IMPROVEMENT IN FUNCTIONAL RECOVERY OF THE ISOLATED GUINEA PIG HEART AFTER HYPERKALEMIC REPERFUSION WITH ADENOSINE

The aim of this study was to examine the effect of initial hyperkalemic reperfusion (HKR), with and without added adenosine, on coronary flow, myocardial function, and endothelium-dependent and endothelium-independent coronary vascular function. Cardioplegic arrest was induced in 40 isolated guinea pig hearts by infusing oxygenated cardioplegic (high in potassium ion) Krebs solution for 5 minutes. Hearts were then stored at room temperature for 3.5 hours. On reperfusion, hearts were divided into four groups of 10 hearts each: control, reperfusion with regular Krebs solution (4.6 mmol/L potassium chloride); base hyperkalemic reperfusion, initial reperfusion with 37° C oxygenated, cardioplegic Krebs solution for 5 minutes; hyperkalemic reperfusion with addition of 1 mmol/L adenosine during HKR; and hyperkalemic reperfusion with addition of 5 mmol/L adenosine. Coronary reserve (adenosine bolus 2 mmol/L) and responses to acetylcholine (1 μ mol/L) and nitroprusside (100 μ mol/L) were examined before and after ischemia and reperfusion. Flow did not return to preischemic values in any group after reperfusion. Adenosine treatment during initial reperfusion increased coronary flow (percentage of baseline $\pm$ standard error of the mean) from 57% $\pm$ 4% in control and 45% $\pm$ 3% in hearts with hyperkalemic reperfusion to 79% $\pm$ 3% and 83% $\pm$ 5% in hearts with hyperkalemic reperfusion also treated with, respectively, 1 mmol/L adenosine and 5 mmol/L adenosine ($p < 0.05$). At 30 and 60 minutes of reperfusion, however, flow remained elevated only in the group treated with 5 mmol/L adenosine. Coronary reserve and responses to acetylcholine and nitroprusside were equivalently depressed in all groups after reperfusion. Recovery of left ventricular systolic and diastolic function was improved in all groups after hyperkalemic reperfusion (54% $\pm$ 4% of preischemic value) compared with control (39% $\pm$ 3%), and recovery was further enhanced in the group treated with 5 mmol/L adenosine (60% $\pm$ 4%). In this *ex vivo* model, hyperkalemic reperfusion improved myocardial function after cardioplegic arrest and the addition of 5 mmol/L adenosine improved coronary flow. Adenosine may counteract the potassium chloride-induced vasoconstriction that occurs during hyperkalemic reperfusion and may thus improve coronary flow and myocardial function. Postischemic depression of endothelium-dependent or endothelium-independent vascular functions, however, was not alleviated by hyperkalemic reperfusion with or without adenosine. (J THORAC CARDIOVASC SURG 1996;111:74-84)

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Warm cardioplegic blood reperfusion was long ago demonstrated to reduce reperfusion damage in a canine model of ischemia and reperfusion.^{1,2} It was found that by lowering calcium and increasing pH, potassium, and osmolarity in the initial 500 ml of blood reperfusate, left ventricular performance as measured by left ventricular pressure and maximum pressure changes (dp/dt_{max}), which was depressed by about 40% in control hearts, could be nearly restored. Arresting the heart was thought

to reduce oxygen demand during this initial reperfusion period, making the delivered oxygen available for reparative work and replenishment of energy stores before the onset of cardiac pump function. A whole series of consecutive experimental studies, aimed at further improving the method, corroborated the beneficial effects of warm cardioplegic blood reperfusion,³⁻⁷ which seemed to be effective in human beings as well as in animals.^{8,9}

Although the benefits of hyperkalemic reperfusion (HKR) for recovery of myocardial function are well documented, data concerning coronary vascular function are less conclusive. Potassium is a well known vasoconstrictor and is regularly used in isolated vessel preparations to constrict vessel rings. A high potassium concentration reduces myocardial oxygen demand by reducing cardiac work, but it also limits oxygen delivery by restricting coronary flow so that this ratio is reduced.¹⁰ Flow during HKR was not reported in Follette and colleagues' initial study,² and oxygen supply, as well as demand, may decrease during HKR. Regional blood flow was measured in the beating working heart before and after ischemia and reperfusion, however, and a marked decrease (almost 25%) in coronary flow was observed in the group receiving only the high-potassium perfusate.² In human beings, coronary flow during HKR was found to either decrease⁸ or increase,⁹ possibly depending on previous damage to the coronary vasculature. Moreover, the effect of metabolically mediated flow changes could not be determined because coronary sinus oxygen was not measured. In this study, we investigated the effect of HKR on coronary flow and myocardial oxygen balance in an *ex vivo* model of cardiac ischemia and reperfusion. The isolated, Langendorff-perfused guinea pig heart preparation allows careful control of extrinsic variables and precise on-line measurement of global coronary flow and oxygen extraction during all phases of the experiment. The preparation is also suitable for testing endothelium-dependent and endothelium-independent vascular responsiveness and release of metabolic byproducts. Our aim was to improve recovery of myocardial function by augmenting coronary flow during HKR through the addition of the coronary vasodilator adenosine to the initial cardioplegic perfusate. An improvement in the oxygen nutrient supply caused by adenosine, coupled with depression of contractility by high potassium during HKR, might result in improved cardiac performance after ischemia.

Methods

After Animal Studies Committee approval was obtained, 40 albino English short-haired guinea pigs (250 to 350 gm) were injected intraperitoneally with 20 mg ketamine and 1000 U heparin. The animals were decapitated when unresponsive to noxious stimuli. All animals received humane care 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" published by the National Institutes of Health. Our methods have been published in detail.^{10,12} After thoracotomy, the aorta was cannulated distal to the aortic valve and the venae cavae and pulmonary artery were cut. Each heart was immediately perfused retrogradely through the aorta and excised. All hearts were perfused at a controlled perfusion pressure of 55 mm Hg, as measured at the aortic root. The perfusate, a modified Krebs-Ringer solution, was filtered in line (5 μ m pore size, Astrodisc; Gelman Scientific, Ann Arbor, Mich.) and contained the following: 137 mmol/L sodium ion, 4.5 mmol/L potassium ion, 1.2 mmol/L magnesium ion, 2.5 mmol/L calcium ion, 134 mmol/L chloride ion, 15.5 mmol/L bicarbonate, 1.2 mmol/L dihydrogen phosphate, 11.5 mmol/L glucose, 2 mmol/L pyruvate, 16 mmol/L mannitol, 0.05 mmol/L ethylenediaminetetraacetic acid, and 5 U/L insulin. Cardioplegic Krebs solution has an equivalent osmolarity, 300 mosm/L, but differs in concentrations of sodium ion (123 mmol/L), potassium ion (28 mmol/L), and calcium ion (1.25 mmol/L). Perfusate and bath temperatures were maintained at 37° C by means of a thermostatically controlled water circulator (VWR 1130; Preston Industries Inc., Niles, Ill.). Both solutions were equilibrated with a gas mixture of 97% oxygen and 3% carbon dioxide. Left ventricular pressure (LVP) was measured isovolumetrically with a transducer (DTX; Spectramed, Oxnard, Calif.) connected to a flexible, saline solution-filled latex balloon (Hugo Sachs Elektronik, KG, March-Hugstetten, Germany) inserted into the left ventricle through the mitral valve by way of a cut in the left atrium. Balloon volume was adjusted to maintain an end-diastolic LVP of 6 mm Hg. Positive and negative maximum instantaneous pressure changes (dp/dt_{max}) were determined electronically with an analog differentiator.

Electrograms were recorded with pairs of bipolar electrodes (plastic-coated silver, diameter 125 μ m) placed on the right atrial appendage and on the right ventricular pulmonary conus. The electrode signals were amplified and displayed continuously on a digital oscilloscope (Nicolet 310; Nicolet Instrument Corporation, Madison, Wis.) and audially monitored. Electrogram intervals for determination of heart rate and atrioventricular conduction time were measured on-line by digital timer systems that allowed instantaneous interval and rate analyses. Ventricular tachycardia was defined by the presence of uniform or multiform ventricular waveforms and faster ventricular than atrial rate. Ventricular fibrillation was defined by the presence of erratic activity in the ventricular electrogram and by the absence of pressure generation by the left ventricle.

Coronary flow was measured on-line with an ultrasonic flow probe (T106 small animal blood flowmeter; Tran-

sonic Systems, Inc., Ithaca, N.Y.). Coronary sinus effluent was collected by a cannula placed in the right ventricle through the pulmonary artery after ligation of the venae cavae. Coronary outflow oxygen tension (P_{O_2}) was measured continuously on-line (Instech 203B; Instech Laboratories, Plymouth Meeting, Pa.). The temperature-controlled Clark electrode was recalibrated periodically by means of a bypass circuit with perfusate gassed with room air. Both inflow and outflow P_{O_2} were verified off-line with a self-calibrating analyzer system (ABL-300; Radiometer, Copenhagen, Denmark). Oxygen delivery was calculated from the inflow P_{O_2} times oxygen solubility (24 μ l/ml saline solution/760 mm Hg) times coronary flow per gram dry heart tissue weight. Myocardial oxygen consumption (MVO_2) was calculated as follows: oxygen solubility times coronary flow per gram times the difference between inflow and outflow P_{O_2} . Percentage of oxygen extraction was calculated as 100 times the difference between inflow and outflow P_{O_2} divided by inflow P_{O_2} . Efficiency of oxygen utilization in performing contractile work was defined as systolic LVP times heart rate divided by MVO_2 .

All directly measured electronic signals were displayed on a high-resolution eight-channel recorder (Astro-Med Dash 8; Astro-Med Inc., West Warwick, R.I.), tape-recorded for back-up (Vetter D1; Vetter Co., Rebersburg, Pa.), and stored on floppy discs by a personal computer (model 310; Hewlett-Packard Co., Palo Alto, Calif.) equipped with a 12-bit analog-to-digital converter sampling at 10 Hz (AD 200; Infotek Systems, Anaheim, Calif.). Each value is the mean of a 10-second data segment that was visually inspected to ensure absence of artifacts.

The time constant of isovolumic pressure decline, τ , which reflects the time required for LVP to fall about one third of its value at peak $-dP/dt_{max}$, was calculated as described previously.¹³ Peak $-dP/dt_{max}$ was used as zero time, and pressure was recorded for 60 msec per beat for 3 to 4 consecutive beats. The natural log of pressure at time t ($\ln P_t$) was plotted against the t -axis, and τ was defined as the negative reciprocal of the slope of the linear section of the curve, according to the equation $\ln P_t = -t/\tau + \ln P_0$, where P_t is LVP at time t and P_0 is LVP at peak $-dP/dt$. This method has been successfully applied to the Langendorff-perfused guinea pig heart by using a fluid-filled left ventricular balloon system connected to a pressure transducer by a short tube of stiff polyethylene material.¹⁴

Protocol and statistical analysis. After completion of the preparation and a 10-minute period of stabilization, adenosine (0.2 ml of a 2 mmol/L stock solution) was injected to determine maximal coronary reserve. Only hearts that increased flow by 100% or more were included. After 30 minutes of further stabilization, baseline values of all measured variables were obtained and hearts were assigned to one of four groups: control ($n = 10$), HKR ($n = 10$), HKR with 1 mmol/L adenosine (HKR+A1, $n = 10$), and HKR with 5 mmol/L adenosine (HKR+A5, $n = 10$).

Cardioplegic arrest was induced in all hearts by perfusing oxygenated cardioplegic Krebs solution for 5 minutes at room temperature (23° C). The left ventricular balloon was then deflated, and hearts remained unperfused and

submerged in the same cardioplegic Krebs solution at room temperature for 3.5 hours. Control hearts were then reperfused with regular 37° C Krebs solution for 90 minutes. Hearts in the HKR group were initially reperfused with 37° C oxygenated cardioplegic Krebs solution for 5 minutes before reperfusion with regular Krebs solution. In hearts in the HKR+A1 and HKR+A5 groups, 1 and 5 mmol/L adenosine, respectively, were added to the cardioplegic Krebs solution during the initial 5 minutes of reperfusion only. The left ventricular balloon was re-inflated to 6 mm Hg end-diastolic pressure at 15 minutes of reperfusion, and all hearts were bolus-infused with lidocaine (0.05 ml of a 1% solution) at 20 minutes of reperfusion to convert any hearts not already in sinus rhythm. Subsequently, lidocaine was only given to those hearts that reverted to ventricular fibrillation. Coronary flow was measured during induction of cardioplegia, during hyperkalemic reperfusion, and at 1, 2, 3, 4, 5, 15, 30, and 60 minutes of reperfusion. Measurements of all other variables were made at 30 and 60 minutes of reperfusion. Before and after ischemia and reperfusion, coronary reserve (adenosine bolus, 2 mmol/L) and responses to acetylcholine (1 μ mol/L) and nitroprusside (100 μ mol/L) perfusion were assessed. Before ischemia and at 10 and 60 minutes of reperfusion, coronary effluent was collected and immediately frozen at -70° C for later analysis of adenosine and inosine levels by high-pressure liquid chromatography.¹⁵

All data are expressed as mean $\pm$ standard error of the mean. Statistical differences among groups were obtained by two-way analysis of variance for repeated measurements (SuperANOVA; Abacus Concepts Inc., Berkeley, Calif.). Differences were considered significant at $p < 0.05$.

Results

Coronary flow. Baseline coronary flow, measured in milliliters per minute, and dry tissue weight, measured in grams, did not differ among groups (Fig. 1). In all groups, flow remained essentially unchanged during induction of cardioplegic arrest. In control hearts, peak reflow within 1 minute of reperfusion reached $85\% \pm 8\%$ of 52 ± 2 ml/min/gm preischemic flow and stabilized at $57\% \pm 4\%$ after 3 minutes. In hearts in the HKR group, peak reflow was $93\% \pm 7\%$ of 58 ± 3 ml/min/gm preischemic flow but stabilized at $45\% \pm 3\%$ until the end of the 5-minute hyperkalemic reperfusion. During perfusion with regular Krebs solution, coronary flow reached $58\% \pm 2\%$ of baseline within 5 minutes and remained at this level until 60 minutes of reperfusion. In hearts in the HKR+A1 group, in which 1 mmol/L adenosine was present during the 5 minutes of HKR, peak reflow slightly exceeded preischemic baseline flow ($112\% \pm 7\%$ of 57 ± 3 ml/min/gm basal flow). At the end of HKR, flow had stabilized at $79\% \pm 3\%$ of the preischemic value.

Fig. 1. The time course of coronary flow (in milliliters per minute per gram dry weight) is illustrated for four groups: control, HKR, HKR+A1, and HKR+A5. Measurements were taken before ischemia and reperfusion at baseline (BL) and at the end of 5 minutes of perfusion with cardioplegic Krebs solution (CP). After 210 minutes of ischemia (ISCH), further measurements were obtained at peak reflow (PRF), the end of the 5 minutes of initial HKR, and after 1, 2, 3, 4, 5, 15, 30, and 60 minutes of reperfusion after the start of perfusion with regular Krebs solution. For better identification of single data points, the initial 15 minutes of reperfusion were stretched on the x-axis scale. Means $\pm$ standard error of the mean. Symbols indicate *p* values: *, *p* < 0.05 vs control; #, *p* < 0.05 vs HKR; \$, *p* < 0.05 vs HKR+A1.

Subsequently, flow slowly decreased to $76\% \pm 5\%$ and $65\% \pm 3\%$ at 15 and 30 minutes, respectively, but did not change thereafter ($64\% \pm 3\%$ at 60 minutes). Reperfusion with 5 mmol/L adenosine in hyperkalemic Krebs solution did not further increase peak reflow ($123\% \pm 9\%$ of the 60 ± 2 ml/min/gm baseline flow), nor did it affect steady-state perfusion at the end of HKR ($83\% \pm 5\%$ of initial flow). In contrast to hearts in the HKR+A1 group, however, coronary flow did not decrease during perfusion with regular Krebs solution without adenosine and it remained significantly less depressed ($83\% \pm 5\%$ and $81\% \pm 6\%$ at 30 and 60 minutes, respectively) compared with flows in all other groups.

Heart rate and cardiac rhythm. Baseline heart rate was about 230 beats/min in all four groups and increased slightly (by 5% to 20%) on reperfusion. There were no differences among groups. Most of the hearts were dysrhythmic or fibrillated on reperfusion. After 60 minutes, only 20% of control hearts but more than 50% of the hearts treated with HKR performed at stable sinus rhythm, defined as fewer

than five ventricular extrasystoles or periods of ventricular tachycardia per minute. In all hearts, hemodynamic and metabolic measurements were obtained during periods of sinus rhythm.

Left ventricular systolic and diastolic functions. Left ventricular contractile function was assessed by measuring isovolumetric LVP and $dP/dt_{\max}$. In this preparation, preload and afterload factors are held constant. In control hearts ischemic arrest and 60 minutes of reperfusion decreased developed (systolic minus diastolic) LVP (LVDP) and $dP/dt_{\max}$ to $39\% \pm 3\%$ and $45\% \pm 3\%$ of preischemic values, respectively. HKR significantly improved both parameters (LVDP $54\% \pm 4\%$; $dP/dt_{\max}$ $62\% \pm 5\%$). Addition of 1 mmol/L adenosine to the initial cardioplegic reperfusate had no additional effect on recovery of left ventricular contractile function. Addition of 5 mmol/L adenosine to the cardioplegic reperfusate, however, further increased LVDP and $dP/dt_{\max}$ ($60\% \pm 4\%$ and $78\% \pm 8\%$, respectively; Fig. 2). Left ventricular diastolic function was assessed by determining $-dP/dt_{\max}$, which reflects events that occur very early during LVP decline, and

Fig. 2. The baseline data and the data for 30 and 60 minutes of reperfusion are given for LVDP, $+dP/dt_{\max}$ ($+dP/dt$), $-dP/dt_{\max}$ ($-dP/dt$), and τ (Tau). Left ventricular systolic and diastolic functions were markedly depressed after ischemia and reperfusion. Recovery of left ventricular function was improved by HKR alone and was further enhanced by addition of adenosine to the initial cardioplegic reperfusate. Means $\pm$ standard error of the mean. Symbols indicate p values: *, $p < 0.05$ vs control; #, $p < 0.05$ vs control and HKR.

by determining τ , which represents later events in the course of isovolumetric LVP decrease. In control hearts, $-dP/dt_{\max}$ decreased to $35\% \pm 3\%$ of -1483 ± 70 mm Hg/sec before cardioplegic arrest. HKR improved $-dP/dt_{\max}$ throughout the reperfusion period ($52\% \pm 5\%$ of the preischemic values at 60 minutes). Addition of 1 or 5 mmol/L adenosine had no significant effect on recovery of $-dP/dt_{\max}$. The τ value increased to $149\% \pm 5\%$ of baseline in control hearts after 60 minutes of reperfusion and

did not statistically differ in hearts in either HKR or HKR+A1 groups. Only the high concentration of adenosine in HKR+A5 hearts significantly reduced the time constant of relaxation, to $116\% \pm 5\%$ of baseline (Fig. 2).

Oxygen metabolism. MVO_2 decreased to $60\% \pm 3\%$ of baseline after 60 minutes of reperfusion in control hearts but was maintained significantly better in hearts in the HKR ($81\% \pm 5\%$), HKR+A1 ($80\% \pm 3\%$) and HKR+A5 ($89\% \pm 8\%$) groups

Fig. 3. The baseline data and the data at 30 and 60 minutes of reperfusion are given for the arterial to coronary venous oxygen difference ($AVDO_2$), coronary flow, MVO_2 , and the efficiency of oxygen utilization (efficiency). In control hearts, coronary flow was depressed to a degree similar to that of MVO_2 , suggesting an essentially intact oxygen balance. HKR increased MVO_2 with no effect on flow, resulting in an increased oxygen extraction ($AVDO_2$). Addition of 5 mmol/L adenosine to the initial reperfusate increased flow and reduced $AVDO_2$ to nearly baseline values. Efficiency remained equivalently depressed in all groups. Means $\pm$ standard error of the mean. Symbols indicate p values: *, $p < 0.05$ vs control; #, $p < 0.05$ vs control, HKR, and HKR+A1.

(Fig. 3). Arterial-venous oxygen difference, which in this preparation with constant arterial PO_2 also reflects oxygen extraction, increased in all groups but was significantly higher in hearts in the HKR group ($133\% \pm 7\%$) than in control hearts ($111\% \pm 3\%$) or hearts in the HKR+A5 group ($116\% \pm 7\%$; Fig. 3). The efficiency of the myocardium in converting consumed oxygen into mechanical work was

equally decreased in all groups, to about 75% of baseline (Fig. 3).

Coronary reserve and vascular function. Before ischemia and reperfusion, coronary reserve, as evaluated by bolus injection of adenosine, amounted to well above 100% of baseline flow in all groups. After ischemia and reperfusion, coronary reserve was equally depressed in all groups, to only a 30%

Fig. 4. Flow responses to coronary vasodilators before and after cardioplegic arrest and reperfusion. *C1* through *C5*, Resting flow immediately before the respective vasodilator stimulation; *ADE*, 2 mmol/L adenosine bolus; *ACH-M*, maximal flow response to acetylcholine infusion (1 μ mol/L); *ACH-P*, steady-state flow during acetylcholine infusion and pacing the heart at the rate before infusion of the vasodilator; *NP*, nitroprusside (100 μ mol/L); *BL*, baseline; *30* and *60*, respective periods of reperfusion in minutes; *ISCH*, ischemia (210-minute period). The increased postischemic basal flow in the HKR+A5 group did not result in improved responses to the vasodilators. Means $\pm$ standard error of the mean. All stimulations caused statistically significant flow increases; all postischemic responses were significantly less pronounced than respective preischemic responses.

increase above the depressed postischemic resting flow. Postischemic flow responses to perfusion with nitroprusside and acetylcholine were similarly depressed in all groups (Fig. 4). The increased postischemic resting flow in hearts in the HKR+A5 group had no effect on the respective flow responses.

Adenosine and inosine levels. Because adenosine and inosine concentrations were not determined in all experiments, data from hearts in the control and HKR groups, which did not differ between groups, were pooled for analysis. As shown in Fig. 5, inosine concentrations increased dramatically on reperfusion but approached baseline values after 60 minutes of reperfusion in all groups. Adenosine in coronary effluent increased only slightly in hearts in the control, HKR, and HKR+A1 groups at 10 minutes of reperfusion. The pronounced increase of adenosine in the HKR+A5 group, to almost 2000 nmol/L, mainly reflects the results of a single experiment in which adenosine increased from 2 to 9350 nmol/L. This is in contrast to more moderate increases from 18 to 405 nmol/L in the remaining

hearts in the HKR+A5 group. At 60 minutes of reperfusion, adenosine levels were significantly elevated only in hearts in the HKR+A5 group.

Discussion

We used a model of cardioplegic arrest and reperfusion in isolated perfused guinea pig hearts to demonstrate the following: (1) Compared with normal Krebs solution reperfusion, HKR improved recovery of myocardial function but not of coronary flow. (2) Addition of 5 mmol/L adenosine during only the initial 5 minutes of hyperkalemic reperfusion significantly increased coronary flow and additionally enhanced myocardial function for up to 60 minutes of reperfusion. (3) The postischemic coronary flow responses to both endothelium-dependent and endothelium-independent vasodilators were similarly depressed in all groups.

The finding of improved myocardial function after HKR confirms the results of Follette and co-workers,^{1,2} who first described the beneficial effects of warm cardioplegic blood reperfusion in dogs. Because this group added potassium to the perfusate without

Fig. 5. Adenosine and inosine levels in coronary effluent at baseline before ischemia (BL) and at 10 minutes and 60 minutes of reperfusion. Data did not differ between control (C) and HKR groups and were therefore pooled for statistical analyses. Inosine concentrations increased similarly in all groups at 10 minutes of reperfusion. Ischemia and reperfusion caused a moderate increase in the release of endogenous adenosine from control and HKR hearts groups at 10 minutes of reperfusion. Both concentrations of adenosine during initial HKR increased effluent adenosine levels after 10 minutes of reperfusion, but after 60 minutes of reperfusion adenosine remained elevated only in the HKR+A5 group. Control, $n = 3$; HKR, $n = 2$; HKR+A1, $n = 5$; HKR+A5, $n = 5$. Means $\pm$ standard error of the mean. Symbols indicate p values: *, $p < 0.05$ vs BL; #, $p < 0.05$ vs C and HKR; \$, $p < 0.05$ vs HKR+A1.

adjusting osmolarity to normal, it is not entirely clear whether the observed beneficial effect of warm cardioplegic blood reperfusion was caused by cardioplegic arrest or rather by hyperosmolarity. In our isolated heart study, the osmolarity of the cardioplegic Krebs solution was adjusted to 300 mosm by reducing sodium chloride in exchange for the increase in potassium chloride. Improved LVDP, dP/dt_{max} , and $-dP/dt_{max}$ after HKR treatment clearly indicate that myocardial systolic and diastolic functions are both better preserved solely by HKR. Moreover, markedly improved myocardial function was achieved without the antioxidative capacity of whole blood, as in warm cardioplegic blood reperfusion,^{4, 6, 16} and also without pharmacologic free-radical scavengers. In our study, improved myocardial recovery with HKR is probably caused by reduced oxygen demand during initial reperfusion. At 37° C, oxygen demand of the arrested and decompressed heart is reduced by 80% compared with the beating or fibrillating heart.¹⁶ Reduced demand may

allow myocardial tissue to use the redelivered oxygen more efficiently for processes not associated with the output of mechanical work.⁶

If the improved oxygen balance is indeed crucial for the beneficial effects of HKR, then the well-known vasoconstricting effect of elevated potassium chloride during HKR might limit the effectiveness of this therapy. Indeed, after ischemia and reperfusion, coronary flow during HKR without adenosine decreased to $45\% \pm 3\%$ of its preischemic baseline. The increased arterial-venous oxygen difference until 60 minutes of reperfusion in hearts in the HKR group demonstrates inadequate myocardial perfusion for oxygen demand and may indicate either more pronounced vascular damage (decreased oxygen delivery) or increased oxygen demand as a result of the enhanced metabolic demand.

These data allow two conclusions: (1) Because hyperkalemia reduces the coronary vascular conductance during and for several minutes after HKR,

oxygen delivery to the myocardium is limited during HKR. (2) HKR may not have aggravated damage to the coronary vasculature, but rather may have increased the metabolic demand as function improved.

In previous experimental studies coronary flow during warm cardioplegic reperfusion was not well documented, and clinical studies investigating coronary vascular resistance during cardioplegic reperfusion remain inconclusive.^{8,9} We believe that if HKR did not limit oxygen supply, myocardial performance could be further improved. Various experimental studies have shown that vasodilators improve postischemic coronary flow and myocardial function well beyond the period of drug infusion.^{11, 17-19}

Among the various cardiac effects of adenosine,²⁰ the adenosine₂ receptor-mediated increase in vascular conductance and oxygen delivery may be the pivotal mechanism during reperfusion.^{11, 17, 18, 21} The two tested concentrations of adenosine, 1 mmol/L and 5 mmol/L, have been successfully used during hyperkalemic cardioplegic arrest.^{22, 23} In our study, both concentrations (administered during HKR only) increased flow during initial HKR, but only 5 mmol/L adenosine resulted in sustained enhancement of coronary flow and myocardial function during the entire reperfusion period. Improvements in initial flow and oxygen delivery during initial reperfusion, which were equivalent for both adenosine concentrations, cannot be the pivotal mechanism of the sustained effects of 5 mmol/L adenosine.

We can only speculate on the mechanisms of the beneficial effects of 5 mmol/L adenosine. Our data (Fig. 5) indicate that effective concentrations of adenosine were present in the myocardial tissue of hearts in the HKR+A5 group at 60 minutes of reperfusion. The subsequent vasodilation may have increased oxygen delivery and allowed better myocardial performance without affecting the mechanisms responsible for the depression of cardiac efficiency in converting oxygen to mechanical work. In a previous study from our laboratory,¹⁵ washout of endogenously produced adenosine after ischemia and reperfusion in isolated hearts persisted for longer than 10 minutes but was complete after 40 minutes of reperfusion. This correlates well with our inosine and adenosine levels in the control, HKR, and HKR+A1 groups, but it does not explain the elevated adenosine levels in HKR+A5 hearts after 60 minutes of reperfusion. The coronary vascular endothelium has a high metabolic rate for adenosine,²⁴ which may explain why 5 minutes of 5

mmol/L but not 1 mmol/L adenosine reperfusion could load the myocardial tissue with sufficient adenosine to delay complete washout of the drug for more than 1 hour. According to an alternative hypothesis, during high-dose adenosine reperfusion in the presence of abundant oxygen and energy substrates, some of the exogenously administered adenosine may be phosphorylated to produce adenosine triphosphate (ATP) in excess of energy demand.²⁵ Such rapid ATP synthesis from exogenous adenosine was demonstrated in isolated cardiac muscle cells.²⁶ After washout of exogenous adenosine, these intracellular ATP stores might be dephosphorylated to provide additional energy for enhanced cardiac function and release of adenosine for the observed flow increase.

None of the treatments tested in our study had any effect on the attenuated coronary reserve response during reperfusion (Fig. 4). While a bolus injection of adenosine increased flow by about 120% to 150% before ischemia in all groups, flow increases were only 26% to 34% over postischemic resting flow. Because endothelium-dependent (acetylcholine) and endothelium-independent (nitroprusside) vasodilators elicited similar significant flow responses, the postischemic depression of responses to both vasodilators indicates vascular smooth muscle damage rather than specific deterioration of endothelial function.²⁴ Possibly perivascular edema limited the flow responses after reperfusion.

Although whole-animal experiments with blood as coronary perfusate are certainly much closer to the clinical setting, the isolated heart preparation allows control of preload and afterload factors, excludes neural and exocrine influences, and allows more precise on-line measurement of ventricular function indexes, coronary flow, and MVO₂, thus providing useful data. We conclude that HKR for 5 minutes improves subsequent myocardial function, probably by reducing oxygen demand for electromechanical work during early reperfusion. Increasing flow during HKR by administering up to 1 mmol/L adenosine provides no sustained additional benefits for either coronary flow or myocardial function. In contrast, 5 mmol/L adenosine during 5 minutes of HKR improves flow and myocardial function for up to 60 minutes of reperfusion. Delayed washout of adenosine or accelerated resynthesis of ATP during HKR and subsequent dephosphorylation, with consequent prolonged adenosine₁ receptor- and adenosine₂ receptor-mediated effects beyond the period of adenosine-enriched HKR, may account for the beneficial

effects of the higher dose of adenosine in our model. Although postischemic resting flow was improved in the HKR+A5 group after ischemia, the depression of coronary reserve was not affected by either HKR or adenosine.

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