Contraction Uncoupling With Butanedione Monoxime Versus Low Calcium or High Potassium Solutions on Flow and Contractile Function of Isolated Hearts After Prolonged Hypothermic Perfusion

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Background Normal ionic perfusate containing butanedione monoxime (BDM), a reversible myofilament inhibitor, could be better than either a high potassium (KCl) or a low calcium (CaCl₂) perfusate for long-term cardiac preservation. This hypothesis was tested in 70 isolated guinea pig hearts.

Methods and Results Three groups—time control (8 hours, 37°C), cold control (22 hours, 3.8°C), and cold+BDM (22 hours)—were perfused with typical Krebs-Ringer solution (2.5 mmol/L CaCl₂ and 4.5 mmol/L KCl). Two other groups were cold perfused for 22 hours either with 2.5 mmol/L CaCl₂+20 mmol/L KCl (high) or with 0.5 mmol/L CaCl₂ (low)+4.5 mmol/L KCl. These changes were maintained from 20 minutes before cold perfusion until 30 minutes after rewarming to 37°C. Coronary vasodilator reserve was tested before cold perfusion and 2 hours after warm reperfusion with adenosine (Ade), acetylcholine (Ach, endothelium dependent), and nitroprusside (NP, endothelium independent). Each treatment decreased left ventricular pressure (LVP) by more than 80% before cold perfusion. During warm reperfusion, LVP was lower in cold control (−72±5%), high KCl (−76±4%), and low CaCl₂ (−80±4%) groups than in BDM (−38±3%) or time control (−18±4%) groups; coronary flow (CF) was lower in high KCl (−67±4%) and low CaCl₂ (−54±7%) groups than in cold control (−37±6%), BDM (−30±5%), or time control (+2±3%) groups; and percent oxygen extraction (controls, 62±4%) was higher in the high KCl group (83±6%) than in cold control (72±3%), BDM (73±3%), low CaCl₂ (72±5%), or time control (63±3%) groups. CF responses to Ade, Ach, and NP (+103±7%, +24±5%, and +34±5% before cold) were attenuated (+76±6%, +18±5%, and +23±4%) in the time control group (5 hours later), were reduced but present in the BDM group (+10±5%, −5±5%, and −5±5%), and were absent in both low CaCl₂ and high KCl groups after 2 hours of reperfusion.

Conclusions Normal ionic BDM solution better preserves cardiac function and basal CF after prolonged cold perfusion than do cold control, high KCl, and low CaCl₂ solutions. Vasodilatory capacity is markedly diminished after perfusion with either the high KCl or the low CaCl₂ solution. (Circulation. 1994;89:2412-2420.)

Keywords • contractility • butanedione monoxime • hypothermia • transplantation

Improved methodology to preserve donor hearts for longer periods is a crucial goal in cardiac transplantation research.1 Induced depression of cardiac work and metabolism by hypothermia alone is not adequate for long-term cardiac protection. The typical experimental approach to reduce myocardial reperfusion damage is to flush the heart with a cold high potassium (KCl cardioplegic) solution to arrest the heart. Another approach is to decrease contractile function and metabolism by infusing a reduced calcium solution. A potential problem with either of these techniques to suppress metabolism is that intracellular stabilization of K⁺ and Ca²⁺ ions may be slowed or ineffective during reperfusion with normal ionic solution because of impaired Na⁺–K⁺-ATPase pump and Na⁺-Ca²⁺ pump activity.2-4 When ionic gradients are disturbed, dysrhythmias can occur, and myocardial contractility, vascular smooth muscle function, and endothelial cell responsiveness are impaired.

Our objective was to reduce myocardial damage during hypothermic preservation using a normal, ie, extracellular, ionic solution. Several potential advantages of a normal ionic solution for preservation are (1) better maintenance of ionic gradients and ion pump function; (2) prevention of intracellular Ca²⁺ overload, or underload, and membrane depolarization causing depressed cardiac function and dysrhythmias; and (3) shortening of time to reestablish equilibration with normothermic reperfusion. We proposed that butanedione monoxime (BDM), contained in a normal ionic solution, protects isolated guinea pig hearts better than a high KCl solution or a low CaCl₂ solution when these changes are made before, during, and initially after hypothermic perfusion for 1 day. We have reported that BDM protects isolated
guinea pig hearts when BDM is added to a normal ionic Krebs-Ringer solution before, during, and initially after either 30 minutes of coronary occlusion or 1 day of hypothermic perfusion. In both situations, BDM enhanced functional recovery and decreased the incidence and severity of dysrhythmias. The major effect of BDM is to decrease myofibrillar Ca$^{2+}$ sensitivity, with a lesser effect of altering the uptake or release of Ca$^{2+}$ from the sarcoplasmic reticulum. BDM is also a vasodilator. BDM may afford protection not only by reducing metabolic stress, but by improving coronary vasodilatation. Ketamine and 1000 U heparin and decapitated pigs (400 to 600 g) were injected intraperitoneally with 10 mg ketamine and 1000 U heparin and decapitated when unresponsive to noxious stimulation. Isolation and cannulation of the distal inferior and superior venae cavae were performed, and a cannula was placed in the aortic valve. Each heart was immediately perfused retrogradely through the aorta and aortic valve. The aortic root was incised and connective tissue excised. All 70 hearts were perfused initially at a constant aortic root perfusion pressure of 55 mm Hg. The standard perfusate, a modified Krebs-Ringer solution, was filtered (5-µm pore diameter) in-line (Astrodisc, Gelman Scientific) and had the following control composition (in mmol/L): Na$^+$ 137, K$^+$ 5, Mg$^{2+}$ 1.2, Ca$^{2+}$ 2.5, Cl$^-$ 134, HCO$_3^-$ 15.5, H$_2$PO$_4^-$ 1.2, pyruvate 2, glucose 11.5, mannitol 16, and EDTA 0.05 and insulin 5 U/L. Perfusion and bath temperature were maintained initially at 36.7±0.1°C using a thermostatically controlled water circulator (model 800, Fisher Scientific).

Systolic left ventricular pressure (LVP) was measured isovolumetrically with a transducer connected to a thin, saline-filled latex balloon inserted into the left ventricle through the mitral valve from a cut in the left atrium. Balloon volume was adjusted to maintain a diastolic LVP of 0 mm Hg during the control period. Two pairs of bipolar electrodes (Teflon-coated silver; diameter, 125 µm) were placed in each heart to monitor intracardiac electrograms from which spontaneous sinoatrial rate and atrioventricular conduction time were measured. Electrode signals were amplified and displayed continuously on an image-storing oscilloscope. Atrial rate was determined from the right atrial beat-to-beat interval, and atrioventricular conduction time was determined from the superior right atrial to right ventricular pulmonary conus beat-to-beat interval. Electrogram intervals were measured instantaneously by digital timer systems that allow on-line interval and rate analyses. Description and identification of dysrhythmias have been described in detail previously.

Coronary sinus effluent was collected by placing a cannula into the right ventricle through the pulmonic valve after ligating the venae cavae. Coronary inflow (aortic) was measured at constant temperature with an electromagnetic flowmeter calibrated daily by 4-point, timed collections into a volumetric cylinder over the flow range of 0 to 24 mL/min. Calibration curves for each group were best-fit by regression analysis. Zero inflow was periodically established by temporarily bypassing the flow transducer. Coronary inflow and outflow (coronary sinus) O$_2$ tensions were measured continuously on-line (model 203B, Instech) and verified immediately off-line with an intermittently self-calibrating analyzer system (model 813, Instrumentation Labs). The in-line, temperature-controlled, miniature Clark oxygen electrodes were calibrated periodically using a constant-flow bypass circuit in which perfusate was gassed with 100% N$_2$, 21% O$_2$, and 96% O$_2$ to adjust O$_2$ tension to 20, 150, and 650 mm Hg, respectively. Because these hearts depend solely on the crystalloid solution from which to extract dissolved oxygen, O$_2$ delivery was calculated from the inflow O$_2$ tension times O$_2$ solubility (24 µL·mL saline$^{-1}$·760 mm Hg$^{-1}$) times coronary flow per gram of wet heart tissue. Percent O$_2$ extraction was calculated as 100 times the difference between inflow and outflow O$_2$ tensions divided by inflow O$_2$ tension. Myocardial O$_2$ consumption was calculated as O$_2$ solubility times coronary flow per gram times (inflow O$_2$ minus outflow O$_2$ tension difference). Relative cardiac efficiency was calculated as the product of heart rate (beats per minute) and developed isovolumetric LVP (systolic minus diastolic LVP, in mm Hg) divided by O$_2$ consumption (mL·min$^{-1}$·g$^{-1}$) and expressed as (mm Hg·beat)/(0.1 µL·O$_2$·g$^{-1}$). By this index, a relative decrease in the amount of oxygen consumed to perform an isovolumetric contraction indicates improved cardiac efficiency. Electrograms, heart rate, atrioventricular conduction time, outflow O$_2$ tension, coronary flow, LVP, and perfusion pressure were FM tape recorded for later detailed analysis. All measured variables were displayed on a fast-writing (3 kHz), thermal-array eight-channel recorder (model MT9500, Astro-Med). Calculated variables were computed using a software program (Microsoft Excel).

Perfusate and bath temperature were maintained at 37±0.1°C during the initial normothermic perfusion period of 1.5 hours. During the hypothermic perfusion period of 22 hours, perfusate and bath temperature were maintained at 3.8±0.1°C. A switch to hypocorpusion at 3.8°C was accomplished by use of a separate refrigerated jacket and perfusion circuit (VWR 1160) placed in parallel with the warm perfusion circuit. Normothermic perfusion for 4 hours at 37.7±0.1°C after cold perfusion was reinstated by switching back to the warm circuit. Warm and cold perfusion circulation circuits were temperature equilibrated in advance. Time to reach half the temperature fall from 36.7 to 3.8°C was 5 minutes. On lowering temperature, at 15°C, cardiac perfusion was switched from constant pressure to a low constant flow (1.7 mL·g$^{-1}$·min$^{-1}$) in order to maintain baseline normothermic flow during constant pressure perfusion. Perfusion pressure, monitored throughout hypothermia at constant flow, varied over a range of 25 to 40 mm Hg. On raising temperature to 25°C, cardiac perfusion was returned to the constant-pressure mode. Time to reach half the temperature rise from 3.8 to 38°C was 3 minutes. Warm and cold perfusate solutions were equilibrated with a gas mixture of 96% O$_2$ and 4% CO$_2$. For hearts in all groups during the initial normothermic period, mean coronary arterial (inflow) pH averaged 7.47±0.02 (±SEM); PCO$_2$, 25±1 mm Hg; and HCO$_3^-$, 24±1 mm Hg; during the hypothermic period, inflow values, measured at 37°C, were 7.18±0.03, 43±2 mm Hg, and 789±18 mm Hg, respectively. There were no significant differences for these values among the groups at the two given temperatures.

Peak coronary flow responsiveness was tested before and after hypothermic perfusion by maximally vasodilating the vasculature with adenosine (Sigma-Aldrich). Adenosine (0.2 mL of a 200-µmol/L solution) was injected into the aortic (coronary perfusion) cannula. Percent oxygen extraction was measured to assess direct vasodilatory responses as differentiated from those due to an autoregulatory response. Interpretation of these data relies on the assumption that production of local metabolites is proportional to myocardial oxygen consumption and that local metabolites are major factors controlling autoregulation of coronary flow. Thus, an imbalance of oxygen consumption to oxygen delivery reflects a change in coronary vascular tone. A submaximal concentration of epinephrine HCl (0.5 µmol/L) was infused for 2 minutes.
before and after the hypothermic period to test chronotropic and inotropic responsiveness. Coronary responsiveness to 1 μmol/L acetylcholine and 100 μmol/L sodium nitroprusside (Nipride, Abbott Labs), indicators of endothelium-dependent and -independent vasodilation, respectively, were tested during 2-minute infusions 30 minutes before cold perfusion and 60 minutes after rewarming (ie, 30 minutes after reperfusion with standard Krebs-Ringer solution). For acetylcholine, flow changes were measured during initial (maximal) exposure, during the steady-state response to spontaneously slowed heart rates (146±14 beats per minute), and during pacing at 240 beats per minute to approximate intrinsic heart rates. At the end of each experiment, wet and dry hearts were obtained to determine relative changes in cardiac water.

Protocol

Once isolated, hearts were assigned randomly to one of five groups containing 11 to 15 hearts each: group a, time control (normothermia, normal ionic solution); group b, treatment control (hypothermia, normal ionic solution); group c, 10 mmol/L BDM (hypothermia, normal ionic solution with added BDM); group d, 0.5 mmol/L CaCl₂ (hypothermia, low calcium, normal potassium); and group e, 20 mmol/L KCl (hypothermia, high potassium, normal calcium). For groups c through e, changes in perfusate were made 20 minutes before hypothermic perfusion, during 22 hours of hypothermic perfusion, and for 30 minutes after restoring normothermic perfusion. Cardiac protection was defined as a decrease in dysrhythmias and improved contractility, coronary flow, and efficiency of oxygen utilization. A preliminary study showed that cardiac function was improved equally after hypothermic perfusion after treatment with 10 and 20 mmol/L BDM, that 10 mmol/L BDM produced improvement over that of 5 mmol/L BDM, and that the two lower concentrations had no significant effects on heart rate or atrioventricular conduction. BDM was prepared daily in Krebs-Ringer solution.

All variables were measured in the steady state before the subsequent change in protocol. Because rewarming provoked ventricular dysrhythmias in many hearts at about 25°C, each heart received prophylactically one bolus injection of 0.1 mL of 10 mg lidocaine HCl during rewarming at 25°C to reduce the occurrence of such dysrhythmias. If after treatment with lidocaine a ventricular dysrhythmia persisted for longer than 60 minutes, lidocaine was administered again.

Statistical Analysis

All data are expressed as mean±SEM. Mean values were considered significant at P<.05. Significance was determined by ANOVA with repeated measures; if the F test was significant, Fisher's least significant difference test was used to compare means (STATVIEW, Abacus Concepts). Derived variables and changes from control were computed (Microsoft EXCEL). Among-group (treatment effects) comparisons were used to assess differences among the five groups for a given variable at a specific time or test interval. The following groups were statistically compared for each variable examined at a specific time interval: 10 mmol/L BDM versus time control, treatment control versus 10 mmol/L BDM, 0.5 mmol/L CaCl₂ versus treatment control, and 20 mmol/L KCl versus 0.5 mmol/L CaCl₂. Changes in flow and oxygen extraction responses to vasodilator testing after normothermic reperfusion (postcontrol, C2) are also displayed in two other figures. Software programs were run (Macintosh SE30 and Apple). Significance for the incidence and duration of dysrhythmias was determined by x² and t tests, respectively. The expected incidence of dysrhythmias was assumed to be zero.

Results

Atrial Rate, Atrioventricular Conduction, and Cardiac Rhythm

Initial atrial rate (C) was similar in each group and averaged 228±5 beats per minute for all groups. Atrial rate increased similarly with epinephrine infusion to 302±6 beats per minute for all groups. Before and initially after hypothermic perfusion, ie, during changes in solution, atrial rate slowed in the CaCl₂ group to 203±7 beats per minute and ceased in the KCl group. Atrial rate was slower only initially with warm reperfusion in the BDM (176±8 beats per minute) and treatment control (174±6 beats per minute) groups and was unchanged with time (227±6 beats per minute) and with epinephrine infusion (291±6 beats per minute) among all groups during normothermic reperfusion with normal ionic solution. Between approximately 17 and 12°C, during cooling to 3.8°C, atrial and ventricular electrical and mechanical activities ceased in each hypothermia group without fibrillation after slowing of pacing and development of atrioventricular dissociation. Hearts remained quiescent during 22 hours of hypothermia, and slow atrial activity began to occur earlier in the cold treatment control group than in the remaining groups at approximately 20°C during rewarmin. Lidocaine was given prophylactically to all hearts at 25°C, and all hearts were temporarily arrested.

Control atrioventricular time was similar for all groups (65±2 milliseconds). Lowering CaCl₂ slightly increased atrioventricular time before hypothermic perfusion to 86±4 milliseconds and greatly increased atrioventricular conduction time to 118±3 milliseconds while causing atrioventricular dissociation in some hearts after normothermic reperfusion. Increasing KCl caused atrioventricular dissociation initially on reperfusion with normal ionic solution. In all groups except the time control and BDM groups, there were severe cardiac ventricular dysrhythmias (ventricular fibrillation, tachycardia, and electromechanical dissociation) not converting to sinus rhythm with repeated lidocaine injection on rewarmin. The incidence of ventricular fibrillation compared with sinus rhythm was significant (P<.05) in treatment control (36%) and CaCl₂ (38%) groups but not in the KCl group (22%). All hearts remained in or had reverted to sinus rhythm by 2 hours of warm reperfusion.

Coronary Flow and Oxygen Extraction

Coronary flow (Fig 1) was similar in each group initially (C). Initial adenosine injection increased flow maximally about twofold in each group. On initial treatment with high KCl perfusate (at 1 hour), coronary flow markedly decreased in the arrested hearts. There were no significant changes in flow for other groups on changing solution (at 1 hour). On normothermic reperfusion (23 hours), flow was unchanged during low CaCl₂ perfusion but reduced in all other hypothermia groups both initially and throughout the reperfusion period with normal perfusate. Of the hypothermic groups, flow remained lowest in the high KCl group and was highest in the BDM group throughout the reperfusion period.

Changes in coronary flow were approximately inversely proportional to changes in percent oxygen extraction (Fig 2). Before hypothermia at 1 hour, percent oxygen extraction marked decreased only in low CaCl₂ and BDM groups. Initially after hypothermia, with continued treatment, percent oxygen extraction remained lower than initial controls (C) in low CaCl₂ and BDM groups, whereas it was elevated above controls in treatment control and KCl groups (statistics not shown). During reperfusion with normal ionic perfusate, oxygen...
extraction increased in each hypothermia group compared with the time control group; these values were higher than prehypothermic controls and were not further increased by infusion of epinephrine. Oxygen extraction was highest in the KCl group in which coronary flow was lowest.

Changes in coronary flow and percent oxygen extraction responses to adenosine, acetylcholine, and nitroprusside before and after hypothermic perfusion are summarized in Figs 3 and 4. With a few exceptions, flow increased similarly with these vasodilators in the initial (prehypothermic) period in each group. In the nonhypothermia, time control group, responses to these vasodilators persisted but were attenuated 5 hours later. One hour (C2) after returning to normal ionic perfusate, flow was reduced in all hypothermia groups—most in the KCl group and least in the BDM group. There were no additional significant changes in flow with adenosine, acetylcholine, or nitroprusside testing in treatment control, CaCl₂, and KCl groups except for a slight relative increase in flow with adenosine in the treatment control group. Compared with its posthypothermia control flow (C2), however, flow increased with adenosine, acetylcholine (maximal response), and nitroprusside testing in the BDM group. Initially, percent oxygen extraction was reduced equally in each group by acetylcholine and nitroprusside (Fig 4). In the time control group, the decreases in oxygen extraction were attenuated but persisted with adenosine and nitroprusside testing 5 hours later. In the BDM group, oxygen extraction decreased with acetylcholine and nitroprusside testing compared with posthypothermia (C2) control. Oxygen extraction did not change in the other hypothermia groups from their posthypothermia (C2)
control values with adenosine, acetylcholine, or nitroprusside testing.

**LVP, Oxygen Consumption, and Cardiac Efficiency**

Developed (systolic minus diastolic) LVP (Fig 5), initially similar in each group, increased about 25% with epinephrine infusion in each group, decreased similarly by 82±1% in CaCl₂ and BDM groups, and decreased by 100% (arrested) in the KCl group. Maximal cold-induced contractures occurred between 20 and 22°C. Changes in LVP from normothermia to hypothermia, ie, between 25°C and 20°C, were cold control, 97±6 to 89±6 mm Hg (nonsignificant); BDM, 18±2 to 18±2 mm Hg (nonsignificant); CaCl₂, 20±2 to 59±3 mm Hg (P<.05); and KCl, 0 mm Hg. Diastolic LVP was elevated similarly (P<.05) to about 7±2 mm Hg in all hypothermia groups except the KCl group at 20°C. After reperfusion, diastolic LVP was continuously elevated in cold treatment control (8±3 mm Hg), CaCl₂ (13±5 mm Hg), and KCl (9±3 mm Hg) groups but not in the BDM group. Developed (systolic minus diastolic) LVP was initially greatly depressed with normothermic reperfusion during treatment in all hypothermia groups. With discontinuation of treatment after 23 hours, LVP remained depressed in all groups compared with the time control group, but LVP was highest in the BDM group. Compared with the prehypothermia perfusion period, the responsiveness to epinephrine was blunted in all groups.

The decreases in LVP with low CaCl₂ or addition of BDM were accompanied by marked increases in coronary sinus oxygen tension and no change in flow, whereas cardiac arrest (LVP, 0 mm Hg) with high KCl was accompanied by marked decreases in coronary sinus oxygen tension and flow. Oxygen consumption (Fig 6) roughly paralleled the changes in LVP before hypothermic perfusion in all groups. Treatment with BDM, low CaCl₂, or high KCl before hypothermia equally reduced the rate of oxygen consumption. Initially after hypothermia, oxygen consumption was lowest in the KCl group and highest in the treatment control group. Oxygen consumption during the normothermic reperfusion period was highest in the BDM group and lowest in CaCl₂ and KCl groups.

Relative cardiac efficiency (Fig 7) was similar for all groups initially and decreased more during CaCl₂ treatment than with BDM treatment before hypothermic perfusion. During the period of normothermic reperfusion, relative cardiac efficiency was highest in the BDM group but was lower for all hypothermia groups than for the time control group (statistics not shown).

Dry heart weight, expressed as percent of wet heart weight, was not significantly different in the time control (13.4±0.5%), BDM (13.6±0.4%), treatment control (13.4±0.4%), low CaCl₂ (13.1±0.5%), or high KCl (13.1±0.3%) groups.

**Discussion**

Our major objective was to compare three methods of cardiac depression before long-term hypothermic perfusion to determine their ability to preserve cardiac
function with reperfusion. Preservation was indicated by a reduced incidence of dysrhythmias and by improved LVP development, oxygen extraction, cardiac efficiency, and flow responses after 22 hours of hypothermic perfusion. Overall, our results indicate that low-flow cardiac perfusion with a normal ionic solution containing BDM better protects in vitro hearts than either a high KCl or a low CaCl₂ solution; moreover, neither altered ionic solution protects better than the normal extracellular solution. For hypothermia groups,
we observed that atrial rate and atrioventricular conduction returned similarly in all groups; ventricular dysrhythmias were frequent in each group except the BDM group; developed LVP and cardiac efficiency were significantly higher in the BDM group and were similar in the cold treatment control, high KCl, and low CaCl₂ groups; coronary flow and oxygen consumption were reduced in all groups but were highest in the BDM group and lowest in the high KCl group; and percent oxygen extraction increased in all groups but was highest in the KCl group. Although baseline coronary flow was depressed after hypothermic preservation in each group, it was least depressed in the BDM group. Moreover, flow responses to endothelium-dependent and -independent vasodilators acetylcholine and nitroprusside, respectively, disappeared in all except the BDM group, wherein responses, although present, were blunted. The response to adenosine after cold perfusion was also greatest in the BDM group. There was no relative uptake or loss of cardiac water among the groups.

Most experimental approaches to long-term cardiac preservation depend on depression of contractile function by induced ionic cardioplegia, eg, high KCl or low CaCl₂ solutions, or by membrane receptor-blocking agents. In our model, the best approach was to decrease intrinsic contractile mechanism during hypothermic preservation with BDM. Many metabolic inhibitors such as deoxyglucose, an inhibitor of glycolysis, or cyanide, an inhibitor of respiratory chain enzymes, have toxic or irreversible effects. BDM is a small hydrophobic molecule that causes a marked depression of contractility with little change in electrical activity in isolated hearts. BDM does not decrease intracellular Ca²⁺ tran-
sients in papillary muscle at concentrations up to 10 mmol/L. BDM’s major effect is to decrease responsiveness of troponin C to Ca++, as in addition to its major effect on the contractile system proteins, BDM appears to have effects on other intracellular and membrane sites; for example, BDM has been shown to also depress mobilization of sarcoplasmic reticular Ca++ in the heart. In addition to its negative inotropic effect, BDM is a vasodilator. The mechanism of this effect is not known but could involve mechanisms similar to those observed in cardiac muscle cells. Interestingly, BDM also inhibited cold contractures during initial cooling and diastolic contracture with normothermic reperfusion. If hypothermia causes cold contractures by reducing sarcoplasmic reticular Ca++ reuptake or by slowing ion pumps to increase myoplasmic Ca++, then BDM might attenuate contractures by acting as a sort of intracellular pharmacomechanical uncoupler to reduce sensitivity of myofilaments to Ca++ without a major direct effect on transsarcolemmal Ca++.

A goal was to depress contractile function equally before inducing hypothermia. BDM, high KCl, and low CaCl2 similarly decreased contractile function (LVP) and oxygen consumption before hypothermic perfusion. BDM and low CaCl2 treatments are most readily compared because they equally depressed LVP without affecting atrial rate or coronary flow before hypothermic perfusion. High KCl treatment caused cardiac arrest but also a marked decrease in coronary flow with no net reduction in extracted oxygen. On the other hand, perfusion with BDM or low CaCl2 caused a relative vasodilation before and after hypothermic perfusion as shown by similar decreases in percent oxygen extraction, i.e., an increased oxygen supply-to-demand ratio in these groups. Because BDM or low CaCl2 perfusion equally improved oxygen delivery and cardiac work before hypothermia, these hypothermia treatments alone cannot explain the improved responses after hypothermia in the BDM group compared with the CaCl2 group. Thus, BDM appears to afford better protection by a mechanism other than simple depression of cardiac work and metabolism. Interestingly, neither the high KCl nor the low CaCl2 solution protected hearts better than the control ionic solution. It appears that the vasodilatation and higher oxygen extraction ratio caused by the high KCl solution may be detrimental during reperfusion, particularly if intracellular Ca++ is elevated.

Addition of a calcium channel blocker to perfusion solutions may be expected to decrease cardiac work and metabolism by reducing calcium influx and Ca++-induced sarcoplasmic reticular Ca++ release. Calcium channel blockers may protect the heart by reducing cardiac metabolism before, during, and after normothermic ischemia and hypothermia. Because calcium channel blockers act primarily through Ca++-dependent receptor mechanisms located only within the sarclemma, they may not reduce contractility and energy expenditure sufficiently for preservation, so their effectiveness during hypothermia is controversial.

Continuous perfusion with a low CaCl2 solution may have several advantages over administration of a high-concentration calcium channel blocker. Reducing CaCl2 decreases cell membrane Ca++ influx, promotes vascular smooth muscle relaxation, prolongs the cardiac action potential without depolarizing the cell membrane, and greatly decreases cardiac work and metabolism. However, low CaCl2 also prolongs the atrioventricular interval, promotes conduction block, and could cause slower repletion of sarcoplasmic reticular Ca++ stores on reperfusion. Several calcium ion pathways are likely affected by altering extracellular calcium: rates of passive transsarcolemmal diffusion and voltage-activated transport, rates of active exchange with sodium and potassium by membrane pumps, and the degree of intracellular buffering and exchange with sarcoplasmic reticular stores. It should be noted that moderate hypothermia (about 20°C) per se increases intracellular Ca++ not only by slowing pump mechanisms but also by facilitating Ca++-induced Ca++ release, as shown by rapid cooling-induced contractures, an indirect method of assessment of sarcoplasmic reticular Ca++ release. Reducing extracellular CaCl2 from 5 to 0.2 mmol/L appears to have little effect on reducing sarcoplasmic reticular Ca++ release because rapid cooling contractures (to 5°C) are similar at both CaCl2 concentrations. Interestingly, we observed nearly a tripling in the magnitude of cold contractures on initial cooling from 37°C to 20°C in 0.5 mmol/L CaCl2 solution but not in 2.5 mmol/L CaCl2 solution. Relaxation (slope of negative derivative of LVP) was also prolonged to a greater extent in the low CaCl2 group than in other groups at 20°C (personal observation). These effects could reflect attenuated Ca++ reuptake by sarcoplasmic reticulum, enhanced release of sarcoplasmic reticular Ca++, or increased sensitivity of myofilaments to Ca++ on cooling. With normothermic reperfusion, it is unlikely that 0.5 mmol/L CaCl2 induces calcium paradox, i.e., pathological intracellular flooding of calcium and sodium, because this occurs only with calcium-free solution. However, the function of calcium-activated membrane pumps may not be optimal with rearming so that calcium homeostasis is delayed or incomplete.

Continuous perfusion with a high KCl solution causes calcium loading of cardiac cells. This is thought to occur because prolonged membrane depolarization promotes calcium influx by activating voltage-sensitive slow calcium channels and voltage-sensitive sodium-calcium exchangers. When the sarcoplasmic reticulum is saturated with Ca++, Ca++ from extracellular fluid may bypass the sarcoplasmic reticulum to potentiate contraction and to cause diastolic contracture. Indeed, high KCl–arrested hearts have a higher basal oxygen consumption, which is probably calcium dependent, than do very low CaCl2–arrested hearts. Enhanced contracture is not observed because of cardiac cell depolarization with KCl but is observed on washout of the high KCl solution as an elevation of diastolic pressure. If intracellular Ca++ is high on reperfusion, resulting abnormal repolarization potentials can allow random reentrant pathways to develop into ventricular fibrillation. KCl-induced vasoconstriction can result in inadequate coronary flow and oxygen delivery, even with cardiac arrest and reduced metabolic demand.

A major factor retarding full return of contractile function may be inadequate coronary vasodilator capacity. We have shown that BDM is useful for temporarily depressing cardiac function and for attenuating deleterious functional effects of prolonged hypothermic perfusion. Moreover, the relative amount of potential work
(isovolumetric developed LVP times heart rate) for a given rate of oxygen consumption was considerably improved by BDM compared with the other treatments. This may be accounted for by the improved coronary flow with BDM and by the absence of diastolic contracture after hypothermic perfusion. We found that although contractility was improved best after treatment with BDM (65% of control versus 20% to 30% in other hypothermic groups), coronary flow remained about 50% below control during reperfusion after hypothermic perfusion. In cold-treated hearts, when coronary perfusion pressure is increased sufficiently above control to increase coronary flow to the prehypothermic levels, contractile function increases [unpublished finding]. We have reported that pharmacological vasodilation improves cardiac function after hypothermia. Moreover, improved flow likely underlies the observed improvement in relative cardiac efficiency, ie, potential cardiac work per rate of oxygen consumption.

Reduced production of EDRF could underlie, at least in part, the relative vasoconstriction observed during reperfusion after prolonged hypothermia. The present study indicates that coronary responsiveness to both endothelium-dependent (acetylcholine) and endothelium-independent (nitroprusside) vasodilators as well as a mixed-effect vasodilator (adenosine) is severely blunted after long-term hypothermic perfusion regardless of whether the extracellular potassium or calcium is altered. Only the BDM-treated group exhibited any coronary flow responsiveness to adenosine, acetylcholine, and nitroprusside after warm reperfusion. Although these responses were small, this suggests a continued release of nitric oxide by vascular endothelium to induce vasodilation, as well as a small direct vasodilator response to nitroprusside after prolonged hypothermia. However, because the adenosine and nitroprusside responses were greatly attenuated, we could not discern whether the defect was greater in vascular muscle or in vascular endothelium. Because there was no relative uptake of water in the treated groups compared with the time control group, global myocardial edema is unlikely to be a cause of the meager vascular responsiveness. However, the distribution of water may have been altered, so cellular-versus-interstitial edema cannot be ruled out.

In conclusion, high KCl or low CaCl2 solutions afford no better protection against mechanical dysfunction in vitro hearts after long-term hypothermic perfusion than a normal extracellular solution. Moreover, the vasoconstriction induced by high KCl may also be detrimental to restoring function. Perfusion with normal extracellular solution containing BDM to reduce intracellular Ca2+ activity, however, affords better protection than normal or altered KCl or CaCl2 solutions. A limitation of our study was the use of an isolated heart preparation to examine cardiac preservation. The in vitro crystallloid perfused heart has adequate coronary and mechanical reserves, although these reserves are less than in the in vivo, blood-perfused heart. Also, bloodborne factors, such as platelets, may play a significant role in reperfusion injury after hypothermia. Moreover, only potential (pressure) work was measured, and kinetic (volume) work may not be as well preserved. It will be important to examine such approaches for long-term cardiac preservation using in vivo animal models to validate the potential success of transplanting the human donor heart harvested many hours earlier.

Acknowledgments

This work was supported in part by grants from the American Heart Association, Wisconsin affiliate (88-GA-06); the National Institutes of Health (HL-34708); and an Anesthesiology Research Training Grant (GM-08377). We are especially grateful to James S. Heisner for his excellent technical skills, to Robert Mehner for laboratory assistance, and to Miriam Mick and Edith Sulzer for literature retrieval and secretarial assistance.

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Circulation. 1994;89:2412-2420
doi: 10.1161/01.CIR.89.5.2412

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