**Reduction of Cytosolic Ca**\(^{2+}\) Loading and Improved Cardiac Function After Cardioplegic Cold Storage of Guinea Pig Isolated Hearts**

David F. Stowe, MD, PhD; Srinivasan G. Varadarajan, MD; Jianzhong An, MD; Steven C. Smart, MD

**Background**—Hypothermia is cardioprotective, but it causes Ca\(^{2+}\) loading and reduced function on rewarming. The aim was to associate changes in cytosolic Ca\(^{2+}\) with function in intact hearts before, during, and after cold storage with or without cardioplegia (CP).

**Methods and Results**—Guinea pig hearts were initially perfused at 37°C with Krebs-Ringer’s (KR) solution (in mmol/L: Ca\(^{2+}\) 2.5, K\(^{+}\) 5, Mg\(^{2+}\) 2.4). One group was perfused with CP solution (Ca\(^{2+}\) 2.5, K\(^{+}\) 18, Mg\(^{2+}\) 7.2) during cooling and storage at 3°C for 4 hours; another was perfused with KR. LV pressure (LVP), dP/dt, O\(_2\) consumption, and cardiac efficiency were monitored. Cytosolic phasic [Ca\(^{2+}\)] was calculated from indo 1 fluorescence signals obtained at the LV free wall. Cooling with KR increased diastolic and phasic [Ca\(^{2+}\)], whereas cooling with CP suppressed phasic [Ca\(^{2+}\)] and reduced the rise in diastolic [Ca\(^{2+}\)]. Reperfusion with warm KR increased phasic [Ca\(^{2+}\)] 86% more after CP at 20 minutes and did not increase diastolic [Ca\(^{2+}\)] at 60 minutes, compared with a 20% increase in phasic [Ca\(^{2+}\)] after KR. During early and later reperfusion after CP, there was a 126% and 50% better return of LVP than after KR; during later reperfusion, O\(_2\) consumption was 23% higher and cardiac efficiency was 38% higher after CP than after KR.

**Conclusions**—CP decreases the rise in cardiac diastolic [Ca\(^{2+}\)] observed during cold storage in KR. Decreased diastolic [Ca\(^{2+}\)] and increased systolic [Ca\(^{2+}\)] after CP improves function on reperfusion because of reduced Ca\(^{2+}\) loading during and immediately after cold CP storage. (*Circulation. 2000;102:1172-1177.*)

**Key Words:** contractility ● cardioplegia ● calcium ● hypothermia

**Heart preparation for transplant by arrest in vivo with a cold cardioplegic (CP) crystalloid solution.** After removal, they are stored near freezing for up to 4 to 5 hours and reperfused initially with cold CP solution during cardiac anastomoses before warm blood perfusion. A major adverse effect of long hypothermic storage (HS) is reduced left ventricular (LV) diastolic compliance and contractility on reperfusion. The time course of changes in diastolic and systolic Ca\(^{2+}\) and LV function during and after HS has not been quantified in intact hearts. One aim was to examine whether myoplasmic Ca\(^{2+}\) overloading occurs during and after HS in normal ionic solution and whether this is temporally associated with diastolic dysfunction, and consequently, reduced contractility and contractile efficiency on reperfusion.

CP solutions may be protective, in part because the high K\(^{+}\) concentration maintains cardiac arrest on cooling and rewarming.\(^{1,2}\) Excess Mg\(^{2+}\) concentration in some CP solutions is believed to counteract Ca\(^{2+}\) loading by reducing Ca\(^{2+}\) influx and myoplasmic Ca\(^{2+}\) release.\(^{5,6}\) Both extracellular ionic changes may supplement the metabolic sparing effects of hypothermia per se and thus improve function on warm reperfusion. However, it is not known whether CP reduces diastolic and systolic Ca\(^{2+}\) loading during as well as after HS or whether this improves reperfusion function. A second aim was to determine whether a high-Mg\(^{2+}\), high-K\(^{+}\) CP solution better restores the temporal association between Ca\(^{2+}\) and cardiac function after HS. To test this, LV [Ca\(^{2+}\)] was measured simultaneously with indices of mechanical and metabolic function in intact hearts.

**Methods**

**Isolated Heart Preparation and Measurements**

Our preparation and measurements have been described in detail.\(^{4,7-10}\) Guinea pig hearts were perfused (55 mm Hg) in Langendorff fashion with a Krebs-Ringer’s (KR) solution (pH 7.39±0.01, Pa\(_O_2\) 560±10 mm Hg) containing (in mmol/L): Na\(^+\) 137, K\(^+\) 5, Mg\(^{2+}\) 2.4, Ca\(^{2+}\) 2.5, Cl\(^-\) 134, HCO\(_3^-\) 15.5, H\(_2\)PO\(_4^-\) 1.2, glucose 11.5, pyruvate 2, mannitol 16, EDTA 0.05, probenicid 0.1, and insulin (5 U/L). Isovolumetric LV pressure (LVP), spontaneous heart rate (HR), coronary inflow (CF), and pulmonary artery outflow O\(_2\) tension were measured continuously. Percent O\(_2\) extraction (%O\(_2\)E) was calculated as 100 × (Pa\(_O_2\)−Pa\(_O_2\)/Pa\(_O_2\); myocardial O\(_2\) consump-

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tion (MV2O2) as CF/g · (PaO2−PvO2) · 24 μL O2/mL at 760 mm Hg; and cardiac efficiency as systolic minus diastolic LVP · HR/MV2O2.

**Kd of Free Ca2+ at Different Temperatures**

Calibration techniques have been described.19 The dissociation constant (Kd) of indo 1 was 149±8 nmol/L at 37°C. Emission scans, after autofluorescence correction, were conducted with the same technique in 4 additional homogenates at 27°C, 17°C, and 7°C to determine Kd for calculating [Ca2+]. Free indo 1 reduced the fluorescence ratio F385/F456 in a nearly linear fashion by 0.30, 0.23, and 0.16 per 10°C fall. Kd increased 28% at 27°C (205 nmol/L), 44% at 17°C (254 nmol/L), and 67% at 7°C (285 nmol/L). The linear relationship (y=mx+b) for temperature and Kd was Kd = 4.6°C+323.8 (r²=0.99). The calculated Kd at 3°C was 300 nmol/L. Similarly, Liu et al11 reported that at 1 μmol/L [Ca2+], Kd increased from 139 nmol/L (37°C) to 255 nmol/L (15°C) and to 297 nmol/L (5°C).

**Loading Indo 1 and Recording Ca2+ Transients**

As we have reported,10 a trifurcated fiber-optic cable was placed against the LV free wall to excite and record Ca2+ signals. In a non–indo 1 vehicle group (n=6), F385 and F456 signals were recorded over time, as in indo 1 studies, and individual autofluorescence signals were subtracted from indo 1 signals at the corresponding time. Background autofluorescence was also determined for each heart before indo 1 loading and after initial perfusion and equilibration at 37°C. Thereafter, 16 hearts were loaded with indo 1-AM until F385 and F456 intensities increased by 10-fold. Indo 1 loading and washout reduced contractility by ~25%, if, ie, from ~105 to 78 mm Hg; this was a more a result of pluronic acid than indo 1 buffering. Signals were filtered (Corion) at 385 and 456 nm. Background fluorescence but not indo 1 dye fluorescence is influenced by tissue oxygenation state at these 2 isosbestic wavelengths.12,13 Solutions contained probenecid to retard leakage of indo 1. At 37°C, emission fluorescence remained ~5-fold greater than background for ≥3 hours after washout of extracellular indo 1-AM. Hypothermia markedly retarded the parallel declines in F385 and F456 over time, so that F385/F456 remained unchanged. At each sampling interval, F385, F456, F385/F456, and LVP were recorded over 8 to 9 cardiac cycles every 10 ms for 2.5 seconds. Data were later background-corrected and converted to [Ca2+] by mathematical routines we developed.

**Calculation of Compartmental Ca2+ Concentrations**

Calibration curves were derived according to protocols by Brandes et al12,13 with modifications of a standard equation for fluorescence indicators.14 Total (t) [Ca2+] was calculated from the F385/F456 ratio (R), Rmin (SbfH for >100 μmol/L Ca2+), Rmax (Rmax×SbfH/R385 for 0 Ca2+), S456 (R385/R456 at 456 nm emission), and Kd according to the equation

\[ [Ca^{2+}] = S_{456} \times K_d \times \left( \frac{R - R_{\text{min}}}{R_{\text{max}} - R} \right), \]

where S456=(1–SbfH)/(1–SbfH) and bH is the slope (b) of F385 as a function of F385/F456 Rmin was 5.986 and Rmax was 0.059.

Noncytosolic (nc) fluorescence, primarily mitochondrial, was assessed after each experiment by perfusing hearts with MnCl2 to quench fluorescence derived from the cytosolic (c) compartment.15,16 As described earlier,10 Mn2+ and Mn2+ signals were calculated at each time point by multiplying residual mitochondrial fluorescence fractions (f385 and f456) by total end-diastolic fluorescence. In other studies (n=9), we observed that continuous Mn2+ quenching reduced average F456/F385 and F456/F385 signals, respectively, to 3.9 and 10.7 times the indo 1 unloaded baseline at time zero, and to 2.5 and 5.4 times baseline after 120 minutes, indicating that indo 1 fluorescence ratio is not altered appreciably over time, so that noncytosolic Ca2+ does not also become quenched. Unstimulated endothelium does not contribute significantly to total [Ca2+].14 Maximally stimulated endothelial Ca2+ has a moderate effect on F385 and F456 as shown in Figure 1.

**Protocol**

Initial background (unloaded) measurements were obtained after 20 minutes of stabilization and determination of maximal flow with adenosine. After loading and residual washout of indo 1, recordings were obtained every 1 to 5 minutes during normothermia. Perfusate and bath were maintained at 37°C before and after hypothermia by a heated water circulator and at 3°C by a parallel, refrigerated water circulator. Bradykinin (BK, 10 nmol/L) was infused for 5 minutes before cooling to maximally stimulate endothelial and/or vascular Ca2+. N⁵-Nitro-L-arginine methyl ester (L-NAME), 100 μmol/L, and sodium nitroprusside (SNP), 100 μmol/L, were given to 60 to 70 minutes after reperfusion at 37°C to assess basal endothelial or vascular [Ca2+] as estimated from the change in myocardial [Ca2+]. MnCl2 was then given to quench cytosolic Ca2+ transients.

LVP, CF, and PO2 were measured continuously before and after HS. Extracellular pH decreases from 7.4 to ~6.9 in 4% CO2 gassed solution at 3°C.4,7,8,10 But hypothermia alone induces a relative intracellular alkalosis.18 [Ca2+], and systolic-diastolic LVP were recorded continuously at all temperatures to 3°C and back to 37°C. Hearts were randomized into KR and CP groups. CP solution differed from KR solution in that KCl was 18 mmol/L and MgCl2 was 7.2 mmol/L. In the CP group, perfusion was switched from KR to CP just before the onset of cooling (at 85 minutes) and switched back to KR 10 minutes into the rewarming phase at 22°C to 25°C (at 355 minutes).

The appropriate Kd was used to calculate nmol/L [Ca2+] at each temperature. F385, F456, F385/F456 Ca2+ transients, LVP, and dP/dt were displayed and digitized simultaneously with proprietary software. F signals were calibrated to nmol/L [Ca2+] with algorithms that (1) corrected for autofluorescence over time and conditions, (2) selected the appropriate Kd, and (3) adjusted for noncytosolic Ca2+ after quenching of cytosolic Ca2+. LVP, CF, and raw metabolic data were also recorded with Ca2+ transient data and were later analyzed together. Variables measured or calculated were systolic (sys), diastolic (dia), and phasic systolic-diastolic (sys-dia), ie, released [Ca2+], sysLVP, diaLVP, sys-diaLVP, and dP/dtmax.

All data were expressed as mean±SEM and compared by Tukey's comparison of means tests after 2-way ANOVA (between KR and CP groups) and 1-way ANOVA for repeated measures (within each group). Differences among means were considered significant when P<0.05.

**Results**

Figure 1 displays drug effects on [Ca2+] at 37°C in KR-perfused hearts before and after HS. There was no significant
Effects of 4 Hours of Cold (3°C) Storage of Isolated Hearts in KR Solution (5.0 mmol/L K⁺, 1.2 mmol/L Mg²⁺) or CP Solution (18.0 mmol/L K⁺, 7.2 mmol/L Mg²⁺) on [Ca²⁺], and Mechanical and Metabolic Variables

<table>
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<th>Variable</th>
<th>KR</th>
<th>Control</th>
<th>1-min RP (22°C)</th>
<th>20-min RP</th>
<th>60-min RP</th>
<th>Control</th>
<th>1-min RP (22°C)</th>
<th>20-min RP</th>
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<td>356</td>
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<td>dia[Ca²⁺], mmol/L</td>
<td>94±3</td>
<td>538±74</td>
<td>108±74</td>
<td>117±8</td>
<td>92±3</td>
<td>256±26†</td>
<td>101±6</td>
<td>99±5†</td>
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<td>sys[Ca²⁺], mmol/L</td>
<td>440±40</td>
<td>900±79</td>
<td>337±21</td>
<td>518±98</td>
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<td>349±37†</td>
<td>541±40</td>
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<td>362±80</td>
<td>235±20</td>
<td>401±97</td>
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<td>38±10</td>
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<td>600±240</td>
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<td>125±14†</td>
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<td>Venous pH</td>
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<td>7.13±0.03</td>
<td>7.15±0.02*</td>
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<td>48±4</td>
<td>53±3</td>
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<td>62±4†</td>
<td>66±6†</td>
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<td>79±3</td>
<td>76±3</td>
<td>82±2</td>
<td>78±3</td>
<td>75±4</td>
<td>74±4</td>
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<td>%O₂E</td>
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<td>21±6</td>
<td>47±6</td>
<td>38±4</td>
<td>0.1±0.6†</td>
<td>41±9†</td>
<td>65±9†</td>
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</table>

RP indicates reperfusion (min) after HS; CE, cardiac efficiency = sys-diaLVP · HR/MVO₂. Temperature at 1 min reperfusion is 22±1°C and at other times, 37°C. Myoplasmic [Ca²⁺] values are corrected for temperature, autofluorescence, and noncytosolic Ca²⁺.

*p<0.05 vs Control pre-HS; †p<0.05 for CP vs KR.

Difference in sys, dia, or sys-dia[Ca²⁺] between the initial control at 60 minutes and the final control at 385 minutes.

SNP and L-NAME had no effect on [Ca²⁺], whereas BK, which increases endothelial Ca²⁺ to stimulate NO synthesis, increased the mmol/L equivalent of dia[Ca²⁺] and sys[Ca²⁺] by 22±1% and 29±2%, respectively. BK and SNP, respectively, increased CF by 64±2% and 78±2% and decreased %O₂E by 43±3% and 45±3%; L-NAME had no cardiac effects. Mn⁴⁺ had no effect on contractility but decreased cellular [Ca²⁺] and abolished phasic [Ca²⁺]; this non–cytosolic compartment [Ca²⁺] was taken into account in calculating cytosolic [Ca²⁺] (see Methods).

The Table displays effects of 4 hours of storage at 3°C of hearts perfused with either KR (n=8) or CP (n=8) solution on Ca²⁺ handling and cardiac function. Both groups had similar initial values (at 70 minutes) before HS. In KR at 1 minute of reperfusion (at 356 minutes), dia[Ca²⁺] and sys[Ca²⁺] were markedly elevated, and diaLVP was increased and each index of function (except %O₂E) was depressed; during CP, elevations in dia, sys, and dia-dia[Ca²⁺] were much lower, and cardiac function remained nil because of cardiac perfusion. After 20 minutes of warm reperfusion with KR in both groups (at 385 minutes), the KR group exhibited elevated values for dia[Ca²⁺] and depressed values for sys and sys-dia[Ca²⁺] and function; after CP, there was no change from control for each index of [Ca²⁺] and higher values for sys and sys-dia[Ca²⁺] and function compared with KR.

After 60 minutes of reperfusion (at 425 minutes), values had returned to control levels for each variable after KR except for dia[Ca²⁺], which remained elevated, and MV O₂, which remained lower than before HS; after CP, all variables returned to control levels except for dP/dt and cardiac efficiency, which were higher than hypothermia control values (Table). CF was unchanged in both groups except for a lower value after KR at 1 minute of reperfusion. Coronary sinus pH was lower after KR and HS. HR was initially lower in both groups during early reperfusion. There were no dysrhythmias other than transient 2° block after CP; after KR, there were occasional premature ventricular extrasystoles during the initial 10 minutes of reperfusion.

Figures 2 and 3 display associations between [Ca²⁺] and function in more detail at discrete periods before, during, and after HS. Figure 2A plots the association between −dP/dtmax (relaxation) and dia[Ca²⁺]. Dia[Ca²⁺] rose more after KR than after CP during cooling, storage, and rewarming; on warm KR reperfusion, the KR group exhibited slower relaxation (−dP/dtmax) than the CP group, Figure 2B displays the relationship between sys-dia[Ca²⁺] and sys-diaLVP. LVP decreased moderately during cooling with KR, but was nil after CP; there was no sys-diaLVP or sys-dia[Ca²⁺] during cooling with CP, but sys-dia[Ca²⁺] increased markedly during cooling with KR. On initial rewarming (at 356 minutes), sys-dia[Ca²⁺] again rose transiently after KR but not after CP. During normothermic KR reperfusion, sys-dia[Ca²⁺] was higher after CP than after KR at 20 minutes of reperfusion, but this difference declined over the next 80 minutes. The CP-treated group, however, exhibited better recovery of sys-diaLVP throughout KR reperfusion.

Figure 3A plots sys[Ca²⁺] with +dP/dtmax. These results are qualitatively similar to those of Figure 2B in that sys[Ca²⁺] rose, whereas +dP/dtmax decreased, during cooling after KR, and CP caused arrest; on rewarming after washout of CP,
sys[Ca\textsuperscript{2+}] again increased markedly after KR but not after CP. During 80 minutes of KR reperfusion, sys[Ca\textsuperscript{2+}] and +dp/dt\textsubscript{max} were higher after CP than after KR. Figure 3B shows that changes in sys-dia[Ca\textsuperscript{2+}] were qualitatively similar to, but of lesser magnitude than, those for sys[Ca\textsuperscript{2+}]. MVO\textsubscript{2} was only moderately reduced during cooling and rewarming after CP and was higher during 80 minutes of KR reperfusion after CP than after KR.

**Discussion**

This study is the first to demonstrate in intact hearts (1) the temporal association between cardiac function and dia and sys[Ca\textsuperscript{2+}] before, during, and after 4 hours of HS and (2) the effects of CP to attenuate Ca\textsuperscript{2+} loading and improve cardiac function. CP reduced myoplasmic Ca\textsuperscript{2+} loading on cooling, storage, and rewarming after storage, with improved function on reperfusion better than storage alone (KR). Dia[Ca\textsuperscript{2+}] was reduced by CP during hypothermia, and myocardial contracture during hypothermia was eliminated. On reperfusion after CP, sys and sys-dia[Ca\textsuperscript{2+}] were higher and dia[Ca\textsuperscript{2+}] was lower; contractility, relaxation, MVO\textsubscript{2}, and cardiac efficiency were improved. This study demonstrates the utility of CP to better maintain myocardial Ca\textsuperscript{2+} homeostasis and so to improve cardiac function.

Ionized Ca\textsuperscript{2+} exists primarily in cardiac myoplasm, but also in mitochondria and in endothelial and smooth muscle cells. It is important to know whether these compartments contribute substantially to Ca\textsuperscript{2+} signals recorded in intact hearts. L-NAME and SNP had no overall effect on total cellular [Ca\textsuperscript{2+}] under control conditions, so it is unlikely that basal endothelial or vascular [Ca\textsuperscript{2+}] contributed significantly to myoplasmic [Ca\textsuperscript{2+}] transients. The fact that BK, which stimulates NO synthesis via increased endothelial [Ca\textsuperscript{2+}], significantly increased fluorescence suggests that stimulated endothelial Ca\textsuperscript{2+} may contribute to total Ca\textsuperscript{2+}. But a change in endothelial Ca\textsuperscript{2+} is unlikely to contribute to total [Ca\textsuperscript{2+}], because CF was unaltered on reperfusion. Conversely, Mn\textsuperscript{2+}, by quenching primarily cytosolic [Ca\textsuperscript{2+}] bound to indo 1, decreased cellular [Ca\textsuperscript{2+}] by \( \approx 25\% \).

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**Figure 2.** A, Relationship of dia[Ca\textsuperscript{2+}] and -dp/dt\textsubscript{max} before, during, and after 4 hours of storage in KR- and CP-perfused hearts. Note greater rise in dia[Ca\textsuperscript{2+}] during storage and initial reperfusion in KR group. -dp/dt\textsubscript{max} but not dia[Ca\textsuperscript{2+}] was higher after CP during 80 minutes of reperfusion. B, Relationship of sys-dia[Ca\textsuperscript{2+}] (phasic) and sys-diaLVP (phasic). Note a greater rise in initial sys-dia[Ca\textsuperscript{2+}] and sys-diaLVP during cooling and higher sys-dia[Ca\textsuperscript{2+}] during storage and initial reperfusion after KR. Sys-diaLVP was higher during 80 minutes of reperfusion after CP, and sys-dia[Ca\textsuperscript{2+}] was higher up to 20 minutes of reperfusion after CP. Significance between groups at specific time points is given in Table.

**Figure 3.** A, Relationship of sys[Ca\textsuperscript{2+}] and +dp/dt\textsubscript{max} before, during, and after 4 hours of storage in KR- and CP-perfused hearts. Note greater rise in initial sys[Ca\textsuperscript{2+}] and +dp/dt\textsubscript{max} during cooling and higher sys[Ca\textsuperscript{2+}] during storage and initial reperfusion in KR group. +dp/dt\textsubscript{max} was higher during 80 minutes of reperfusion after CP, and sys[Ca\textsuperscript{2+}] was higher during initial 20 minutes of reperfusion. B, Relationship of sys-dia[Ca\textsuperscript{2+}] and MVO\textsubscript{2}. Note greater rise in initial sys-dia[Ca\textsuperscript{2+}] and sys-diaLVP during cooling and higher sys-dia[Ca\textsuperscript{2+}] during initial reperfusion after KR. MVO\textsubscript{2} was higher during 80 minutes of reperfusion after CP, and sys-dia[Ca\textsuperscript{2+}] was higher during initial 20 minutes of reperfusion. See Table for significance at specific time points.
Hypothermia to 3°C to 5°C is widely used to protect donor hearts during storage before transplantation.1,2 Increasing duration of storage beyond 3 to 5 hours leads to worsened mechanical and metabolic function on reperfusion. Severe hypothermia is key, because it decreases metabolism and delays degradation of intracellular enzymes. Enzymatic activity decreases ≈50% for each 10°C fall in temperature,19 so even at 3°C, MV02 is ≈10% of that at 37°C. Reperfusion with an oxygenated, normal-ionic solution after storage triggers rapid ATP regeneration and partially or completely restores ion pump activities, action potentials (AP), and mechanical function.

At >15°C, myocardial cells preserve ion homeostasis, because resting membrane potential (Eₘ) and AP phase 0 dV/dt are maintained even though AP duration is markedly increased.20 At <15°C, Ca²⁺ loading and diastolic contracture occur,7,8,10 probably because of attenuated temperature-dependent Ca and Na pump activities, as evidenced by AP “flattening.”21 At 3°C, Eₘ remains depolarized, and repolarization cannot occur.22 In the peri-hypothermic period, high [K⁺] arrests the heart and reduces metabolism by cell depolarization, whereas high [Mg²⁺] is proposed primarily to attenuate cellular [Ca²⁺] by displacing Ca²⁺ from binding sites in the Ca²⁺ channel and by reducing sarcoplasmic reticular (SR) Ca²⁺ release via increased [Mg²⁺].5,23 Ca²⁺ release increases with high-K⁺ and/or high-Mg²⁺ solutions before and after storage reduces Ca²⁺ accumulation and improves reperfusion function.5,6 Our results show more specifically that improved function after hypothermic arrest not only reduces dia[Ca²⁺] loading during storage but also improves sys-dia[Ca²⁺] on later reperfusion. Dia[Ca²⁺] increased during storage and markedly on reperfusion in the KR group; this was associated with diastolic contracture and diastolic dysfunction.

Interrelated ionic disturbances underlie hypothermia-induced Ca²⁺ loading during HS and dysfunction associated with Ca²⁺ overloading on reperfusion.25–33 Hypothermia most likely prolongs Ca²⁺ channel influx and alters ion exchangers that trigger enhanced SR Ca²⁺-induced Ca²⁺ release. During hypothermia, depressed Ca and Na pump activities may lead to Na and Ca overload via Na-H exchange and reversed Na-Ca exchange.27,31,34 It is known that Na and Ca pump inhibitors can depolarize diastolic Eₘ toward the Na equilibrium potential, enhance net Ca²⁺ entry mediated by a prolonged depolarized state through L-type Ca channels with Na⁺ influx in exchange for Ca²⁺ influx, and/or increase Na⁺ loading via Na⁺ influx in exchange for H⁺ efflux.35,36 Excessive Na⁺ entry can elevate cell [H⁺] via Na-H exchange, and importantly, Ca²⁺ entry through slowed or reversed Na-Ca exchange,36 because repolarization is slowed, particularly if the Na pump is incapable of reversing the Na load. Thus, contractile dysfunction early after cold storage stems in large part from uncorrected ion homeostasis by these ion pumps.

During severe hypothermia when Eₘ can be nearly depolarized because of Na⁺ pump inhibition, the increased [Na⁺] may shift the reversal potential for Na-Ca exchange toward a less negative Eₘ and thus promote greater Ca²⁺ influx by the exchanger to increase [Ca²⁺], ie, enhanced “reversed” mode operation. Exposure of isolated embryonic myocytes to 10°C hypothermia for 6 hours increased total cell sodium by 2- to 3-fold and total cell calcium by 50%.31 However, there was insignificant Na-Ca exchange activity during severe hypothermia if Na-H exchange was also inhibited.31 At constant pH, hypothermia increases myocardial pH by 0.17 units per 10°C fall.18 This probably occurs because metabolism is reduced and Na⁺ influx is enhanced at the expense of H⁺ efflux, causing increased pH. Both extracellular acidosis and Na-H exchange inhibitors can reduce the rise in total cell sodium in myocytes at 10°C.31

Hypothermia increases [Ca²⁺] largely by attenuating SR Ca²⁺ reuptake because of reduced SR Ca pump activity.32,33 SR Ca uptake activity was reduced 50% on 4 hours of storage at 4°C compared with 37°C controls, and Ca pump activity was decreased to 50% at 24 hours at 4°C.33 The contributions of Ca pumps and Na-Ca exchange to contractility appear to be depressed equivalently during cooling,27 but Ca²⁺-induced Ca²⁺ release triggered by Na-Ca exchange may be reduced more than that triggered by Ca²⁺ influx.34 Thus, reduced pump activity may disrupt Ca²⁺ homeostasis and thus impede myofilament relaxation and excitation-contraction coupling. We have reported10 that moderate hypothermia increases total phasic [Ca²⁺] and phasic contraction; here, we report that severe hypothermia increases dia[Ca²⁺], abolishes sys-dia[Ca²⁺], and causes contracture. The hypothermia-induced increase in Ca²⁺ loading may be attenuated by Mg²⁺ via an effect to reduce voltage-dependent Ca²⁺ influx or to reduce SR Ca²⁺ release.5,24

Our study suggests that hypothermia also causes mitochondrial Ca²⁺ loading, which can damage mitochondria and impair ATP synthesis. Excess mitochondrial Ca²⁺ could reduce contractile efficiency, because mitochondrial Ca²⁺ plays a pivotal regulatory role linking cardiac mechanics and energy production.37,38 Inhibiting mitochondrial Ca²⁺ uptake was shown to attenuate mechanical dysfunction after warm ischemia and reperfusion without altering cytosolic [Ca²⁺].37 Hypothermia helps to preserve mitochondrial function during subsequent ischemia.39

Hypothermia-induced changes in Na⁺, Ca²⁺, K⁺, and H⁺ homeostasis largely protect the heart. However, Ca²⁺ overload during cooling, storage, or rewarming is probably responsible for dysfunction on reperfusion. Knowledge of specific time-dependent changes in myoplasmic [Ca²⁺] in normal and CP solutions before, during, and after hypothermia should aid development of improved methods for heart preservation.

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Reduced Cytosolic Ca\textsuperscript{2+} Loading and Improved Cardiac Function After Cardioplegic Cold Storage of Guinea Pig Isolated Hearts

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