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Temperature Threshold and Modulation of Energy Metabolism in the Cardioplegic Arrested Rabbit Heart[1]

Xue-Han Ning, Cheng-Su Xu, Ying C. Song, Keith F. Childs, Yun Xiao, Steven F. Bolling, Flavian Mark Lupinetti, and Michael A. Portman

Division of Cardiology, Department of Pediatrics, and the Division of Cardiovascular Surgery, Department of Surgery, University of Washington, Seattle, Washington 98195; and the Division of Thoracic Surgery, Department of Surgery, University of Michigan Medical Center, Ann Arbor, Michigan 48109, U.S.A.

Hypothermia protects ischemic tissues by reducing ATP utilization and accumulation of harmful metabolites. However, it also reduces ATP production, which might cause deterioration in the energy supply/demand ratio. Modulation of energy supply/demand according to temperature has not been previously studied in detail. In this study, isolated, perfused rabbit hearts ($n = 60$) were used to determine the effects of various temperatures on myocardial energy metabolism and function during cardioplegic arrest. Ischemia was induced by crystalloid cardioplegic solution at 4, 18, 30, and 34°C for 120 min, respectively. At each temperature, the hearts were divided into a glucose-treated group which contained 22 mM glucose in cardioplegic solution as the only substrate and a control group which contained 22 mM mannitol to keep same osmolarity. Following 15 min reperfusion, recovery of left ventricular developed pressure (DP), $\pm dP/dt_{max}$, and the product of heart rate and DP were significantly higher in 30, 18, and 4°C groups than those in 34°C control group. The functional recovery was also significantly higher in the 34°C glucose-treated group than that in the 34°C control group, but there was no difference between those groups at 30°C and the temperature below 30°C. Myocardial ATP concentration was significantly lower in 34°C control group than those in other groups. There is a close relationship between myocardial ATP concentration and functional recovery ($R^2 = 0.90$). The accumulations of lactate and CO_2 were significantly higher at 34°C in glucose-treated group than those in the control group. However, there was no significant difference between these two groups at 30°C and the temperature below 30°C. These results indicate that under these study conditions: (1) a marked decrease in energy supply/demand occurs above 30°C, implying that a temperature threshold exists; and (2) this can be ameliorated by provision of glucose as substrate in cardioplegia solution.

Key Words: ATP; cardioplegia; CO_2; glucose; hypothermia; lactate; myocardial ischemia; myocardial reperfusion; temperature; threshold.

Hypothermia protects ischemic myocardium by reducing metabolic demands and injurious catabolite production (3, 6, 12–14, 25, 27, 28). These beneficial effects are negated somewhat by a reduction in high-energy phosphate synthesis, which might offset the balance between energy demand and supply. Separate from these energy metabolic considerations, profound hypothermia can also induce temperature-dependent injury through various mechanisms including enhancement of cellular calcium entry, which leads to muscle contracture (1, 4, 8, 9, 15–17, 23, 26). Cardioplegia enhances the protective effects of hypothermia (12, 13). Glucose addition to crystalloid solutions further strengthens these cardioprotective effects by promoting anaerobic glycolysis, which itself is temperature dependent (19–21, 24). These multiple factors could influence functional recovery during reperfusion after ischemia. This study's principal objective was to determine that specific temperature range, which minimizes injurious effects of hypothermia, while maintaining the heart energy supply/demand ratio. The data indicate that this optimal temperature range is fairly broad. However, energy supply/demand ratio deteriorates rapidly above 30°C, implying that a temperature threshold exists for myocardial protection. Additionally, the threshold can be modified by substrate provision, which in-

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creases energy supply during increased energy demand at higher temperatures.

MATERIALS AND METHODS

Preparation of Isolated Heart

White New Zealand rabbits (male or female, 2.2–2.7 kg body wt) were anesthetized with sodium pentobarbital (45 mg/kg, intravenously) and heparinized (700 U/kg, intravenously). Median sternotomy was performed and the heart was rapidly excised and immersed momentarily in ice-cold physiological salt solution (PSS), pH 7.4, containing 118.0 mmol/L NaCl, 4.0 mmol/L KCl, 22.3 mmol/L $NaHCO_3$, 11.1 mmol/L glucose, 0.66 mmol/L KH_2PO_4, 1.23 mmol/L $MgCl_2$, and 2.38 mmol/L $CaCl_2$. Previous studies have shown that myocardial H_2O content was stable ($\leq$85%) in isolated perfused rabbit hearts perfused at 80–90 mm Hg with PSS using the same protocol (19–21). The aorta was cannulated in the Langendorff mode and the heart was perfused with PSS that has been equilibrated with 95% O_2–5% CO_2 at 37°C and passed twice through 3.0-μm pore size filters. Perfusion pressure was maintained at 90 mm Hg. An incision was made in the left atrium, and a fluid-filled latex balloon was passed through the mitral orifice and placed in the left ventricle. The balloon was connected to a pressure transducer for continuous measurement of left ventricular pressure (LVP) and the first derivative of LVP (dP/dt). The caudal vena cavum, the left and right cranial vena cava, and the azygous vein were ligated (19). The pulmonary artery was cannulated for collection of coronary flow, measured with a flow meter (T201, Transonic Systems Inc., Ithaca, NY).

The analog signals were continuously recorded on a pressurized ink chart recorder (Gould, Inc., Cleveland, OH) and an on-line computer (Macintosh, Biopac Analog Signal Acquisition System). To characterize cardiac function, developed pressure (DP) is defined as peak systolic pressure (PSP) minus end-diastolic pressure (EDP). The product of heart rate (HR) and DP (PRP, mm Hg/min) was calculated to provide an estimate of myocardial work. Myocardial oxygen consumption (MV_{O_2}) was calculated as the expression $MV_{O_2} = CF \times [(Pa_{O_2} - Pv_{O_2}) \times (c/760)]$, where CF is coronary flow (ml/min), $(Pa_{O_2} - Pv_{O_2})$ is the difference in the partial pressure of oxygen (P_{O_2}, mm Hg) between perfusate and coronary effluent flow, and c is the Bunsen solubility coefficient of O_2 in perfusate at 37°C (22.7 μl $O_2 \times atm^{-1} \times ml^{-1}$ perfusate) (19, 22). Because hearts were quickly placed in liquid nitrogen for metabolite measurements at the end of experiments, the heart was not weighed and the measurements of CF and MV_{O_2} were not normalized by heart weight. Oxygen extraction was calculated as the expression, O_2 EXT = MV_{O_2}/oxygen content in the perfusate. Procedures followed were in accordance with institutional and NIH guidelines on treatment of animals.

Lactate and CO_2 Measurements

The first 1.5 ml of coronary effluent was collected at ischemic flush time (see Experimental Protocols) and at reflow. Lactate concentration was measured with a GM7 Analyser (Analox micro-Stat, London). Concentrations of O_2 and CO_2 were measured with a Radiometer (ABL 3, Copenhagen, Denmark). Difference in CO_2 content (d_{CO_2}) between coronary outflow and inflow was calculated as $d_{CO_2} = (Pv_{CO_2} - Pa_{CO_2}) \times c/V_m$, where $(Pv_{CO_2} - Pa_{CO_2})$ is the difference in the partial pressure of carbon dioxide (P_{CO_2}, mm Hg) between coronary effluent flow and perfusate, c is the solubility coefficient of CO_2 in perfusate at 37°C (0.53 ml $CO_2 \cdot ml^{-1}$ perfusate), and V_m is molar volume (22.4 ml/mmol/L) (20).

ATP and Metabolites

To observe changes in tissue nucleotides (ATP, ADP, AMP, IMP) and nucleosides (adenosine, inosine, hypoxanthine, and xanthine), hearts were quickly frozen in liquid nitrogen and then lyophilized for 48 h at −40°C under 200-Torr vacuum. An aliquot (10 mg) of the dried tissue was homogenized with 800 μl of 0.73 M trichloroacetic acid (TCA). After centrifugation (7000 rpm, 2 min) at 4°C, the

supernatant (400 μl) was removed and added to a new Eppendorf tube containing an equal volume of tri-*n*-octylamine and Freon (1:1, v/v). The sample mixture was then vortexed and centrifuged as before. The aqueous phase was analysed with high-performance liquid chromatography (HPLC) (5, 19). Mobile phase was prepared as follows: buffer A consisted of 1.47 mM tetrabutylammonium phosphate (TBAP) as a pairing ion and 73.5 M potassium dihydrogen phosphate (pdp), and 0.0% acetonitrile; buffer B consisted of 10% acetonitrile in distilled, deionized water, 1.33 mM TBAP, and 66 M pdp. The final concentration of acetonitrile was adjusted by a two-pump control method for achieving optimum peak resolution and separation of nucleotides (3%) and nucleosides (0.5%) at pH 3.05. Standard curves were generated from serial dilutions of ATP, ADP, AMP, IMP, adenosine, hypoxanthine, xanthine, and inosine (Sigma Chemical Co., St. Louis, MO) at 10, 25, 50, 100, and 500 μmol/L. A Water 484 UV absorbance detector was used for nucleotide and nucleoside determinations. Peak areas from samples were integrated and least square curves were plotted.

Experimental Protocols

After completing instrumentation and performing calibrations, left ventricular balloon volumes were varied over a range of values to construct left ventricular function curves. In this manner, it is possible to define a specific balloon volume that is associated with a developed pressure between 100 and 140 mm Hg. This volume was maintained the same during baseline and reperfusion conditions. The intraventricular balloon volumes were not adjusted to produce specific end-diastolic pressures (rather, we defined a level of systolic pressure development), but end-diastolic pressures at baseline greater than 10 mm Hg were not considered acceptable (22). Data from hearts characterized by developed pressures less than 100 mm Hg or greater than 140 mm Hg were not used. Baseline data were obtained after an equilibration period of approximately 30 min. The same procedures were followed in each experiment. During the baseline period, data were obtained with the hearts maintained at 37°C by water circulated through the organ bath. The pulmonary outflow temperature was monitored continuously with a thermal probe to adjust the temperature of the infusion. During ischemia, the PSS infusion was stopped and 60 ml of oxygenated St. Thomas' cardioplegic solution (CP) at 4°C was injected into the aorta at a rate of 1 ml/s to begin the 2-h ischemia. The organ bath temperature was changed to 4, 18, 30, or 34°C during ischemia. Fifteen milliliters of St. Thomas' cardioplegic solution (4°C) was injected every 30 min thereafter. The St. Thomas cardioplegic solution contained 109.0 mmol/L NaCl, 25.0 mmol/L KCl, 21.9 mmol/L $NaHCO_3$, 16.0 mmol/L $MgCl_2$, and 0.8 mmol/L $CaCl_2$. When the 2-h ischemic period was ended, the hearts were reperfused with oxygenated PSS at 37°C and the water bath temperature was increased to 37°C. During the 15 min of reperfusion, hemodynamic data were recorded to compare with baseline data and to determine the degree of functional recovery in each heart. After reperfusion, hearts were quickly placed in liquid nitrogen for metabolite measurements.

A total of 60 hearts were used for this study. The hearts divided into 4, 18, 30, and 34°C ischemic groups. In pilot studies, the functional recovery was similar at 4, 12, 18, 22, 26, and 30°C ischemia, but the recovery was dramatically decreased at 34 and 37°C ischemia. Therefore, we performed experiments at the above four temperatures to simplify the protocol. Each temperature group was divided into two subgroups: control group and glucose-treated group. Twenty-two millimolar glucose was added into cardioplegic solution as substrate to observe the effect of ischemic temperature on myocardial metabolite levels in the glucose-treated group. It has been demonstrated that 22 mM glucose is an adequate concentration of substrate during warm ischemic arrest (19). Mannitol (22 mM) was added into cardioplegic solution as an osmolar control.

Statistical Analysis

Values reported are means ± standard error (SE) in the text, tables, and figures. The Statview 4.5 (FPV) Program (Abacus Concepts, Inc., Berkeley, CA, 1995) was used for statistical analysis. Data were evaluated with repeated measures analysis of variance within groups and single factor analysis of variance across groups. When significant F values were obtained, Scheffe's test was used to distinguish which groups differed from each other significantly. The criterion for significance was taken to be $P < 0.05$ for all comparisons.

RESULTS

Functional Parameters

On baseline. The left ventricular balloon volumes were similar in the groups ($P > 0.05$, Table 1). During baseline conditions, there were no significant differences between control and glucose-treated groups in EDP, DP, $\pm dP/dt_{max}$, HR, PRP, CF, MV_{O_2} and O_2EXT (Table 1).

Functional recovery during reperfusion. In Table 1 and Fig. 1, the data demonstrate that hypothermic hearts provides greater functional recovery than that observed in the 34°C control hearts. There were no significant differences between control and glucose-treated hearts after 4, 18, and 30°C ischemia. The hypothermic hearts were characterized by higher developed pressures, higher dP/dt_{max} values, and lower end diastolic pressure, indicating that the hypothermia improved recovery from ischemic and/or reperfusion injury. Heart function in the hypothermic groups remained improved from 4 to 30°C in both control and glucose-treated hearts.

Ischemic contracture. As noted under Materials and Methods, a specific balloon volume was adjusted and maintained throughout the experiment, allowing comparisons of left ventricular pressure under constant end-diastolic volume. After injecting cardioplegic solution, the left ventricular pressure was always near 0 mm Hg. The beginning of ischemic contracture was defined by the initial rise in left ventricular pressure above 2 mm Hg. Ischemic contracture started in the 34°C control group after 61.5 ± 3.2 min of ischemia but there was no contracture in the hypothermic groups. There was also no contracture in the 34°C glucose-treated group.

Aerobic and Anaerobic Metabolic Products

The difference of CO_2 content (d_{CO_2}) and lactate concentration ($d_{LACTATE}$) between coronary outflow and inflow were dramatically increased in the glucose-treated group at 34°C ischemia, indicating that the aerobic and anaerobic metabolism were elevated at 34°C. Figure 2 summarizes the results. There were no significant differences between control and the glucose-treated groups at 4, 18, and 30°C ischemia.

At 34°C, there was a higher d_{CO_2} and $d_{LACTATE}$ in the glucose-treated group. The functional recovery was better in this group than that of control group. In contrast, at this temperature there were a lower d_{CO_2} and $d_{LACTATE}$ in the control group, and the levels were similar to those at 30°C. However, the functional recovery was obviously poor compared with all other groups.

One molecule of glucose would be broken down to two molecules of lactate or broken down to six molecules of CO_2. The glucose utilization was calculated by the formula (20) Glucose utilization = $(d_{CO_2}/6 + d_{LACTATE})$ mM, where d_{CO_2} and $d_{LACTATE}$ are the differences of CO_2 content and of lactate concentration between coronary effluent and inflow perfusates. The calculated glucose utilization was similar in the control and glucose-treated hearts during 4°C (0.33 ± 0.04 and 0.41 ± 0.02 mM, respectively), 18°C (0.25 ± 0.06 and 0.29 ± 0.03 mM, respectively), and 30°C (0.96 ± 0.06 and 0.99 ± 0.11 mM, respectively) ischemia (Fig. 3). At 34°C the calculated glucose was 1.20 ± 0.06 mM in control hearts but was 2.59 ± 0.11 mM in the glucose-treated hearts.

The ratio of anaerobic metabolism to the total glucose utilization was calculated as glucose broken down to lactate/(Glucose broken down to lactate + Glucose broken down to CO_2). The ratio of anaerobic metabolism to the total glucose utilization is shown in Fig. 4. The ratio of anaerobic metabolism increases with the in-

TABLE 1
Hemodynamics (Mean ± SE)

Group	C4 (n = 7)	G4 (n = 7)	C18 (n = 6)	G18 (n = 6)	C30 (n = 6)	G30 (n = 6)	C34 (n = 11)	G34 (n = 11)
LVV (ml)	1.30 ± 0.06	1.59 ± 0.13	1.30 ± 0.10	1.37 ± 0.07	1.30 ± 0.12	1.40 ± 0.12	1.29 ± 0.11	1.53 ± 0.07
EDP (mm Hg)								
Baseline	1.9 ± 0.63	1.7 ± 0.47	1.8 ± 0.60	2.2 ± 0.17	1.8 ± 0.40	1.5 ± 0.22	2.7 ± 0.41	1.2 ± 0.23
REP	16.9 ± 5.05	19.4 ± 2.68	12.8 ± 1.72	13.3 ± 0.99	9.3 ± 2.55	8.3 ± 0.84	68.5 ± 5.24	23.3 ± 1.95*
DP (mm Hg)								
Baseline	115.1 ± 3.5	114.1 ± 3.0	117.7 ± 5.0	116.7 ± 6.1	119.0 ± 5.9	113.5 ± 5.8	108.1 ± 2.5	109.5 ± 2.4
REP	77.7 ± 8.2	69.8 ± 5.2	88.5 ± 7.4	97.3 ± 5.4	93.2 ± 2.8	97.2 ± 6.0	11.9 ± 2.3	69.9 ± 4.4*
dP/dt_{max}(mm Hg/s)								
Baseline	1577 ± 72	1664 ± 80	1442 ± 42	1475 ± 105	1540 ± 116	1545 ± 149	1549 ± 88	1654 ± 87
REP	900 ± 112	820 ± 83	1172 ± 72	1253 ± 115	1055 ± 46	1223 ± 99	154 ± 25	900 ± 54*
$-dP/dt_{max}$ (mm Hg/s)								
Baseline	977 ± 26	944 ± 48	993 ± 35	1060 ± 65	937 ± 48	983 ± 66	1064 ± 44	1050 ± 32
REP	690 ± 78	687 ± 56	850 ± 60	908 ± 63	760 ± 24	852 ± 50	105 ± 20	698 ± 32*
HR (beats/min)								
Baseline	197.0 ± 4.4	200.9 ± 8.6	163.2 ± 16.5	191.0 ± 12.6	178.7 ± 2.8	196.8 ± 6.4	197.5 ± 10.8	196.1 ± 11.8
REP	200.9 ± 4.8	214.9 ± 8.7	187.5 ± 8.4	190.2 ± 9.0	162.3 ± 5.3	177.0 ± 3.5	175.5 ± 11.2	177.5 ± 11.1
PRP (10^3 mm Hg/min)								
Baseline	22.70 ± 0.85	22.95 ± 1.21	18.83 ± 1.35	22.19 ± 1.78	21.22 ± 0.91	22.41 ± 1.55	21.39 ± 1.32	22.95 ± 1.21
REP	14.96 ± 1.76	14.63 ± 1.24	16.45 ± 1.27	18.49 ± 1.29	15.16 ± 0.80	17.20 ± 1.10	2.11 ± 0.29	11.85 ± 0.67*

Note. The hemodynamic indices were determined in isolated reperfused hearts at baseline and after 15 min of reperfusion (REP) as described under Materials and Methods. Abbreviations used: C, control group; G, glucose-treated group (4, 18, 30, and 34 represents the ischemic temperature at 4, 18, 30, and 34°C, respectively); DP, left ventricular developed pressure; dP/dt_{max}, maximum of the first derivative of left ventricular pressure; EDP, left ventricular end diastolic pressure; HR, heart rate; LVV, left ventricular volume, PRP, product of HR and DP.

* $P < 0.05$, G vs C for same condition.

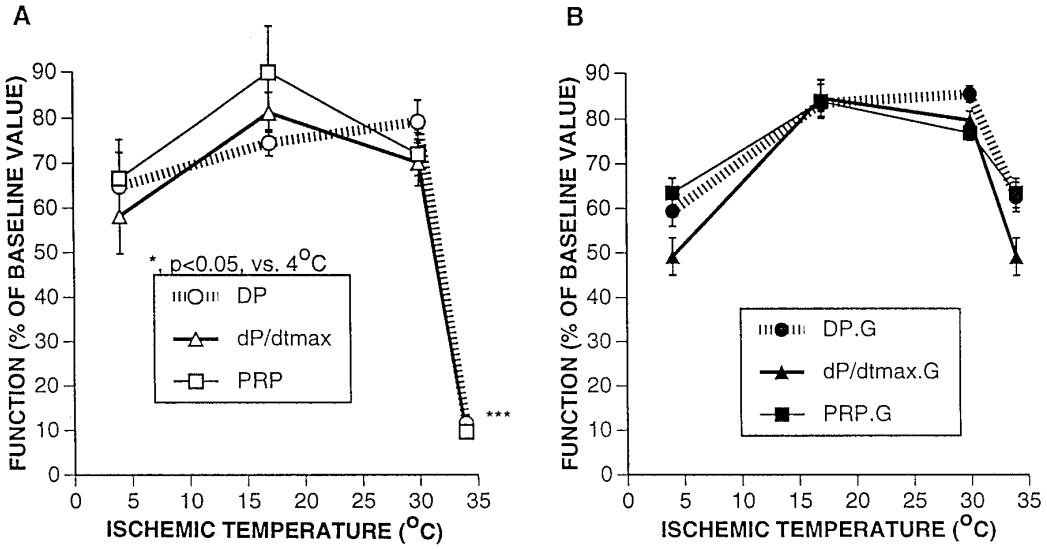

FIG. 1. Effect of hypothermia on functional recovery during reperfusion in isolated hearts. The reperfused cardiac function parameters are plotted as a percentage of baseline (37°C). Abbreviations used: DP, developed pressure; dP/dt_{max}, positive maximum of the first derivative of left ventricular pressure; PRP, product of HR and DP. *$P < 0.05$, versus at 4°C. Hypothermia (4–30°C) increases functional recovery during reperfusion in control hearts (A). The functional recovery is better in the glucose-treated hearts (B) than that in control hearts after 34°C ischemia.

crease in ischemic temperature until 30°C to reach maximal capacity.

Energy Status

At 15 min of reperfusion, the concentrations of myocardial ATP, and total nondiffusible nucleotides (TNN) were much higher in the hypothermic groups than those in the 34°C control group. There were no noticeable differences between glucose-treated hearts and control hearts after 4, 18, and 30°C ischemia. However, a significantly higher concentration of ATP and TNN was observed in the glucose-treated group than those in the control group after 34°C ischemia. The concentration of adenosine and total nondiffusible nucleotides were not significantly different between all the groups at 15 min of reperfusion. Table 2 summarizes the levels in energy status. Reference values for energy metabolites expressed as micromoles per gram of dry tissue in the aerobic heart ($n = 5$; 37°C) are ATP 20.04 ± 0.48, ADP 4.59 ± 0.57, AMP 0.38 ± 0.08, TNN 25.03 ± 0.90, ADO 0.14 ± 0.03, and TDN 0.61 ± 0.07. These reference values differ with ischemic values at all temperatures in control hearts ($P < 0.05$).

DISCUSSION

Temperature optimization for myocardial preservation has been an active focus of investigation. However, temperature modulation of myocardial metabolism in order to improve ischemic resistance has not been studied in detail. A specific characteristic of this modulation is the threshold phenomenon, which occurs above 30°C in control hearts in this study. Marked cardiac dysfunction during reperfusion occurs in these hearts subjected to ischemia at 34°C while substantially milder dysfunction occurs in hearts subjected to ischemia at 30°C and below. The severity of postischemic dysfunction is closely related to myocardial ATP concentration during reperfusion. Figure 5 illustrates the relation between left ventricular developed pressure and ATP. These data are consistent with previous studies which have closely linked ATP preservation with postischemic function (19–21).

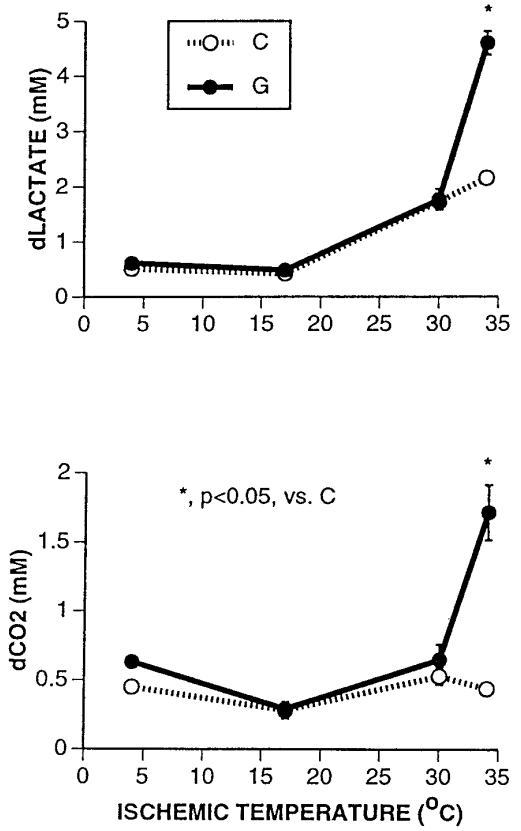

FIG. 2. Effects of hypothermia on cardiac metabolism at 120 min of ischemia. Abbreviations used: C, control group; G, glucose-treated group. Differences in CO_2 content (d_{CO_2}) or in lactate concentration ($d_{LACTATE}$) between coronary effluent and inflow perfusates are plotted versus ischemic temperature. Values were determined as described under Materials and Methods. $*P < 0.05$, G versus C at the same ischemic temperature. d_{CO_2} and $d_{LACTATE}$ are significantly lower in the control group than that in glucose-treated group at 34°C. There are no significant differences between C and G under hypothermic ischemia (4–30°C).

Differences in lactate and CO_2 production between control and glucose supplied hearts do not occur at the three lower temperatures studied. Nor are there temperature related differences in these glycolytic products at 30°C or below. Anaerobic glycolysis with lactate production represents the predominant ATP producing pathway during ischemia in this model. Inequities between ATP production and utilization would result in high energy phosphate store depletion, which in these experiments are indexed by ATP levels. Although anaerobic ATP production increases with temperature as does utilization, the consistency of ATP values implies that energy

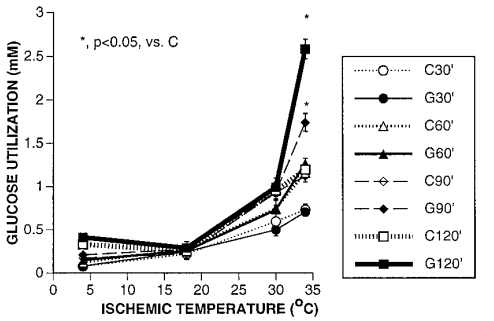

FIG. 3. Relationship between glucose utilization and ischemic temperature. Abbreviations used: C, control group; G, glucose-treated group; 30′, 60′, 90′, and 120′, at 30, 60, 90, and 120 min of ischemia. There are no significantly differences between C and G under hypothermic ischemia (4–30°C). The maximal utilization of glucose in the control group is about 1.2 mM that is reached at 60 min of ischemia under 34°C in both control and glucose-treated groups. This level does not reach under 30°C hypothermia in the 120 min of ischemia. The maximal utilization of glucose in the glucose-treated group is about 2.6 mM at 34°C and the level is higher than all control groups.

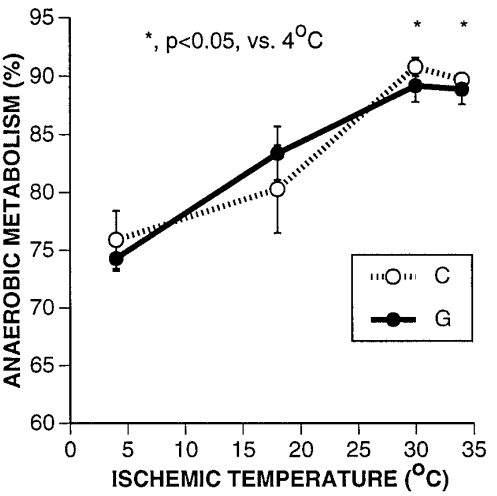

FIG. 4. Relationship between the ratio of anaerobic metabolism and temperature at 120 min of ischemia. Abbreviations used: C, control group; G, glucose-treated group. The ratio of anaerobic metabolism increases with the increase in ischemic temperature until 30°C.

TABLE 2
ATP and Metabolites (μmol/g Dry Tissue, Mean ± SE)

	C4	G4	C18	G18	C30	G30	C34	G34
ATP	14.86 ± 2.73	16.59 ± 0.67	16.21 ± 2.21	14.93 ± 2.73	17.38 ± 1.33	19.14 ± 0.64	2.02 ± 0.38**	10.54 ± 0.40*
TNN	22.88 ± 3.54	24.74 ± 0.73	21.28 ± 2.24	20.07 ± 2.92	21.74 ± 1.47	23.75 ± 1.02	5.23 ± 0.35**	15.03 ± 0.48*,**
ADO	0.31 ± 0.03	0.35 ± 0.05	0.20 ± 0.07	0.21 ± 0.03	0.25 ± 0.05	0.39 ± 0.13	0.57 ± 0.23	0.06 ± 0.01
TDN	2.24 ± 0.42	2.11 ± 0.15	1.21 ± 0.45	0.84 ± 0.12	1.17 ± 0.35	0.81 ± 0.23	1.29 ± 0.28	0.55 ± 0.09

Note. ATP and metabolites were determined in isolated reperfused hearts at 15 min of reperfusion as described under Materials and Methods. Abbreviations used: C, control group; G, glucose-treated group (4, 18, 30, and 34 represents the ischemic temperature at 4, 18, 30, and 34°C, respectively); ADO, adenosine; TNN, total nondiffusible nucleotides; TDN, total diffusible nucleosides. *$P < 0.05$, G vs C for same condition; **$P < 0.05$, control or glucose-treated groups vs C4 or G4, respectively.

supply/demand ratio is constant from 4 to 30°C with or without provision of glucose in cardioplegia. A marked decrease in the energy supply/demand ratio is indexed by the dramatic drop in ATP at 34°C. However, the presence of glucose in cardioplegia ameliorates both cardiac dysfunction and ATP loss in the postischemic hearts subjected to 34°C. This response represents a blunting of the threshold effect as apparent in Fig. 1 and Table 2. Glycogen depletion is known to occur during prolonged ischemia (2) and may result in substrate deprivation in the control hearts. Lack of substrate may result in accelerated ATP depletion and its associated cardiac dysfunction. This metabolic response implies that above 30°C, ATP utilization accelerates and substrate deprivation limits the hearts ability to increase ATP synthesis. This abrupt decrease in energy supply/demand ratio is reduced by substrate provision during ischemia at 34°C.

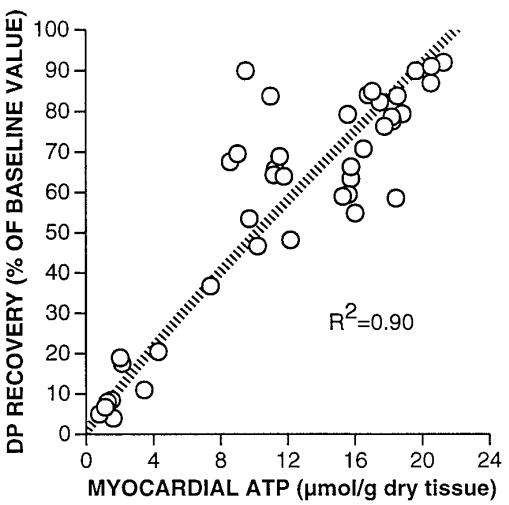

FIG. 5. Relationship between myocardial ATP concentration and functional recovery. The reperfused left ventricular developed pressure is plotted as a percentage of baseline (37°C) at 15 min of reperfusion. Abbreviation used: DP, left ventricular developed pressure.

Although postischemic function appears to be modulated by energy metabolism above 30°C, it does not appear to play a major role below this temperature in the current cardioplegic arrested model. Myocardial dysfunction and damage has been documented during profound hypothermia. The temperature and mode of injury is specific to the model under investigation, but in general is related to enhanced cellular calcium entry leading to contracture (1, 23). This injury occurs well below 20°C (1, 23, 28) and may be enhanced by multidose cardioplegic solution (18). In the present study, although there was a trend, cardiac dysfunction was not significantly different at 4°C from 30 or 18°C.

Previous studies have examined the effects of ischemic temperature on postischemic function and cardiac metabolism. These investigations have also elaborated the adverse effects of temperatures below 20°C (3, 6, 12–14, 25, 27, 28). However, temperature threshold as a factor for myocardial protection has not been systematically examined previously without alteration of

several other important factors among experimental groups (18). The finding that ATP depletion does not accelerate until temperatures reach above 30°C in this particular model employing cardioplegia implies that at least profound hypothermia (<20°C) may not be required during cardiac surgery under similar conditions.

Similarly, the beneficial effects of glucose in cardioplegia have been studied in several laboratories (2, 7, 19, 24). Inconsistent findings among these studies may be related to several factors including ischemic temperature and oxygenation of the cardioplegia solutions under study. Dougherty *et al.* found that the beneficial effect of glucose on postischemic function only occurred in oxygenated cardioplegia (7), similar to that used in the present study. They could define no differences in high energy phosphate levels associated with the superior postischemic function provided by glucose in the oxygenated cardioplegia. However, their study was performed only at 8°C, and glucose modulation of temperature effects on metabolism was not considered. Thus, the present study unlike previous investigations demonstrates that glucose in oxygenated cardioplegia does modulate the temperature threshold.

The significance of these findings rests in the temperature threshold characterization, which has not been previously defined. This includes the observation that this threshold can be modified by glucose provision in cardioplegia. Theoretically, the presence of glucose in cardioplegia could allow an increase in the operative temperature during complex cardiac surgery with relatively long aortic-cross clamp durations. Conceivably this could minimize hypothermia related damage to other organs during cardiac surgery. This approach would require further investigation in an appropriate model *in situ.*

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