<td>Normal bicarbonate buffer pH 7.4</td>
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<td>0</td>
<td>0</td>
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<td>11.9</td>
<td>12.5</td>
</tr>
<tr>
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<td>Buffer + 16mM KCl</td>
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<td>7.1</td>
<td>8.4</td>
</tr>
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<td>Buffer + 16mM K Citrate</td>
<td>45.1</td>
<td>1.8</td>
<td>2.9</td>
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<td>E</td>
<td>Buffer + 16mM KCl</td>
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<td>29.0</td>
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<td>F</td>
<td>Buffer + 16mM MgCl2</td>
<td>44.7</td>
<td>29.7</td>
<td>31.0</td>
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</tbody>
</table>

Table 1. The Effect of the Modification of Anionic and Cationic Balance upon Recovery from Ischemia

![Figure 1. Percent recovery of aortic flow rate in the isolated perfused working rat heart after a 30 min period of total ischemia. Each heart was subjected to a 2 min period of coronary infusion prior to the onset of ischemia. The composition of the infusate was: normal buffer (buffer A); buffer containing 16 mM KCl (buffer B); buffer containing 16 mM K aspartate (buffer C); buffer containing 16 mM K citrate (buffer D); buffer containing 16 mM KCl plus 16 mM MgCl2 (buffer E); buffer containing 16 mM KCl plus 16 mM Mg aspartate (buffer F). Each point represents the mean for six hearts and the bars represent the standard error.](http://circ.ahajournals.org/content/195/2/95/F1)

Absolute values for aortic flow rate (ml/min), peak systolic pressure (cm H2O), and heart rate (beats/min) for the pre-ischemic control values were obtained 2 min prior to the onset of coronary infusion (i.e., 18 min after the onset of the experiment and 4 min prior to the onset of ischemia). Aortic flow rates, peak systolic pressures, and heart rates for the post-ischemic control values were obtained 2 min prior to the onset of coronary infusion (i.e., 4 min prior to the onset of ischemia). The duration of ischemia was 30 min. The post-ischemic recovery values were obtained 5, 10 and 15 min after the termination of arrest. Each value represents the mean for six hearts, t = time period.
jected to 30 min ischemia and after 15 min of the recovery period the hearts had recovered to 68.1 ± 5.7% and 73.6 ± 5.1% of the control aortic flow rate.

These combined results (percent change illustrated in fig. 1; absolute values reported in table 1) illustrate the marked protective action of potassium and also magnesium and show that their effects are additive. However, contrary to the suggestions of Bretschneider 19 and Kirsch 31 the inclusion of aspartate does not significantly improve protection and recovery. The inclusion of citrate, which may have a potential damaging effect through its ability to chelate calcium or inhibit anaerobic glycolysis significantly, reduced the protection afforded by potassium.

Modification of Electrical Activity

High concentrations of procaine and acetylcholine have each been suggested as effective cardioplegic agents 9, 32 and possibly also act as protective agents through their rapid induction of arrest and prevention of ischemic beating. The ability of residual procaine to control any rhythmic disturbance during recovery may add to its potential value. Hearts (N = 6 for each protocol) were therefore infused, prior to ischemia, with perfusion fluid containing either 71 mM acetyl choline (buffer G) or 7.4 mM procaine (buffer H). The recoveries for aortic flow were 22.2 ± 10.2% and 26.6 ± 10.4% respectively. In the absence of these compounds there was no recovery, thus illustrating their potential protective action.

Modification of Metabolic Activity

The controversy over the alleged protective properties of glucose and possibly insulin in the oxygen deficient heart has been well documented. 26, 27 In general it appears that glucose can offer considerable protection to the anoxic or hypoxic heart (where coronary flow is near normal, lactic acid accumulation, acidosis, and the inhibition of glycolysis may not occur) but is less able to offer protection to the ischemic heart where coronary flow and toxic metabolite removal are severely impaired and anaerobic energy production is rapidly inhibited. 31 Studies were therefore carried out to determine whether glucose and insulin would improve the recovery of the ischemic and the ischemic arrested heart.

After perfusion with normal buffer hearts were infused with perfusion fluid containing normal buffer plus the following substances: 11 mM glucose (buffer I); 11 mM glucose plus 16 mM potassium plus 16 mM magnesium (buffer J); 11 mM glucose plus 16 mM potassium plus 16 mM magnesium plus 0.01 I.U./ml insulin (buffer K). The hearts (N = 6 for each group) were subjected to 30 min ischemia and at the end of the 15 min recovery period the recoveries (fig. 2, table 2) were 0%, 66.5 ± 8.1% and 65.8 ± 3.1% respectively. It is clear that the inclusion of glucose or glucose and insulin does not improve the post-ischemic recovery since the control recovery values remained 0% and the recoveries in hearts with addition of electrolytes did not differ in hearts with electrolytes alone (68.1%) and with electrolytes plus glucose (66.5%).

Equally as controversial as the protective properties of glucose and insulin are the alleged protective properties of extracellular high energy phosphates. 29, 33 The potential protective effects of extracellular creatine phosphate and adenosine triphosphate were therefore investigated. Experimental protocols (6 hearts in each experiment) were: 1) pre-ischemic infusions of perfusion fluid containing 16 mM potassium plus 10 mM creatine phosphate (buffer L); and 2) 16 mM potassium plus 10 mM adenosine triphosphate (buffer M). The presence of these high energy phosphates had a marked protective effect and increased the recovery from 29.9% (16 mM potassium only) to 37.4 ± 17.6% and 76.6 ± 4.4% for hearts in which CP and ATP, respectively, had been added to the perfusate.

The large standard error for the creatine phosphate recoveries was due to the fact that while three hearts recovered well to high levels, ventricular fibrillation and/or dysrhythmias that occurred in the other three produced very poor values for final recoveries. The mechanism by which creatine phosphate induced dysrhythmias is unknown; however, inclusion of pyruvate (7.4 mM) in the perfusate containing creatine phosphate prevented the rhythmic disturbances. When hearts (N = 6) were infused with perfusion fluid con-
TABLE 2. Effect of Metabolic Modifications upon Recovery from Ischemia

<table>
<thead>
<tr>
<th>Buffer code</th>
<th>Composition of pre-ischemic infusate</th>
<th>Aortic flow rate (ml/min)</th>
<th>Peak systolic pressure (cm H&lt;sub&gt;2&lt;/sub&gt;O)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Recovery</td>
<td>Control</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>t=18</td>
<td>t=5</td>
<td>t=10</td>
<td>t=15</td>
</tr>
<tr>
<td>Effect of Glucose and/or Insulin</td>
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<td></td>
<td></td>
<td></td>
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<td>I</td>
<td>Buffer + 11mM Glucose</td>
<td>41.9</td>
<td>0</td>
<td>0</td>
</tr>
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<td>E</td>
<td>Buffer + 16mM KCl</td>
<td>44.3</td>
<td>29.0</td>
<td>29.2</td>
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<tr>
<td>J</td>
<td>Buffer + 16mM KCl + 16mM MgCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>39.5</td>
<td>22.0</td>
<td>23.0</td>
</tr>
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<td>K</td>
<td>Buffer + 16mM KCl + 16mM MgCl&lt;sub&gt;2&lt;/sub&gt; + 11mM Glucose</td>
<td>39.0</td>
<td>23.2</td>
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<td>Extracellular High Energy Phosphates</td>
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</tr>
<tr>
<td>B</td>
<td>Buffer + 16mM KCl</td>
<td>43.3</td>
<td>11.9</td>
<td>12.5</td>
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<td>M</td>
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<tr>
<td>N</td>
<td>Buffer + 16mM KCl + 10mM CP + 7.4mM Procaine</td>
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<td>20.2</td>
<td>25.7</td>
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<td>O</td>
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<td>24.7</td>
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<tr>
<td>P</td>
<td>Buffer + 16mM KCl + 16mM MgCl&lt;sub&gt;2&lt;/sub&gt; + 10mM CP + 7.4mM Procaine</td>
<td>39.0</td>
<td>31.3</td>
<td>32.7</td>
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</table>

Absolute values for aortic flow rate (ml/min), peak systolic pressure (cm H<sub>2</sub>O), and heart rate (beats/min) for the pre-ischemic control values were obtained 2 min prior to the onset of coronary infusion (i.e., 18 min after the onset of the experiment and 4 min prior to the onset of ischemia). The duration of ischemia was 30 min. The post-ischemic recovery values were obtained 5, 10 and 15 min after the termination of arrest. Each value represents the mean for six hearts.

Abbreviations: CP = creatine phosphate; ATP = adenosine triphosphate.

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obtained 16 mM potassium, plus 10 mM creatine phosphate and 7.4 mM procaine (buffer N) the dysrhythmias were abolished and the recovery was increased to 63.5 ± 12.7%.

In an attempt to improve further the protection and recovery, magnesium was included in the infusate. When hearts (N = 6 for each group) were infused with perfusion fluid containing either 16 mM potassium plus 16 mM magnesium plus 10 mM adenosine triphosphate (buffer O) or 16 mM potassium plus 16 mM magnesium plus 10 mM creatine phosphate plus 7.4 mM procaine (buffer P) the recoveries were further improved to 86.8 ± 0.9% and 88.6 ± 2.8%, respectively.

These results (fig. 2, table 2), in addition to revealing the striking protective action of extracellular adenosine triphosphate and creatine phosphate, also reveal that the protective effects of potassium, magnesium, procaine, adenosine triphosphate, and creatine phosphate are additive.

Modification of Coronary Vessel Diameter

The degree of coronary vasodilation will affect the extracellular volume and hence the amount of any protective solution that may be trapped in the coronary bed during the ischemic period. In addition, the extent of coronary dilation and consequent coronary flow rate during the immediate post-ischemic recovery period may substantially affect the rate and extent of recovery. The effect of including vasodilating agents in the pre-ischemic infusate was therefore investigated. Hearts (N = 6 for each protocol) were infused with perfusion fluid containing 10 mM adenosine (buffer Q) or 16 mM potassium plus 16 mM magnesium plus 10 mM adenosine (buffer R) or 16 mM potassium plus 16 mM magnesium plus 0.025 mM dipyridamol (buffer S). The inclusion of adenosine in the perfusion fluid increased the recovery from 0% to 31.8 ± 9.2% (fig. 3, table 3) and this was associated with a marked increase (approximately 30%) in the coronary flow during the first few minutes of the recovery period. The inclusion of adenosine or dipyridamol in infusion fluid which already contained high concentrations of potassium and magnesium improved the final recovery from 68.1% to 76.9 ± 4.7% and 81.0 ± 2.4% respectively (fig. 3, table 3). However, despite the increase in overall recovery, the early stages of recovery were not improved, probably explained by the fact that in these examples, there was no significant increase in coronary flow during the early phases of recovery.

The explanation for any protective action noted for adenosine or dipyridamol may not reside solely in their ability to induce vasodilation. During ischemia there is considerable degradation of adenine nucleotides and loss of cellular adenosine. This loss of adenosine may have critical consequences during the anaerobic recovery phase by limiting the amount and rate of adenine nucleotide resynthesis. The presence of high concentrations of adenosine or the presence of dipyridamol may substantially reduce cellular adenosine loss.

Miscellaneous Approaches

Various other potential protective agents were also in-
FIGURE 3. Percent recovery of aortic flow rate in the isolated perfused working rat heart after a 30 min period of total ischemia. Each heart was subjected to a 2-min period of coronary infusion prior to the onset of ischemia. The composition of the infusion was:

- normal buffer (buffer A);
- buffer containing 16 mM KCl plus 16 mM MgCl₂ (buffer E);
- normal buffer plus 10 mM adenosine (buffer Q);
- buffer containing 16 mM KCl plus 16 mM MgCl₂ plus 10 mM adenosine (buffer R);
- buffer containing 16 mM KCl plus 16 mM MgCl₂ plus 0.025 mM dipyridamol (buffer S). Each point represents the mean for six hearts and the bars represent the SE.

FIGURE 4. Percent recovery of aortic flow rate in the isolated perfused working rat heart after a 30 minute period of total ischemia. Each heart was subjected to a 2 min period of coronary infusion prior to the onset of ischemia. The composition of the infusion was:

- buffer containing 16 mM KCl plus 16 mM MgCl₂ plus 10 mM adenosine plus 10 mM ATP (buffer T);
- buffer containing 16 mM KCl plus 16 mM MgCl₂ plus 10 mM CP plus 10 mM ATP plus 7.4 mM procaine (buffer U);
- buffer containing 12 mM KCl plus 16 mM MgCl₂ plus 10 mM CP plus 10 mM ATP plus 7.4 mM procaine (buffer V);
- buffer containing 8 mM KCl plus 16 mM MgCl₂ plus 10 mM CP plus 10 mM ATP plus 7.4 mM procaine (buffer W);
- buffer containing 6 mM KCl plus 16 mM MgCl₂ plus 10 mM CP plus 10 mM ATP plus 7.4 mM procaine (buffer X);
- buffer containing 12 mM KCl plus 16 mM MgCl₂ plus 10 mM CP plus 10 mM ATP plus 1.0 mM procaine (buffer Y);
- buffer containing 12 mM KCl plus 16 mM MgCl₂ plus 10 mM CP plus 10 mM ATP plus 1.0 mM procaine and a maintained hypothermia of 25°C (buffer Z) for the duration of ischemia. Each point represents the mean for six hearts and the bars represent the SE.

Table 3. The Effect of Coronary Vessel Diameter upon Recovery from Ischemic Arrest

| Buffer code | Composition of pre-ischemic infusion | Aortic flow rate (ml/min) | | Peak systolic pressure (mm Hg) | | Heart rate (beats/min) |
|-------------|-------------------------------------|--------------------------|---------------------------|--------------------------|----------------------|
|             |                                     | Control t=18 t=5 t=10 t=15 | Recovery                   | Control t=18 t=5 t=10 t=15 | Recovery |
| A           | Normal bicarbonate buffer pH 7.4     | 43.5 0 0 0                | 189 0 0 0                | 268 0 0 0                |
| E           | Buffer + 16mM KCl                    | 44.3 29.0 29.2 30.3      | 172 159 157 158          | 270 238 236 235          |
| Q           | Buffer + 10mM Adenosine              | 39.0 3.5 8.2 12.2        | 190 95 142 149           | 279 72 167 193           |
| R           | Buffer + 16mM KCl                    | 37.0 16.3 23.2 28.5      | 174 153 162 165          | 329 194 278 252          |
| S           | Buffer + 16mM KCl                    | 50.9 15.9 33.9 41.1      | 181 165 169 170          | 297 215 251 264          |

Table 3 Absolute values for aortic flow rate (ml/min), peak systolic pressure (mm Hg), and heart rate (beats/min) for the pre-ischemic control values were obtained 2 min prior to the onset of coronary infusion (i.e., 18 min after the onset of the experiment and 4 min prior to the onset of ischemia). The duration of ischemia was 30 min. The post-ischemic recovery values were obtained 5, 10, and 15 min after the termination of arrest. Each value represents the mean for six hearts.
TABLE 4. The Additive Effects of Protective Agents upon the Recovery from Ischemia

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<thead>
<tr>
<th>Buffer code</th>
<th>Composition of pre-ischemic infuse</th>
<th>Aortic flow rate (ml/min)</th>
<th>Peak systolic pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
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<tr>
<td>T</td>
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<td>Control t=18: 293 Recovery t=5: 243 t=10: 261 t=15: 279</td>
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<td>U</td>
<td>Buffer + 16mM KCl + 16mM MgCl₂ + 10mM CP + 10mM ATP + 7.4mM Procaine</td>
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<td>44.0 34.3 36.3 38.0 181 192 191 184</td>
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<td>X</td>
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<tr>
<td>Z</td>
<td>Buffer + 12mM KCl + 16mM MgCl₂ + 10mM CP + 10mM ATP + 1.0mM Procaine + 25°C Hypothermia</td>
<td>41.7 38.5 40.2 40.0 190 199 195 194</td>
<td>291 255 249 249</td>
<td></td>
</tr>
</tbody>
</table>

Absolute values for aortic flow rate (ml/min), peak systolic pressure (cm H₂O), and heart rate (beats/min) for the pre-ischemic control values were obtained 2 min prior to the onset of coronary infusion (i.e., 18 min after the onset of the experiment and 4 min prior to the onset of ischemia). The duration of ischemia was 30 min. The post-ischemic recovery values were obtained 5, 10, and 15 min after the termination of arrest. Each value represents the mean for six hearts.

Investigated. In an attempt to increase anaerobic energy production and reduce lactic acid production 5 mM dichloroacetate was included in several infusates. In an attempt to minimize cellular acidosis during ischemia a medium (16 mM TRIS) with high buffering capacity was investigated. In order to prevent cell swelling and consequent tissue damage the inclusion of high concentrations of osmotic protective agents such as mannitol (57 mM and 140 mM) was investigated. The use of low calcium (0.25 mM) or potassium (1.2 mM) concentrations to induce rapid arrest was also evaluated. None of these protocols added significantly to the improvement of recovery induced by a combination of electrolytes and high energy phosphates.

Additive Effects and Minimum Effective Concentrations

The results of studies thus far have indicated that substantial myocardial protection during ischemia can be achieved by the infusion of appropriate substances. Furthermore, the protective action of several agents may be additive. In an attempt to formulate a highly effective protective solution a series of studies was carried out in which various protective agents were sequentially added to or removed from the infusate and the effect upon recovery from 30 min of ischemia at 37°C was assessed. In addition, in order to avoid the inclusion of some components of the infusate (particularly potassium) at a higher concentration than necessary, the concentration of certain components was progressively reduced and the effect upon protection and recovery monitored. Perfusion media containing the following concentrations of protective agents were studied (the percentage recovery of aortic flow at the end of the 15 min recovery periods is given in parentheses at the end of each formulation): 16 mM potassium plus 16 mM magnesium plus 10 mM adenosine plus 10 mM adenosine triphosphate (buffer T) (88.3 ± 1.9%); 16 mM potassium plus 16 mM magnesium plus 10 mM creatine phosphate plus 10 mM adenosine triphosphate plus 7.4 mM procaine (buffer U) (93.1 ± 1.0%); 12 mM potassium plus 16 mM magnesium plus 10 mM creatine phosphate plus 10 mM adenosine triphosphate plus 7.4 mM procaine (buffer V) (89.2 ± 0.7%); 8 mM potassium plus 16 mM magnesium plus 10 mM creatine phosphate plus 10 mM adenosine triphosphate plus 7.4 mM procaine (buffer W) (86.3 ± 3.8%); 6 mM potassium plus 16 mM magnesium plus 10 mM creatine phosphate plus 10 mM adenosine triphosphate plus 7.4 mM procaine (buffer X) (87.0 ± 1.8%); 12 mM potassium plus 16 mM magnesium plus 10 mM creatine phosphate plus 10 mM adenosine...
These results (fig. 4, table 4) confirm the additive effects of the protective agents and show that the concentration of potassium and procaine can be substantially reduced without greatly reducing the protective properties of the solution. In addition, although adenosine is able to improve the recovery of the heart in the absence of any other protective agents (fig. 3, table 3) the use of adenosine (or dipyridamol) in the presence of other protective agents (fig. 4, table 4) results in only a small improvement in recovery. For this reason adenosine was omitted from all solutions.

Hypothermic Protection

In previous studies we have quantitated the striking protective action of topical hypothermia upon the ischemic heart. Preliminary studies were therefore carried out to ascertain whether the protective action afforded by 12 mM potassium plus 10 mM creatine phosphate plus 10 mM adenosine triphosphate plus 1.0 mM procaine (buffer Y) at 37°C could be substantially improved by the use of mild topical hypothermia. Hearts (N = 6) were therefore infused with the above solution and subjected to 30 min ischemia at 25°C (buffer Z). The use of the hypothermia increased the recovery from 82.2% to 96.2 ± 0.9%.

Time and Temperature Relationships

The studies thus far have resulted in the formulation of a relatively simple solution which, if infused into the coronary arteries immediately before a 30 min period of total ischemia, permit hearts which would normally not recover at all to recover to almost 100%.

The next phase of investigation was aimed at determining the maximum time for which effective protection could be maintained and therefore a study was carried out in which the duration of ischemia in the presence of protective agents was related to the subsequent recovery of the heart.

Hearts (N = 6 for each group) were infused for 2 min with a perfusion fluid containing 12 mM potassium plus 16 mM magnesium plus 10 mM adenosine triphosphate plus 10 mM creatine phosphate plus 1.0 mM procaine. The hearts were made ischemic and maintained at 30°C for 30, 40, 50, 60, 70, 80 and 90 min. At the end of the respective recovery period the hearts had recovered to 97.6 ± 2.1%; 87.8%.

Figure 5. The effect of the duration of ischemia upon the final percent recovery of aortic flow rate. All hearts were infused for 2 min prior to ischemia with a buffer containing 12 mM KCl plus 16 mM MgCl₂ plus 10 mM CP plus 10 mM ATP plus 1.0 mM procaine. • 30 min ischemia; ● 40 min ischemia; ▽ 50 min ischemia; ○ 60 min ischemia; □ 70 min ischemia; △ 80 min ischemia; ◆ 90 min ischemia. The hearts were subjected to varying periods of ischemia at 30°C. Each point represents the mean for six hearts and the bars represent the SE.

Figure 6. The effect of the degree of hypothermia upon the final percent recovery of aortic flow rate. All hearts were infused for 2 min prior to ischemia with a buffer containing 12 mM KCl plus 16 mM MgCl₂ plus 10 mM CP plus 10 mM ATP plus 1.0 mM procaine. The hearts were subjected to 60 minutes of ischemia at various degrees of hypothermia. • 4°C; ● 12°C; ▽ 20°C; △ 25°C; □ 28°C; △ 30°C; ◆ 33°C; ◆ 37°C. Each point represents the mean for six hearts and the bars represent the SE.
± 2.4%; 74.8 ± 2.1%; 58.5 ± 7.1%; 51.8 ± 16.1%; 16.8 ± 4.4% and 0% respectively (fig. 5).

Having delineated the effect of the duration of ischemia upon the extent of protection it was decided to investigate the extent to which increasing the degree of topical hypothermia could be used to increase further the extent and duration of protection. Hearts (N = 6 for each protocol) were infused for 2 min with perfusion fluid containing 12 mM potassium plus 16 mM magnesium plus 10 mM creatine phosphate plus 10 mM adenosine triphosphate plus 1.0 mM procaine. The hearts were subjected to 60 min of ischemia at 4°C, 12°C, 20°C, 25°C, 28°C, 30°C, 33°C and 37°C. At the end of the recovery period the hearts had recovered to 95.8 ± 1.2%; 92.4 ± 2.5%; 86.7 ± 3.9%; 83.8 ± 2.4%; 75.1 ± 1.0%; 57.5 ± 7.1%; 9.7 ± 5.0% and 0%, respectively. These results (fig. 6) show how the lower levels of topical hypothermia can substantially improve the duration of ischemia that can be tolerated in the presence of the protective solution. The greater the degree of hypothermia the greater is the protection.

Figure 7 shows the relationship between the final recovery of hearts and the duration of ischemia or the degree of hypothermia. While there is a linear relationship between recovery and duration of ischemia, the curve for recovery against the degree of hypothermia exhibits a sharp inflexion between 26°C and 30°C. Thus at temperatures below 26°C, topical hypothermia can afford a high degree of protection but above 30°C the protective effect rapidly falls off with increasing temperature.

**Discussion**

The concept of attempting to protect the ischemic myocardium through the manipulation of various aspects of its metabolic or functional behavior is well established. Experimental approaches to this problem have usually concentrated on the amelioration of one single aspect of the damage induced by ischemia, for example the alteration of substrate utilization in order to maximize anaerobic energy production or the use of hyperosmolar solutions to prevent cellular edemas. However, myocardial ischemia initiates a multitude of potentially damaging cellular changes, which may not be directly related and as such cannot necessarily be combated with a single intervention. It has been the object of the work reported in this paper to evaluate individually, and in combination, a variety of potentially protective interventions, each aimed at some different aspect of cell damage, with a view to devising an effective means of myocardial protection. The results of the studies reported in this paper stress the considerable potential of this approach to the point that hearts which would normally fail totally to recover after 30 min of ischemia can be made to recover to almost 100% of their pre-ischemic function after 60 min ishemia simply by the use of an appropriate intervention.

Our studies have involved the infusion of various aqueous solutions into the coronary tree just prior to the onset of ischemia. In this way, the contents of the solution are trapped in the coronary vasculature for the duration of the ischemic period. Our results suggest that effective protection can be resolved into two distinct components.

The first component is the rapid induction of cardiac arrest. Although immediately following the onset of myocardial ischemia there is an abrupt reduction of contractile activity, this is not complete and considerable contraction occurs for several minutes and also occurs periodically during ischemia. The use of cardioplegic agents such as potassium, procaine, or acetylcholine to induce instantaneous cardiac arrest, and thereby conserve vital cellular energy supplies for cellular maintenance and subsequent recovery, has a major protective effect.

The second component of effective protection relies on the ability of certain compounds to combat one or more of the deleterious changes which occur as a result of tissue ischemia. Potassium and magnesium may exert their protective effect by reducing intracellular ion losses. Procaine, like potassium, in addition to inducing rapid cardiac arrest, may exert an additional protective action by reducing the incidence of dysrhythmias during the recovery period. The effects of extracellular adenosine triphosphate and creatine phosphate are of particular interest. It is commonly held that cell membranes are impermeable to high energy phosphates but there have been several reports in the literature suggesting that adenosine triphosphate is in fact able to cross the muscle cell membrane. Whether the extracellular high energy phosphates enter the cell or whether they act on the cell membrane is unknown, but their protec-
tive effect is striking and is additive to that of potassium, magnesium and procaine.

Additive studies and dose response studies have allowed us to formulate a solution (Krebs Henseleit bicarbonate buffer pH 7.4 in which potassium is elevated to 12 mM, magnesium to 16 mM and which contains 10 mM adenosine triphosphate, 10 mM creatine phosphate and 1 mM procaine) which will improve recovery of aortic flow from 0% to 82% in a rat heart which is made ischemic for 30 min at 37°C. Using such an infusate there is a linear relationship between the recovery of the heart and the duration of ischemia.

Topical hypothermia for the duration of ischemia, through its ability to reduce metabolic rate, energy consumption, and degradative processes, can be a powerful adjunct to the protective action of various infusates. While the greater the degree of hypothermia the greater the protection, this relationship is not linear. Our results illustrate that there is a sharp decline in protection when the temperature rises above 30°C. If this limit to hypothermic protection applies to the human heart, then surface cooling to 30°C (with the associated nonuniformity of cooling and gradual warm-up) which is often utilized during human heart surgery, may be considerably more effective if it were reduced to 20°C.

In conclusion, these findings, though limited by their observation in the rat heart, emphasize the striking value of pre-ischemic infusions in counteracting the deleterious cellular changes induced by myocardial ischemia. The use of carefully formulated infusates in the human heart during conditions of temporary ischemia such as aortic occlusion during open heart surgery may permit a considerable extension of the duration of ischemia that can be tolerated by the heart while ensuring a complete functional recovery.

References

17. Proctor E: Early sinus rhythm in dog hearts preserved for 96 hours and assessed ex vivo. Transplantation 12: 437, 1972
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