CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

MYOCARDIAL PROTECTION IN THE ACUTELY INJURED HEART: HYPERPOLARIZING VERSUS DEPOLARIZING HYPOTHERMIC CARDIOPLEGIA

Jennifer S. Lawton, MD^a
Peng-Wie Hsia, PhD^a
Cynthia T. Allen, BA^a
Ralph J. Damiano, Jr., MD^a

Objectives: The superiority of hyperpolarized arrest with adenosine triphosphate-sensitive potassium channel openers over standard hyperkalemic depolarizing cardioplegia during normothermic ischemia has been documented. This study examined the hypothesis that pinacidil would provide superior protection in a more clinically relevant model of an acutely injured heart and hypothermic cardioplegic arrest. **Methods:** In a blood-perfused, parabiotic, rabbit heart Langendorff model, hearts underwent 15 minutes of unprotected global normothermic ischemia before the administration of 50 ml of cardioplegic solution at 4° C, followed by 50 minutes of hypothermic (15° C) ischemia and 30 minutes of reperfusion. The cardioplegic solutions administered consisted of Krebs-Henseleit solution alone ($N = 6$), Krebs-Henseleit solution with pinacidil (50 $\mu\text{mol/L}$; $N = 10$), Krebs-Henseleit solution with pinacidil (50 $\mu\text{mol/L}$) and glibenclamide (a potassium channel blocker, 10 $\mu\text{mol/L}$; $N = 8$), or St. Thomas' Hospital solution ($N = 8$). The percent recovery of developed pressure, linear diastolic pressure-volume relationships, and coronary blood flow were compared. **Results:** The percent recovery of developed pressure was $32.8\% \pm 2.8\%$, $43.0\% \pm 4.3\%$, $46.5\% \pm 2.2\%$, and $49.3\% \pm 2.7\%$ for the Krebs-Henseleit, the Krebs-Henseleit with pinacidil and glibenclamide, the St. Thomas' Hospital, and the Krebs-Henseleit with pinacidil groups, respectively. No hearts had ventricular fibrillation on reperfusion. **Conclusions:** During hypothermic hyperpolarized arrest, as opposed to normothermic ischemia as in our previous studies, there was neither an increased incidence of ventricular fibrillation nor prolonged electrical activity when compared with results during traditional hyperkalemic arrest. Myocardial protection by St. Thomas' Hospital solution and pinacidil was superior ($p = 0.009$) to that with Krebs-Henseleit solution alone. The protection provided by pinacidil was lost with the addition of glibenclamide, indicating that the drug has adenosine triphosphate-sensitive potassium channel activity during hypothermia. (J Thorac Cardiovasc Surg 1997;113:567-75)

From the Departments of Surgery of the Medical College of Virginia, Richmond, Va.,^a and The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pa.^b

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Address for reprints: Ralph J. Damiano, Jr., MD, Hershey Medical Center, Pennsylvania State University, P.O. Box 850, Hershey, PA 17033.

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The myocyte action potential is generated by the opening and closing of ion channels in the plasma membrane.¹ During myocardial ischemia, there are profound changes in the action potential including a decrease in the resting membrane potential, a decrease in the rate of rise of the action potential upstroke, and a decrease in the action potential duration.^{2,3} A unique metabolically sensitive potassium channel has been shown to be responsible for many of these electrophysiologic changes.³ Noma⁴ first described this potassium channel in guinea pig and rabbit ventricular myocytes and demonstrated that it was inhibited by intracellular adenosine

triphosphate (ATP) and opened during times of ischemia. The opening of this ATP-sensitive potassium (K_{ATP}) channel causes an outward potassium current that hyperpolarizes the cell membrane. This results in a significant shortening of the duration of the plateau phase of the action potential, during which most of the inward calcium transport occurs. This reduction in calcium influx causes a decrease in contractility. Activation of K_{ATP} channels has been shown to underlie the contractile failure seen during periods of ischemia and metabolic inhibition.⁵ This decrease in mechanical activity conserves ATP and thus is cardioprotective.⁴

Previous work from our laboratory has demonstrated the benefit of the ATP-sensitive potassium channel openers (PCOs) pinacidil and aprikalim as cardioplegic agents during normothermic ischemia in our blood-perfused, isolated rabbit heart model. During normothermic ischemia, aprikalim was shown to provide superior myocardial protection compared with 20 mmol KCl in Krebs-Henseleit solution, and pinacidil was shown to provide equivalent myocardial protection compared with 20 mmol KCl in Krebs-Henseleit solution and with St. Thomas' Hospital solution.⁶⁻⁸ The optimal dose of pinacidil was determined to be 50 $\mu\text{mol/L}$.⁷ A shortcoming of these studies was that they examined potassium channel opener cardioplegia only during normothermic global ischemia in normal hearts. However, many cardiac surgeons still use some degree of hypothermia to protect the heart during cardioplegic arrest. Operations also are often complicated by acute ongoing ischemia, such as seen after angioplasty failure. In an effort to evaluate pinacidil in a more clinically relevant model, the current study was designed to compare myocardial protection with pinacidil (50 $\mu\text{mol/L}$), pinacidil with glibenclamide (10 $\mu\text{mol/L}$; a potassium channel inhibitor), and St. Thomas' Hospital solution during hypothermic ischemia in an acutely injured heart.

Material and methods

Experimental preparation. Adult New Zealand White rabbits of either sex weighing 3.0 to 5.0 kg were anesthetized intramuscularly with acepromazine (1 mg/kg) and xylazine (17.5 mg/kg), followed by ketamine (62.5 mg/kg). Each study involved a support animal and a study animal. The support animal was heparinized (2500 U) via an ear vein and underwent cannulation of the left carotid artery, the left internal jugular vein, and the left femoral artery. The femoral cannula was connected to a pressure transducer (model P231D, Gould Inc., Cleveland, Ohio) and blood pressure was monitored continuously on a Gould

ES1000 system. The carotid arterial cannula was attached to silicone rubber tubing (internal diameter 0.125 inch, Baxter Scientific Products, McGaw Park, Ill.) and the blood actively pumped (Masterflex model 7013 pump, Cole-Palmer Int., Chicago) to perfuse a modified Langendorff apparatus, which has been described previously.⁸ The height of the column was 80 cm H_2O . The venous return from the Langendorff column was returned to the internal jugular vein of the support animal with use of a Travenol pump (model 5M1155, Travenol Laboratories, Inc., Deerfield, Ill.). The lungs of the support animal were ventilated (model 683, Harvard Apparatus, South Natick, Mass.) via a tracheostomy with 100% O_2 throughout the experiment.

After support animal preparation was completed, the donor animal was heparinized and the lungs ventilated via a tracheostomy. The animal then underwent rapid sternotomy and cardiectomy. The aorta was cannulated and coronary perfusion instituted via the Langendorff column. The support animal was given indomethacin (1 mg/kg) intravenously to promote blood pressure stability.⁹ The systolic arterial blood pressure of the support animal was maintained higher than 65 mm Hg by transfusion with Plasma-Lyte solution (Baxter Healthcare Corp., Deerfield, Ill.) or blood collected from the donor animal at the time of cardiectomy. Values of arterial blood gases, electrolytes, and the hematocrit of the support animal were monitored at regular intervals and maintained within physiologic limits. The support animal was given supplemental anesthesia intramuscularly as needed throughout the experiment. Heparin (500 U) was given to the support animal at hourly intervals.

After aortic cannulation, a left atriotomy was done and a vent (polyethylene tubing, internal diameter 0.86 mm, Clay Adams, Parsippany, N.J.) was placed into the left ventricle. A fluid-filled latex balloon was placed into the left ventricle and secured with a purse-string suture in the mitral annulus. The balloon was connected to a pressure transducer (model 42559-01, Abbott Laboratories, North Chicago, Ill.) and to a Gould amplifier (model 134615-50, Gould). The zero pressure reference was set at the level of the aortic valve. Two right atrial electrodes were positioned and connected to a pacemaker (model DTU101, Bloom Associates Ltd., Reading, Pa.). The heart was paced at a constant rate (180 to 240 beats/min) throughout the study. Two left ventricular epicardial bipolar electrodes were positioned and connected to a preamplifier (model 11-G5407-58, Gould) and to a universal amplifier (model MU13-4615-58, Gould) and filtered between 0.05 and 1000 Hz.

The pressure and electrogram waveforms were displayed continuously on an oscilloscope (Gould ES1000) and digitized on-line with use of an AT-CODAS system (DATAQ Instruments, Akron, Ohio) at a sampling rate of 1000 Hz.

The heart was enclosed in an air bath surrounded by a glass-jacketed water beaker. Myocardial temperature was monitored throughout the experiment with a temperature probe (model 0112, Shiley Inc., Irvine, Calif.) placed in the right ventricle and was controlled by adjusting the water bath temperature (model 71, Polyscience, Niles, Ill.). Myocardial temperature was maintained at 37°C

throughout the experiment except during the 50-minute ischemic period when the heart was maintained between 12.5° and 16.6° C with use of a second in-line water bath (model 9010, Fisher Scientific, Pittsburgh, Pa.). Coronary flow was monitored with an in-line flow probe (model 2N, Transonic Systems, Ithaca, N.Y.) and an ultrasonic blood flow meter (model 101, Transonic Systems).

Experimental protocol. Hearts were excluded from study if they did not obtain a developed pressure of 80 mm Hg at an end-diastolic pressure of 10 mm Hg or if the baseline developed pressure did not remain stable during the 30 minutes after instrumentation.

After a 30-minute equilibration period, the left ventricular pressure-volume relationships were measured. A wide range of volumes was infused into the intraventricular latex balloon to generate end-diastolic pressures (EDPs) of 0, 2.5, 5, 10, 15, and 20 mm Hg. After baseline data acquisition, fluid was adjusted in the latex balloon to obtain an EDP of 5 mm Hg before the ischemic period.

All hearts underwent a 15-minute period of unprotected, global, normothermic ischemia. This was followed immediately by infusion of cardioplegic solution. Thirty-two hearts were randomly assigned to receive a different cardioplegic solution for myocardial protection during a 50-minute period of global hypothermic ischemia. Hyperpolarizing cardioplegic solution consisted of Krebs-Henseleit solution (in millimolars per liter distilled water: NaCl, 118.5; NaHCO₃, 25.0; KCl, 3.2; MgSO₄, 1.2; KH₂PO₄, 1.2; glucose, 5.5; CaCl₂, 2.5) with pinacidil (50 μmol/L, *N* = 8). Depolarizing cardioplegic solution consisted of St. Thomas' Hospital solution (*N* = 8). A control group received Krebs-Henseleit solution alone (*N* = 6), and a final group received Krebs-Henseleit solution with pinacidil (50 μmol/L) and glibenclamide (a potassium channel blocker, 10 μmol/L; Sigma Chemical Co., St. Louis, Mo.; *N* = 8). Heparin (12.5 U/ml) was added to each of the cardioplegic solutions. Pinacidil was provided by Leo Pharmaceuticals, Ballerup, Denmark. St. Thomas' Hospital solution was provided by Abbott Laboratories. Sodium bicarbonate (8.4%) was added to the St. Thomas' Hospital solution before each infusion of cardioplegic solution (0.5 ml/50 ml) to correct the pH to 7.8.

After the 15-minute period of global, normothermic ischemia, 50 ml of hypothermic (4° C) cardioplegic solution was infused from a height of 80 cm H₂O via a separate column. The cardioplegic solution effluent was collected and discarded. Both the time until cessation of mechanical activity and the time until electrical quiescence were recorded. Cessation of mechanical activity was defined as the absence of a developed pressure.

After 50 minutes of hypothermic global ischemia, the Langendorff column was unclamped and the heart reperused for 30 minutes. If ventricular fibrillation persisted after reperfusion, the heart was defibrillated (model D84, Electrodyne Co., Inc., Westwood, Mass.). Electrolyte, hematocrit, and arterial blood gas measurements of the support animal were repeated to ensure stability. After 30 minutes of reperfusion, data were collected at the identical balloon volumes used during baseline preischemic data acquisition. At the conclusion of the experiment, a sample of the left ventricle was excised, blotted, and weighed to obtain the wet weight. The sample was dried until a

constant dry weight was achieved. Percent tissue water (%TW) was determined by the equation %TW = (wet weight - dry weight)/wet weight.

All animals received humane care in American Association for Accreditation of Laboratory Animal Care-accredited (No. 00036), United States Department of Agriculture-registered (No. 52-R-007) facilities in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Data analysis. Left ventricular systolic and diastolic pressures were determined from the digitized data files with the use of software developed in our laboratory.

End-systolic pressure. The end-systolic pressure (ESP) of a beat was defined as the maximum point of the digitized pressure waveform. The average ESP was calculated by averaging the ESPs of 10 beats. Average ESP values were obtained for each balloon volume at baseline and postreperfusion. The ESP versus balloon volume (BV) data for baseline and postreperfusion were fitted to the linear end-systolic pressure-volume relationship with the use of a least squares linear regression algorithm:

$$ESP = E_{\max} \times (BV) + k$$

where $E_{\max}$ is the slope and k is the y-intercept.

EDP. The EDP of a beat was defined as the point at which the increase in slope of the pressure waveform exceeded a threshold of 0.5 mm Hg/msec. This was visually confirmed for each beat. The average EDP was calculated by averaging the EDP values of 10 beats and was obtained for each balloon volume at baseline and postreperfusion. The EDP versus balloon volume (BV) data were fitted to the linear end-diastolic pressure-volume relationship with the use of a least squares linear regression algorithm:

$$EDP = m(BV - BV_0)$$

where m is the slope and BV_0 is the balloon volume corresponding to an EDP of zero, or the x-intercept.^{10, 11} A linear representation of the diastolic pressure-volume relationships was appropriate over the limited range of end-diastolic volumes examined in this model.¹²

Developed pressure. The left ventricular developed pressure was obtained by subtracting the EDP from the ESP for each data point. An average of 10 data points was used for each balloon volume. The developed pressure (DP) versus balloon volume (BV) data for baseline and postreperfusion were fitted to a linear pressure-volume relationship with use of the following linear regression algorithm:

$$DP = ESP - EDP = (E_{\max} \times BV + k) - m(BV - BV_0)$$

Percent recovery of developed pressure. The percent recovery of developed pressure was calculated as the percentage of the postreperfusion average developed pressure to the baseline average developed pressure at the same balloon volume (BV). This was calculated for each of the postreperfusion matched balloon volumes. The

Table I. Mechanical and electrical parameters

Cardioplegic solution	Time to infuse cardioplegic solution (min)	Time until mechanical arrest (min)*	Time until electrical arrest (min)*
Krebs-Henseleit	3.58 ± 2.10	5.58 ± 0.82	18.58 ± 2.10
St. Thomas' Hospital	2.60 ± 0.16	7.86 ± 0.43	15.82 ± 0.13
50 µmol/L pinacidil	1.93 ± 0.39	7.49 ± 0.62	16.93 ± 0.39
Pinacidil plus glibenclamide	1.89 ± 0.36	6.33 ± 0.67	18.53 ± 1.67

Data are represented as mean plus or minus standard error of the mean.

*Time from onset of 15-minute normothermic ischemic period.

average percent recovery of developed pressure (%Rec DP) was obtained by use of the trapezoidal rule¹³ and the following equation:

Average %Rec DP = 100

$$\times \int_{BV_{\min}}^{BV_{\max}} \frac{DP \text{ postreperfusion } dBV/BV_{\max} - BV_{\min}}{DP \text{ baseline}}$$

where $BV_{\max}$ is the maximum postreperfusion matched balloon volume and $BV_{\min}$ is the minimum postreperfusion matched balloon volume.⁸

Statistical analysis. Values are represented as the mean plus or minus the standard error of the mean. Analysis of variance was used for comparison of multiple groups. A Student's *t* test (or Mann-Whitney test when appropriate) was used to compare two means. A paired Student's *t* test was used to compare means before and after an intervention. Differences were considered statistically significant when $p < 0.05$. Statistical analysis was done with Sigma Stat software (version 1.01, Jandel Corp., San Rafael, Calif.).

Results

Delivery of cardioplegic solution. The mean time to infuse the cardioplegic solution ranged between 1.89 ± 0.36 and 3.58 ± 2.10 minutes and was not different among the groups (Table I).

Temporal aspects of the development of electro-mechanical arrest. The mean times for the cessation of electrical and mechanical activity are represented in Table I for each group. Mechanical arrest occurred in all instances during the period of unprotected, global, normothermic ischemia and was not different among groups. Electrical quiescence was not achieved until after the administration of cold cardioplegic solution in all groups. Electrical quiescence was achieved most rapidly in the St. Thomas' Hospital group, but this was not significantly shorter than in the other groups.

Postischemic recovery of systolic function: depolarized versus hyperpolarized arrest. The percent recovery of developed pressure in the hearts pro-

tected with St. Thomas' Hospital solution, pinacidil, pinacidil with glibenclamide, and Krebs-Henseleit cardioplegic solution was $46.4\% \pm 2.2\%$, $49.3\% \pm 2.4\%$, $43.0\% \pm 4.3$, and $32.8\% \pm 2.8\%$, respectively (Fig. 1). The percent recovery of developed pressure was significantly higher in both the pinacidil and St. Thomas' Hospital groups when compared with that in the Krebs-Henseleit group ($p = 0.009$).

Postischemic recovery of diastolic properties: depolarized versus hyperpolarized arrest. The slopes of the diastolic pressure-volume relationships at baseline and after 30 minutes of reperfusion were compared (Table II). There was a statistically significant increase in slope after reperfusion compared with baseline values in all groups (St. Thomas' Hospital, $p < 0.001$; pinacidil, $p = 0.002$; pinacidil with glibenclamide, $p = 0.002$; and Krebs-Henseleit, $p = 0.017$). In addition, the Krebs-Henseleit group had a significantly higher reperfusion slope as compared with that of all other groups ($p = 0.022$).

Reperfusion arrhythmias. On reperfusion, no hearts underwent ventricular fibrillation.

Reperfusion coronary blood flow. The mean baseline coronary blood flow before ischemia at an EDP of 5 mm Hg ranged between 7.0 ± 1.0 and 9.1 ± 1.7 ml/min and was not significantly different among groups (Fig. 2). Coronary blood flow immediately on reperfusion was significantly increased compared with baseline flow in the St. Thomas' Hospital, pinacidil, and pinacidil with glibenclamide groups ($p = 0.014$, $p < 0.001$, and $p = 0.002$, respectively). The coronary blood flow on reperfusion was significantly lower in the Krebs-Henseleit group compared with that in all other groups ($p = 0.004$).

After 5 and 10 minutes of reperfusion, the coronary blood flow in the pinacidil group remained significantly higher than both the flow at baseline and the flow in the Krebs-Henseleit group ($p < 0.001$ and $p = 0.021$, respectively). The coronary

Fig. 1. Percent recovery of developed pressure (DP) for the Krebs-Henseleit (*Krebs*), pinacidil with glibenclamide (*Pinacidil + Glib*), St. Thomas' Hospital (*St. Thomas*), and pinacidil groups. Values are represented as mean plus or minus the standard error of the mean. * $p < 0.05$ versus Krebs-Henseleit group.

blood flow then returned to baseline levels after 15 minutes of reperfusion. The coronary blood flow in the glibenclamide with pinacidil group remained elevated compared with its baseline value until 10 minutes of reperfusion ($p = 0.042$).

The coronary blood flow in the St. Thomas' Hospital group returned to the baseline level after 5 minutes of reperfusion. After 30 minutes of reperfusion, the coronary blood flow in the St. Thomas' Hospital and Krebs-Henseleit groups declined to a level significantly lower than baseline ($p = 0.003$ and $p = 0.019$, respectively).

Myocardial tissue water. The mean percent tissue water was $80.4\% \pm 0.3\%$, $80.7\% \pm 0.3\%$, $80.7\% \pm 0.5\%$, and $80.4\% \pm 0.1\%$ for the St. Thomas' Hospital, pinacidil, pinacidil with glibenclamide, and Krebs-Henseleit groups, respectively. There was no statistically significant difference in percent tissue water among groups.

Discussion

PCOs pharmacologically open K_{ATP} channels, causing a potassium efflux from the cell, a reduction in the action potential duration, and a reduction in

Table II. Diastolic function

Cardioplegic solution	Baseline slope (mm Hg/ml)	Reperfusion slope (mm Hg/ml)
St. Thomas' Hospital	17.85 ± 1.47	$29.26 \pm 3.17^{*\dagger}$
50 μ mol/L pinacidil	25.71 ± 1.73	$34.98 \pm 2.47^{*\dagger}$
Pinacidil plus glibenclamide	27.73 ± 3.35	$35.85 \pm 3.49^{*\dagger}$
Krebs-Henseleit	27.34 ± 6.22	$53.22 \pm 6.88^*$

Data are represented as mean plus or minus standard error of the mean.

* $p < 0.05$ versus baseline value.

$\dagger p < 0.05$ versus Krebs-Henseleit group.

calcium influx. The resultant contractile arrest has a potent cardioprotective effect during periods of ischemia.^{1, 2, 14} When given before myocardial ischemia, PCOs have been demonstrated to improve recovery of ventricular function, decrease infarct size, and limit high-energy nucleotide loss in various animal models.^{6, 15-17}

The inherent cardioprotective effects of PCOs offer advantages over traditional depolarized arrest with hyperkalemic cardioplegia. The ongoing metabolic processes and derangements in transmembrane ionic gradients that occur at depolarized

Fig. 2. Reperfusion coronary blood flow for the Krebs-Henseleit (*Krebs*), pinacidil with glibenclamide (*Pinacidil + Glib*), St. Thomas' Hospital (*St. Thomas*), and pinacidil groups. Values are represented as mean plus or minus the standard error of the mean. Baseline is represented at time -5, reperfusion is represented at time 0, and so on, in minutes after reperfusion on the x axis. ** $p < 0.05$ pinacidil, St. Thomas' Hospital, and pinacidil with glibenclamide groups versus Krebs-Henseleit group; ‡ $p < 0.05$ pinacidil and pinacidil with glibenclamide groups versus Krebs-Henseleit group; * $p < 0.05$ pinacidil versus Krebs-Henseleit group.

potentials represent major shortcomings of traditional potassium cardioplegia.^{18,19} These metabolic processes include energy-requiring calcium and sodium pumps that continue to turn over and deplete critical energy supplies.^{18,19} Hypothermia became a standard part of many myocardial protection strategies to decrease this ongoing metabolic demand.

The natural resting state of the myocardium is at a more negative membrane potential (hyperpolarized), and at this potential, transmembrane ion gradients are largely balanced and net ion flux is minimal. In addition, few voltage-gated ion channels are open.¹ Thus energy demand is minimized. By providing ischemic protection at a more physiologic membrane potential, PCOs may be more ideal cardioplegic agents than traditional depolarizing hyperkalemic solutions.

Previous work from our laboratory has documented the effectiveness of pinacidil as a cardioplegic agent when compared with hyperkalemic cardioplegia consisting of Krebs-Henseleit solution with 20

mmol/L KCl during normothermic ischemia and has established the optimal dose of pinacidil (50 μ mol/L) in an isolated rabbit heart model.⁷ However, there has been some question as to the efficacy of these PCOs under hypothermic conditions. Strong experimental evidence is available to support this question. The shortening of the action potential duration by pinacidil has been shown to be more rapid and more complete at 37° C than at 23° C.²⁰ Moreover, the outward potassium current activated by pinacidil has been shown to cease at 19° to 21° C in isolated guinea pig myocytes.²¹

However, previous work has suggested that PCOs may be effective as hypothermic cardioplegic agents. PCO cardioplegia with pinacidil has been shown to provide superior protection compared with hyperkalemic cardioplegia during hypothermic ischemia in an intact porcine model.²²

This study was designed to examine the effectiveness of myocardial protection during surgical global hypothermic ischemia in the acutely injured heart

with pinacidil and to compare this protection with that of the standard hyperkalemic cardioplegia, St. Thomas' Hospital solution. K_{ATP} channel activity was examined under hypothermic conditions with use of the potassium channel inhibitor, glibenclamide.

Postischemic functional recovery: depolarized versus hyperpolarized arrest. Pinacidil and St. Thomas' Hospital solution provided superior protection during the 50 minutes of hypothermic arrest in the acutely injured heart when compared with Krebs-Henseleit solution alone. However, there was no statistical difference between pinacidil and St. Thomas' Hospital solution. Although this is in contrast to findings of a previous study,²² this may be explained by the different experimental models and protocols and species variation. The improved protection with pinacidil was lost with the addition of the potassium channel inhibitor glibenclamide. This indicates that some K_{ATP} channel activity by this drug during hypothermia is responsible for the observed cardioprotection.

The lack of statistical difference in percent recovery of developed pressure between the pinacidil and the pinacidil with glibenclamide groups may be related to a suboptimal dose of glibenclamide.

Postischemic recovery of diastolic properties. The end-diastolic pressure-volume relationships were linear over the range of volumes tested in our experiments, as has been described by other investigators.^{11, 12} The mean linear regression coefficient for the pressure-volume relationships was 0.99 ± 0.00 , 0.99 ± 0.00 , 0.99 ± 0.01 , and 0.98 ± 0.01 for the Krebs-Henseleit, Krebs-Henseleit with pinacidil, Krebs-Henseleit with pinacidil and glibenclamide, and St. Thomas' Hospital groups, respectively. All cardioplegia groups showed a significant rise in the slope of the pressure-volume relationship after ischemia, indicating a decrease in ventricular compliance. The reperfusion slope in the Krebs-Henseleit group was significantly greater than that of any other group, which suggests a more severe diastolic injury in this unprotected group.

The Krebs-Henseleit group had a more severe diastolic injury compared with that in the pinacidil with glibenclamide group. This may be because of the inability of the $10 \mu\text{mol/L}$ dose of glibenclamide to saturate and block all of the K_{ATP} channels in the presence of $50 \mu\text{mol/L}$ pinacidil or because of a non- K_{ATP} channel-mediated mechanism of protection by pinacidil.

The development of electromechanical arrest. Mechanical arrest occurred at approximately the same time in all groups. This occurred during the 15-minute period of unprotected normothermic ischemia and therefore no difference would have been expected among groups. This is indicative of a similar response of the hearts in all groups to unprotected ischemia.

There was no significant difference among groups in the time until electrical arrest. This is probably because hypothermia alone is effective in eliminating electrical activity and this overcame the previously observed inability of pinacidil to achieve rapid electrical asystole.

Reperfusion coronary blood flow. Reperfusion coronary blood flow in the pinacidil group was greater than the coronary blood flow in the Krebs-Henseleit group for the initial 10 minutes of reperfusion. The coronary blood flow immediately on reperfusion was greater in the St. Thomas' Hospital group compared with that in the Krebs-Henseleit group but this was not different from that seen with pinacidil. These data are in contrast to our previous findings after normothermic ischemia in which the coronary blood flow was significantly higher in the PCO-protected groups than in the St. Thomas' Hospital group.⁷ This may be explained by the greater ischemic insult in this protocol (15 minutes of normothermic unprotected plus 50 minutes of hypothermic protected ischemia) compared with that in our previous work under normothermic conditions (30 minutes of protected ischemia). The increased coronary blood flow in this study in the St. Thomas' Hospital group, as opposed to that in the previous study, may be the result of a greater ischemic insult that resulted in reperfusion hyperemia. This would be consistent with the poorer recovery of developed pressure in this study compared with our previous results and the significant diastolic injury seen in each group.

The pinacidil with glibenclamide group exhibited some hyperemia on reperfusion and after 5 minutes of reperfusion, which suggests some K_{ATP} channel activity and resultant vasodilation despite the $10 \mu\text{mol/L}$ dose of glibenclamide or a non- K_{ATP} channel-mediated endothelial protection in this group. The shortened period of reactive hyperemia in the pinacidil plus glibenclamide group compared with that in the pinacidil group is consistent with the inhibition of K_{ATP} channel activity by glibenclamide under these hypothermic conditions.

The lack of reactive hyperemia in the Krebs-Henseleit group alone is also different from that in our previous work under normothermic conditions. This suggests that the endothelium in the Krebs-Henseleit group was unable to provide the expected hyperemic response because of the magnitude of the injury in this protocol and a no-reflow response.

However, the changes noted in reperfusion coronary blood flow in these experiments may be reflective of many factors not limited to the vasodilatory properties of pinacidil and reactive hyperemia. It is likely that the cardioprotective properties of pinacidil are independent of the vasodilatory properties, and further research is indicated to elucidate these findings.

Incidence of ventricular fibrillation on reperfusion. In contrast to our previous findings with the use of PCOs during normothermic ischemia, no hearts in this study fibrillated on reperfusion.⁷ PCOs have been demonstrated to be proarrhythmic by decreasing action potential duration and, as a result, decreasing the refractory period.^{16, 23} This finding after hypothermic ischemia may be related to the decreased efficacy of pinacidil at lower temperatures to open K_{ATP} channels and decrease action potential duration. Alternatively, it may represent an electrophysiologically protective effect of hypothermia itself. Further studies will be needed to clarify this issue.

However, both the absence of reperfusion ventricular fibrillation and the lack of prolonged electrical activity by PCO cardioplegia in our study suggest a different electrophysiologic effect of pinacidil on K_{ATP} channels under these hypothermic conditions.

Model shortcomings. The advantages and disadvantages of the blood-perfused isolated rabbit heart Langendorff model have been previously described.^{7, 8} This model provides a closer approximation to the clinical situation than crystalloid-perfused models. There are many advantages of blood over crystalloid perfusion in the investigation of ischemia/reperfusion phenomena. These include a higher oncotic pressure, improved oxygen-carrying capacity, the histidine/imidazole buffering capacity, the presence of free radical scavengers, and improved capillary flow distribution. The isolated rabbit model has been shown to be quite stable over 120 minutes of aerobic perfusion as compared with that of other animal species.²⁴ As always, care should be taken when extrapolating these data to the clinical situation.

In addition, the optimal dose of pinacidil (50 μ mol/L) used in these experiments was determined

with use of a dose-response curve generated with normothermic ischemia.⁷ This dose may not be the optimal choice under the conditions of hypothermia used in the present experiments.

Summary. Pinacidil (50 μ mol/L) provided comparable myocardial protection to that of St. Thomas' Hospital solution during hypothermic ischemia in the blood-perfused rabbit heart model. This protection was superior to that of Krebs-Henseleit solution. The superior myocardial protection provided by pinacidil was lost when glibenclamide was added to the cardioplegic solution, indicating K_{ATP} channel activity during hypothermia despite previous results in isolated myocytes.^{20, 21}

In addition, the previously noted proarrhythmic nature of pinacidil seen in our normothermic experiments was absent under the present hypothermic experimental conditions. This observation raises questions as to the nature of the action of pinacidil under hypothermic conditions. This finding is advantageous for the future clinical use of these agents in that reperfusion ventricular fibrillation, previously noted with normothermia, did not occur after hypothermic global ischemia protected with pinacidil.

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