Acidemia and Hypernatremia Enhance Postischemic Recovery of Excitation-Contraction Coupling

Kazumasa Harada, Alvin Franklin, Robert G. Johnson, William Grossman, James P. Morgan

Abstract

The purpose of the present study was to determine whether Na\(^+\)-H\(^+\) and Na\(^+\)-Ca\(^{2+}\) exchanges modulate postischemic recovery of excitation-contraction coupling. Experiments were performed in 43 isolated isovolumic dog hearts perfused with blood (pH 7.40, 141 mmol/L Na\(^+\), 34\(^\circ\)C, paced at 2 Hz). A 3x3-mm region at the left ventricular (LV) apex was loaded with aequorin for monitoring [Ca\(^{2+}\)], simultaneously with LV pressure. No-flow ischemia for 2 to 3 minutes was followed by 20 minutes of aerobic reperfusion with (1) unmodified control blood (141 mmol/L Na\(^+\), pH 7.40), (2) acidic blood (141 mmol/L Na\(^+\), pH 6.60, at 0 to 3 minutes of reperfusion), (3) hypernatremic blood (149 or 157 mmol/L Na\(^+\), pH 7.40, at 0 to 20 minutes of reperfusion), or (4) hyperosmotic blood (141 mmol/L Na\(^+\)+30 mmol/L mannitol, pH 7.40, at 0 to 20 minutes of reperfusion). Reperfusion with unmodified control blood was immediately followed by an increase in [Ca\(^{2+}\)] and LV systolic and diastolic pressure that persisted for 2 to 3 minutes before returning to or below baseline. Ventricular arrhythmia occurred during this period (>80%). This transient increase of [Ca\(^{2+}\)] was attenuated by acidic or hypernatremic perfusate. With acidic or hypernatremic reperfusion, recovery of LV developed pressure at 20 minutes was more complete than with unmodified control reperfusion: acidic blood (n=7), 93±3% (P<.01); hypernatremic blood (149 mmol/L Na\(^+\), n=7), 89±2% (P<.02); hypernatremic blood (157 mmol/L Na\(^+\), n=4), 91±2% (P<.01); and unmodified control blood (n=17), 80±2%. With hyperosmotic reperfusion, recovery of LV developed pressure at 20 minutes was not improved (82±3%). From these results we conclude that (1) an increase in intracellular Ca\(^{2+}\) occurs transiently after no-flow ischemia and may cause arrhythmia and decreased Ca\(^{2+}\) responsiveness of the contractile elements, (2) acidic and hypernatremic reperfusion ameliorates postischemic dysfunction by preventing the increase in intracellular Ca\(^{2+}\), suggesting that (3) Na\(^+\)-H\(^+\) and Na\(^+\)-Ca\(^{2+}\) exchange may play important modulatory roles during reperfusion. (Circ Res. 1994;74:1197-1209.)

Key Words: aequorin • postischemic recovery • Ca\(^{2+}\) overload • Ca\(^{2+}\) responsiveness • acidosis • hypernatremia • Na\(^+\)-Ca\(^{2+}\) exchange • Na\(^+\)-H\(^+\) exchange

Constrictile dysfunction after brief periods of ischemia has been attributed to increased intracellular Ca\(^{2+}\) (Ca\(^{2+}\) overload),\(^\text{10}\) and acidic reperfusion may modulate recovery by preventing Ca\(^{2+}\) influx into cells.\(^\text{10}\) Koretsune and Marban\(^\text{1}\) showed the possible involvement of decreased sensitivity of the myofilaments to intracellular Ca\(^{2+}\) as a mechanism for hypoxic contractile dysfunction. Moreover, we have previously reported decreased Ca\(^{2+}\) responsiveness of the myofilaments after brief periods of no-flow ischemia.\(^\text{12}\)

Despite the recent accumulation of information in this area, results have often appeared to be contradictory,\(^\text{13}\) and no definitive experiments have been done to directly demonstrate increased intracellular Ca\(^{2+}\) (Ca\(^{2+}\) overload) by means of [Ca\(^{2+}\)], monitoring on a beat-to-beat basis or its possible modulation by acidosis. This is mainly due to difficulties in detecting transient changes of intracellular Ca\(^{2+}\) by available methodologies, such as nuclear magnetic resonance (NMR).\(^\text{8,11}\)

An increase of cytosolic Na\(^+\) at the end of 20 minutes of global ischemia and during the early minutes of reperfusion has been shown by radioisotope study using rat hearts,\(^\text{14}\) and cytosolic Ca\(^{2+}\) accumulation following the increase of cytosolic Na\(^+\) appeared to correlate with deterioration of left ventricular (LV) function. Although Tani and Neely\(^\text{1}\) proposed the possible involvement of Na\(^+\)-H\(^+\) and Na\(^+\)-Ca\(^{2+}\) exchange from this observation, they could not exclude the possibility that deterioration of LV pressure may be caused by a decrease of peak intracellular Ca\(^{2+}\) (abnormal intracellular Ca\(^{2+}\) modulation). To test the possible involvement of Na\(^+\)-H\(^+\) and Na\(^+\)-Ca\(^{2+}\) exchange in modulating [Ca\(^{2+}\)], during reperfusion and to clarify how an increase in [Ca\(^{2+}\)] may decrease the responsiveness of the myofilaments to intracellular Ca\(^{2+}\), we studied two sets of interventions in contrast to global ischemia with unmodified control blood perfusion. Experimental protocols include no-flow ischemia for 2 to 3 minutes followed by 20 minutes of aerobic reperfusion with (1) acidic blood (at 0 to 3 minutes of reperfusion) or (2) hypernatremic blood (at 0 to 20 minutes of reperfusion). To exclude the possibility that hyperosmolarity rather than hypernatremia may modulate LV function, we performed supplementary experiments with hyperosmotic perfusate (at 0 to 20 minutes of reperfusion) produced by mannitol infusion. The nature of each intervention, its rationale, and timing is presented in detail in “Materials and Methods.”
Materials and Methods

Isolated Heart Preparation

Isolated blood-perfused hearts were prepared from 43 mongrel dogs as previously described. Each dog was anesthetized with a mixture of 500 mg/kg urethane and 50 mg/kg chloralose and placed on mechanical ventilation. The heart was prepared by insertion of cannulas into the right ventricle (RV) (through the superior vena cava) and the carotid or subclavian arteries. The heart was then isolated by ligation of the superior and inferior venae cavae and descending thoracic aorta and was removed from the thorax. The lungs were removed, and the pulmonary artery was ligated. Coronary perfusion of the isolated heart was maintained by perfusion of the coronary arteries, via the carotid cannula, with a mixture of physiological salt solution plus blood obtained from the animal during preparation of the isolated heart (pH 7.4; Po2, 600 mm Hg; PCO2, 45 mm Hg; hematocrit, 14.7±0.5%) and circulated by a roller pump through a bubble trap, flowmeter, and heat exchanger (with temperature maintained at 34°C rather than 37.5°C to lessen consumption of aequorin). Coronary perfusion pressure was maintained at 80 to 100 mm Hg throughout the experiment. Coronary venous blood was drained from the RV to minimize ventricular interactive effects. An intramyocardial thermistor was placed in the RV to record temperature. A LV drain was inserted through a stab wound in the LV apex to drain thethesian flow. The left atrium was opened, and an inflatable balloon was placed in the LV through the mitral valve and secured just below the annulus. A balloon size was chosen so that balloon volume would be greater than LV diastolic volume to ensure that pressure increments would reflect LV rather than balloon tension changes. EKG electrodes and pacing electrodes were secured to the surface of the myocardium.

Light-Collecting System for Aequorin Luminescence

For collection of the aequorin light signal from the surface of the LV, the entire isolated heart preparation was positioned in a light-tight box. The coronary perfusion line distal to the pressure control reservoir and the venous drainage line were positioned in the box, and the rest of the system was located outside the box and connected by light-tight junctions in the baseboard. Electrical wires (including pacing cables, thermistor, and 3F Millar catheter for recording pressure within the LV) were also passed through light-tight junctions. A fluidtight, specially equipped photomultiplier tube (9635QA, Thorn EMI) was positioned in proximity to the aequorin-loaded myocardial surface. The interface between the aequorin-loaded myocardial surface and the cathode of the photomultiplier tube was held constant by securing a Teflon patch onto the myocardial surface.

Loading Procedure for Aequorin

We used a modification of our recently reported microinjection technique. An aequorin-loading solution composed of 2.0 mg/mL aequorin, 154 mmol/L NaCl, 5.4 mmol/L KCl, 1.0 mmol/L MgCl2, 12 mmol/L HEPES, 11 mmol/L dextrose, and 0.1 mmol/L EDTA (pH 7.40±0.02) was injected into the interstitium of the epicardium, just beneath the epicardium, with a low-resistance glass micropipette. In toto, 5 to 10 μL of the aequorin-loading solution was injected within 3 minutes into a localized apical region of ≈3x3-mm diameter. The loading area was positioned in the center of the photocathode (1-in diameter). We carefully selected avascular regions for loading to keep myocytes in contact with aequorin as long as possible. Modification of [Ca2+]i or coronary perfusion pressure was not necessary for successful loading.

Signal Recording

Aequorin light signals were recorded from the photomultiplier as anodal current. The dark current was set at zero with the shutter closed. The anodal current was filtered at a time constant of 0.01 second with a variable-analog amplifying device (Fjestad Electronics). The light signal, LV isovolumetric pressure, coronary perfusion pressure, and electrocardiographic signals were then simultaneously recorded both on magnetic tape (A.R. Vetter Co) and on chart strip paper (Gould ES1000).

Calibration of Aequorin Light Signals

The calibration procedure used for conversion of light signals to quantitative [Ca2+]i levels has been described previously. This procedure involves normalization of the aequorin light at each phase of the experiment by the amount of active aequorin in the preparation and then conversion of the normalized light signal to a [Ca2+]i value by use of an in vitro calibration curve. At the end of each experiment, the heart was perfused with a 4% solution of the detergent Triton X-100 in 50 mmol/L CaCl2 at a constant flow rate and 34°C. Within 2 minutes, the myocytes were rendered hyperpermeable and discharged all their remaining aequorin, which quickly reacted with the saturating Ca2+ in the perfusate. The amount of active aequorin remaining in the preparation (Lmax in vivo) at a particular point during the experiment was estimated as described previously. For conversion of fractional luminescence (L/Lmax) into a quantitative [Ca2+]i, a calibration curve was determined in vitro, and then the relation between [Ca2+]i and fractional luminescence was fitted to the following formula:

\[ L/L_{max} = \left[1 + (K_r[K_a][Ca^{2+}])/(1 + K_r + (K_r[Ca^{2+}]))\right] \]

By use of a nonlinear regression analysis with iteration methodology, we obtained the values of two constants: K_r = 4.5×10^(-4) (mol/L) and K_a = 130.0. This formula was used for conversion of fractional luminescence to [Ca2+]i throughout the present study.

Determination of Ca2+ Responsiveness

To determine the responsiveness of the myofilaments to Ca2+, we averaged successive [Ca2+]i transients for a constant period in each stage and compared the relations between peak [Ca2+]i values with peak LV pressures. We also used postextrastolic potentiation to plot the peak [Ca2+]i-LVP curve on a beat-to-beat basis.

Experimental Protocols

In all experiments, after LV pressures and [Ca2+]i signals became steady, the intracellular Ca2+ transient was recorded for 5 minutes, and Ca2+ responsiveness was determined during control perfusion (141 mmol/L Na+, pH 7.4) and at 20 minutes of reperfusion after 2 to 3 minutes of no-flow ischemia. The experiments were divided into 5 protocols: (1) nonischemic control (n=8), (2) unmodified reperfusion (141 mmol/L Na+, pH 7.40, n=17), (3) acidic reperfusion (141 mmol/L Na+, pH 6.60, n=7), (4) hypernatremic reperfusion (149 and 157 mmol/L Na+, pH 7.40, n=7 and 5, respectively), and (5) hyperosmotic reperfusion (141 mmol/L Na+ + 30 mmol/L mannitol, pH 7.40, n=4). For the unmodified protocol, hearts were perfused with unmodified control blood throughout the reperfusion. For the acidic protocol, acidic blood was produced by increasing the PCO2 (respiratory acidosis). Acidemic reperfusion was started immediately after the release of total occlusion of the perfusion line and maintained for 3 minutes. Thereafter, we gradually restored pH to 7.4 to prevent activation of Na+−H+ exchange by the induced difference of [H+]i and [H+], during recovery of the pH2 from acidosis. In four hearts, we verified that transient acidity itself did not produce persistent changes in function. Wallert and Fröhlich showed activation of Na+−H+ exchange by low pH in isolated myocytes from rat hearts. The activation of Na+−H+ exchange (pH, 6.55) is inhibited by >50% after lowering pH, (from 7.4
to 6.6). After 3 minutes of global ischemia, pH is estimated to be ~6.6. Therefore, we expect at least 50% inhibition of Na⁺-H⁺ exchange by our protocol. In addition, the Ca²⁺-aequorin luminescence response relation has been shown not to be significantly affected by pH changes between 7.4 and 6.6. For the hypernatremic protocol, hearts were initially perfused with unmodified perfusate after global ischemia. Within 1 minute of reperfusion, we started arterial infusion of hypernatremic perfusate (2.5 or 5 mmol/L NaCl per minute) for 4 minutes; [Na⁺], reached 155 or 169 mmol/L, respectively, in this period, but the measured final concentration was 149 or 157 mmol/L, respectively, at 20 minutes of reperfusion. The $K_m$ value of Na⁺-H⁺ exchange for extracellular Na⁺ is estimated to be much lower (5 to 40 mmol/L) than the $K_m$ value of Na⁺-Ca²⁺ exchange for extracellular Na⁺ (87.5 mmol/L by Reference 27). This may explain the relative insensitivity of Na⁺-H⁺ exchange to increased extracellular Na⁺. At peak [Na⁺], we expect that the “orthodox mode” of Na⁺-Ca²⁺ exchange (Ca²⁺ efflux) is activated by ~30% (extracellular Na⁺, 155 mmol/L) and 50% (extracellular Na⁺, 167 mmol/L) compared with that produced by unmodified reperfusion (extracellular Na⁺, 141 mmol/L). We will discuss the detailed kinetics of Na⁺-Ca²⁺ exchange in “Discussion.” We also verified that hypernatremia itself did not produce persistent changes in function in three hearts and that hyperosmolality (produced by mannitol) did not substantially change the recovery of LV function in four hearts.

In preliminary studies, we varied the duration of global ischemia (1.5 to 3 minutes) and the pacing rate (1.5 to 2.5 Hz) to test the responses of [Ca²⁺], during reperfusion. We found that neither 2 minutes of global ischemia nor pacing at <2 Hz produced functional damage. We compared the extent of the [Ca²⁺] increase and recovery of LV pressure at every 5-minute sampling point but could not find a significant difference between reperfusion after 2 minutes (n=7) and 3 minutes (n=10) of unmodified global ischemia.

We tried, at most, two periods of global ischemia in each dog heart, but to avoid the possibility that results from the first ischemia might differ from those of the second ischemia by a preconditioning effect, we waited more than 2 hours before the second ischemia. For unpaired comparison, we randomized and balanced the number of the experiments involving a first or second ischemia.

**Statistics**

Results were analyzed by Student’s $t$ test. Data are expressed as mean±SEM. Statistical analysis was performed with paired or unpaired $t$ tests and ANOVA to determine the relations between the maximum increase of diastolic [Ca²⁺], or systolic [Ca²⁺], during reperfusion and mechanical dysfunction 20 minutes after reperfusion. A value of $P<.05$ was considered significant.
Results

Functional Recovery and Intracellular Ca\(^{2+}\) During Reperfusion

Unmodified Reperfusion

The effects of ischemia and reperfusion were studied in 17 of the hearts. During 3 minutes of ischemia, systolic pressure development was rapidly and severely depressed to 17±2% of preischemic values with a significant increase of [Ca\(^{2+}\)]\(_i\), when ischemic conditions were produced by total occlusion of the perfusion line at the descending aorta. The LV diastolic pressure level also decreased to 66±7% when coronary perfusion was interrupted (“turgor effect”), and no increase in LV diastolic pressure was observed during the subsequent period of ischemia. After 2 to 3 minutes of no-flow ischemia, hearts were reperfused with the same oxygenated mixture of physiological salt solution plus blood, as was used for preischemic coronary perfusion. Both systolic and diastolic LV pressure showed initial increases just after reperfusion, with corresponding increases of systolic and diastolic [Ca\(^{2+}\)]\(_i\) (Fig 1, top). The increases of both systolic [Ca\(^{2+}\)]\(_i\), (up to 2 μmol/L) and diastolic [Ca\(^{2+}\)]\(_i\), (up to 1.5 μmol/L) usually occurred during this phase (Fig 1, bottom). The duration of this increase was ≈3 minutes, depending on the peak value of [Ca\(^{2+}\)]\(_i\), but was sustained up to 5 minutes of reperfusion in some cases. Higher peaks of [Ca\(^{2+}\)]\(_i\), elevation were associated with longer durations for the recovery of [Ca\(^{2+}\)]\(_i\), to preischemic values.

This [Ca\(^{2+}\)]\(_i\), elevation during reperfusion was accompanied by ventricular arrhythmias in >80% of the hearts. The initial increase of LV developed pressure during reperfusion was followed by a decrease that stabilized at 20 minutes of reperfusion to a level of 80% (n=17) of that before ischemia (Fig 2). LV developed pressure at 20 minutes of reperfusion was significantly lower than that in the nonischemic control condition.

Acidemic Reperfusion

With acidemic reperfusion, both systolic and diastolic LV pressures were severely depressed, but the increase in [Ca\(^{2+}\)]\(_i\), was much less than that with unmodified control perfusate (Fig 3, top and middle). Because more severe acidemia (pH <6.6) made systolic [Ca\(^{2+}\)]\(_i\), rise to the 2 μmol/L range, we maintained blood pH ≥6.6. During acidemic reperfusion, systolic [Ca\(^{2+}\)]\(_i\), rose to 1.24±0.06 μmol/L and diastolic [Ca\(^{2+}\)]\(_i\), to 0.65±0.14 μmol/L (Fig 4). After 3 minutes of acidemic reperfusion, we gradually restored pHe to 7.4. LV developed pressure slowly recovered within 20 minutes to 93±3% (n=7) of that before ischemia. This recovery was significantly better than that obtained with unmodified reperfusate (unpaired t test, P<.01) (Fig 3, bottom left). Systolic [Ca\(^{2+}\)]\(_i\), returned to the level before ischemia after 20 minutes of reperfusion. We were careful to control the pH of the perfusate when we performed the 20-minute measurement because pH changes may alter the sensitivity of the myofilaments to Ca\(^{2+}\) and affect intracellular Ca\(^{2+}\) modulation. Transient acidity itself or sudden recovery from acidosis may produce persistent changes or rebound in LV function by activating Na\(^+\)-H\(^+\) exchange. The hearts were perfused with acidemic blood as shown in Fig 3, bottom right, but were never made ischemic (n=4). Although low pH (pH 6.6) depressed LV function, only a slight decrease in LV developed pressure was observed at 15 minutes after returning to unmodified control blood. This time course of recovery is consistent with other reports.\(^{10,29}\)

Hypernatremic Reperfusion

We infused hypernatremic perfusate (2.5 or 5 mmol/L NaCl per minute) for 4 minutes, starting within 1 minute of reperfusion; the concentration of Na\(^+\) after 20 minutes of reperfusion was 149 or 157 mmol/L, respectively (Fig 5, top). The initial increase in diastolic LV pressure was slightly (not significantly) attenuated, and the increase in [Ca\(^{2+}\)]\(_i\), was much less than that with unmodified perfusate (Fig 4). LV developed pressure gradually recovered within 20 minutes to 89±2% (n=7) (with 149 mmol/L of hypernatremia) and 91±2% (n=5) (with 157 mmol/L) of that before ischemia (Fig 5, middle); both recoveries were significantly greater than that obtained with unmodified reperfusate (P<.05 by unpaired t test; n=7 and n=5, respectively). Systolic [Ca\(^{2+}\)]\(_i\), returned to the level before ischemia after 20 minutes of reperfusion. We verified that hypernatremia without ischemia does not produce any persistent change in LV function (Fig 5, bottom left). Hearts were perfused with hypernatremic perfusate (5 mmol/L NaCl per minute) for 4 minutes but were never made ischemic (n=3) (Fig 5, bottom left). Although hypernatremia slightly depressed LV function during NaCl infusion, LV developed pressure recovered completely within 10 minutes after infusion. To exclude the possibility that hyperosmolarity rather than hypernatremia may improve recovery in LV function, we infused mannitol at the rate of 7.5 mmol/L per minute for 4 minutes after ischemia at the same times used for the NaCl infusion (Fig 5, middle). Mannitol infusion produced the same amount of hyperosmolarity as produced by NaCl infusion. Mannitol infusion decreased LV developed pressure during early reperfusion; however, LV function at 20 minutes of reperfusion was compatible with unmodified ischemia (82±3%, n=4). Recoveries with hypernatremia (149 and 157 mmol/L Na\(^+\)) were significantly greater than those obtained with hyperosmotic reperfusate (P<.05 by unpaired t test; n=7 and n=5, respectively) (Fig 5, middle).

![Graph showing left ventricular developed pressure (DP) (mean±SEM) as a function of time. Ischemia(+) indicates results from the group with unmodified reperfusion after 3 minutes of global ischemia; ischemia(-), results from the nonischemic control group. Values are compared with values from nonischemic control groups by unpaired t test (**P<.01).](http://circres.ahajournals.org/DownloadedFrom)
**Fig 3.** Top, Representative recording showing the changes in [Ca²⁺]ᵢ and left ventricular (LV) pressure during and after global ischemia with acidemic reperfusion (pH 6.60 for the first 3 minutes of reperfusion by increasing Paco₂, then gradual return of pH to 7.40 within 15 minutes). The experiment shows markedly depressed systolic and diastolic LV pressure during acidemic reperfusion and slight increases of systolic and diastolic [Ca²⁺]ᵢ but no large rise (>1 µmol/L) in [Ca²⁺]ᵢ during reperfusion as occurred in Fig 1. CPP indicates coronary perfusion pressure. Middle, Averaged intracellular Ca²⁺ transient and LV pressure from the same experiment. Bottom left, Graph showing LV developed pressure (DP) (mean±SEM) as a function of time. Results are from the acidemic reperfusion group (n=7) after 3 minutes of global ischemia. LV DP recovered to 93±3% by 20 minutes of reperfusion. Bottom right, Graph showing results from acidemic group without global ischemia (n=4), which indicate only a slight decrease in LV DP 15 minutes after acidemia. Values are compared with values before acidemia by paired t test (**P<.01, *P<.05).
Ca\(^{2+}\) Responsiveness Before and After Global Ischemia and Its Modulation by Acidemic and Hypernatremic Reperfusion

**Unmodified Reperfusion**

With unmodified reperfusion, peak [Ca\(^{2+}\)] and diastolic [Ca\(^{2+}\)] at 20 minutes of reperfusion did not show significant differences from those values before ischemia (Table 1): 0.89±0.05 versus 0.88±0.04 \(\mu\text{mol/L}\) (systolic) and 0.31±0.03 versus 0.29±0.04 \(\mu\text{mol/L}\) (diastolic). Combined with the significant decrease of LV systolic and developed pressure, these results suggest decreased responsiveness of the myofilaments to intracellular Ca\(^{2+}\) and a downward shift of the peak [Ca\(^{2+}\)]–LV pressure curve. This analysis is also supported by the beat-to-beat relation between peak [Ca\(^{2+}\)] and peak LV pressure during postextrasystolic potentiation (Fig 6, top left).

**Acidemic and Hypernatremic Reperfusion**

Averaged values of peak [Ca\(^{2+}\)] and diastolic [Ca\(^{2+}\)] did not show significant differences between those before ischemia and at 20 minutes of reperfusion with either acidemic or hypernatremic reperfusion (Table 1). Data from averaged peak [Ca\(^{2+}\)] and peak LV pressure and data from the beat-to-beat relation between peak [Ca\(^{2+}\)] and peak LV pressure show that acidemic and hypernatremic reperfusion largely prevented the decreased responsiveness of the myofilaments to intracellular Ca\(^{2+}\) seen with control reperfusion (Fig 6, top right and bottom left).

**Transient Systolic and Diastolic [Ca\(^{2+}\)], Increase During Reperfusion as a Cause of Myocardial Dysfunction After Reperfusion**

A transient increase in [Ca\(^{2+}\)] occurs during early reperfusion, and we hypothesized that this might be the cause of postischemic myocardial dysfunction. Therefore, we plotted the percent decrease of LV developed pressure at 20 minutes of reperfusion versus the maximum increase of diastolic [Ca\(^{2+}\)], (Fig 7) and systolic [Ca\(^{2+}\)], during reperfusion. These two factors showed a significant correlation with the percent decrease of LV developed pressure: \(r=-0.73\) (\(P<.01\) versus diastolic [Ca\(^{2+}\)]) and \(r=-0.61\) (\(P<.01\) versus systolic [Ca\(^{2+}\)]). In this figure, we can clearly discriminate the points with interventions from the points without interventions. Unmodified control data here include different rates of pacing and different durations of global ischemia. With acidemic reperfusion, systolic [Ca\(^{2+}\)] increased prominently with pH close to 6.6, but this increase was not followed by postischemic dysfunction, which may explain a rather poor correlation between systolic [Ca\(^{2+}\)] and functional decay.

**Discussion**

There are several important conclusions that can be drawn from the present study. First, we found that postischemic dysfunction after short-term global ischemia is associated with decreased responsiveness of the contractile elements to intracellular Ca\(^{2+}\). Second, the decreased responsiveness may be caused by the intracellular diastolic (and systolic) [Ca\(^{2+}\)], rise during early reperfusion. Third, this early [Ca\(^{2+}\)], rise was ameliorated by extracellular acidemia (pH 6.6) or hypernatremia (149 or 157 mmol/L Na\(^{+}\)) during early reperfusion, and these interventions restored the responsiveness of the contractile elements to intracellular Ca\(^{2+}\), probably by preventing the increase in [Ca\(^{2+}\)], during early reperfusion without changing postischemic [Ca\(^{2+}\)]. Fourth, the protective effects of extracellular acidemia and hypernatremia support the hypothesis that the Ca\(^{2+}\) rise during early reperfusion is caused by Na\(^{+}\)-H\(^{+}\) and Na\(^{+}\)-Ca\(^{2+}\) exchange (or Ca\(^{2+}\)-H\(^{+}\) interaction).

Many studies have demonstrated an accumulation of Ca\(^{2+}\) during reperfusion after global ischemia,\(^1\)\(^-\)\(^9\),\(^14\) the attenuating effect of acidosis on this Ca\(^{2+}\) gain,\(^13\) and improved functional recovery with acidosis.\(^10\) However, some studies showed a discrepancy between acidic attenuation of the increase in [Ca\(^{2+}\)], and functional recovery.\(^13\) So far, a direct relation between acidic attenuation of Ca\(^{2+}\) accumulation and improved functional recovery has not been established. A possible explanation for the apparently contradictory effects of acidosis could be that models may include both reversible ("stunned") and irreversible changes (cell death) because of the prolonged period of ischemia required to demonstrate a significant Ca\(^{2+}\) increase. Smith and Allen\(^30\) suggested a threshold of [Ca\(^{2+}\)], rise during metabolic inhibition beyond which no recovery may occur (2.5 \(\mu\text{mol/L}\)). To avoid these problems, we used
short-term (2 to 3 minutes) global ischemia to produce sustained, but ultimately reversible, effects on the heart.

Using isolated isovolumic dog hearts perfused with blood, we recently developed a new method that provides a qualitative and quantitative estimate of \([\text{Ca}^{2+}]_i\), on a beat-to-beat basis under control and pathophysiological conditions.\(^5\) Our value of \([\text{Ca}^{2+}]_i\) (systolic \([\text{Ca}^{2+}]_i\)/diastolic \([\text{Ca}^{2+}]_i\), 0.87±0.04/0.28±0.10 \(\mu\)mol/L) (Table 2) was consistent with those values reported with the other available techniques and different models, including data reported in the acouquin-loaded ferret heart (0.86±0.11/0.33±0.04 \(\mu\)mol/L),\(^6\) indo 1–loaded rabbit heart (0.609±0.029/0.315±0.025 \(\mu\)mol/L),\(^7\) and data recorded by NMR in the 5F-BAPTA–loaded ferret heart (0.70/0.16 \(\mu\)mol/L).\(^8\) Of interest, values of \([\text{Ca}^{2+}]_i\), assessed by NMR techniques are somewhat lower, perhaps because of the \(\text{Ca}^{2+}\)-buffering properties of 5F-BAPTA, the indicator used in these studies.\(^9\)

**Postischemic Dysfunction Is Caused Not by Abnormal \(\text{Ca}^{2+}\) Modulation but by Decreased Responsiveness of the Contractile Elements**

A rise in \([\text{Ca}^{2+}]_i\), during ischemia has been described in various settings including our previous studies,\(^8,15,16,32\) and the mechanical dysfunction during ischemia is attributed to the accumulation of inorganic phosphate and \(H^+\) as oxidative phosphorylation ceases and high-energy phosphate stores are depleted.\(^11,33\) These studies have largely focused on the mechanical dysfunction during ischemia or hypoxia but not during the reperfu-
TABLE 1. Averaged \([Ca^{2+}]\), and Left Ventricular Pressure Before and After Ischemia and Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>LVP (Systolic/Diastolic), mm Hg</th>
<th>([Ca^{2+}]), (Systolic/Diastolic), (\mu)mol/L</th>
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</thead>
<tbody>
<tr>
<td><strong>Unmodified reperfusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>89±2/10±2</td>
<td>0.88±0.04/0.29±0.04</td>
</tr>
<tr>
<td>Reperfusion (20 minutes)</td>
<td>74±2/11±2</td>
<td>0.89±0.05/0.31±0.03 (n=15)</td>
</tr>
<tr>
<td><strong>Acidemic reperfusion</strong></td>
<td></td>
<td></td>
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<tr>
<td>Preischemia</td>
<td>92±3/6±1</td>
<td>0.81±0.06/0.23±0.04</td>
</tr>
<tr>
<td>Reperfusion (20 minutes)</td>
<td>86±2/5±1</td>
<td>0.83±0.06/0.22±0.04 (n=6)</td>
</tr>
<tr>
<td><strong>Hypernatremic reperfusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>84±7/14±4</td>
<td>0.86±0.11/0.20±0.06</td>
</tr>
<tr>
<td>Reperfusion (20 minutes)</td>
<td>75±6/13±4</td>
<td>0.82±0.11/0.23±0.06 (n=4)</td>
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</table>

LVP indicates left ventricular pressure. Values are mean±SEM. *P<.01 vs corresponding preischemic value by paired t test.

In our experiments, LV pressure development recovered immediately (at 3 minutes) on reperfusion with unmodified perfusate after a brief period of ischemia (Fig 2), which may reflect a rapid clearance of the metabolites responsible for the ischemic LV dysfunction. Therefore, we suspect that the late dysfunction seen at 20 minutes after reperfusion may be caused by a different mechanism from ischemic/hypoxic dysfunction.

We have previously shown that decreased responsiveness \((F_{max}\) in Reference 12) of the myofilaments to \(Ca^{2+}\) is the primary cause of mechanical dysfunction at 20 minutes after 15 minutes of global ischemia in buffer-perfused ferret hearts. In the present study, we show a significant decrease of LV pressure without a decrease in peak \([Ca^{2+}]\), at 20 minutes of reperfusion after global ischemia in dog hearts perfused with blood (Table 1), which confirmed the result with ferret hearts. Although the ideal method of assessing \(Ca^{2+}\)-myofilament interaction is to measure the pressure-[\(Ca^{2+}\)] relation at steady state with tetanus after perfusion of ryanodine, our simpler method nonetheless demonstrates a downward shift of the relation between \([Ca^{2+}]\) and LV pressure or decreased responsiveness \((F_{max})\) of the myofilaments to \([Ca^{2+}]\) (Fig 6, top left; Table 1), as is the case with ferret hearts. Moreover, no significant differences in slopes were found, suggesting that myofilament desensitization did not occur. Acidemic and hypernatremic reperfusion also did not change the averaged value of peak \([Ca^{2+}]\), at 20 minutes of reperfusion after ischemia but did improve the functional recovery. This suggests that these two interventions may improve responsiveness of the myofilaments to \(Ca^{2+}\) after ischemia (Fig 6).

![Graphs showing relation between peak \([Ca^{2+}]\) and LV pressure](http://circres.ahajournals.org/)

**Fig 6.** Top left, Plot showing the relation between peak \([Ca^{2+}]\), and peak left ventricular pressure (LVP) on a beat-to-beat basis during postextrasystolic potentiation before (○) and 20 minutes after (•) global ischemia with unmodified reperfusion. Regression lines are for data before (dashed line) and 20 minutes after (solid line) global ischemia. Top right, Similar plot for data from acidemic reperfusion protocol. Bottom left, Plot for data from hypernatremic reperfusion protocol.
Relation Between \([\text{Ca}^{2+}]_{\text{i}}\) Increase and Decreased Postischemic Responsiveness of the Contractile Elements to \(\text{Ca}^{2+}\)

In the setting of prolonged hypoxic contractile dysfunction, Koretzune and Marban\(^{11}\) demonstrated a rise in intracellular phosphate concentration and a fall in pH, both of which can interfere with \(\text{Ca}^{2+}\)-induced force development by the myofilaments. We cannot apply this result to postischemic dysfunction because metabolites are probably washed out after 20 minutes of reperfusion. Kitakaze and Marban showed a good recovery in intracellular inorganic phosphate and pH at 30 minutes of reperfusion after 15 minutes of global ischemia in the ferret heart.\(^{10}\) We hypothesize that the possible cause of this decreased responsiveness of the myofilaments to \(\text{Ca}^{2+}\) during late reperfusion after ischemia is caused by the \([\text{Ca}^{2+}]_{\text{i}}\) increase not during ischemia but during the early reperfusion period, because the increase in \([\text{Ca}^{2+}]_{\text{i}}\) during the early reperfusion period is higher than that during ischemia. We reported increases of peak systolic and diastolic \([\text{Ca}^{2+}]_{\text{i}}\) during short-term ischemia as 1.04±0.07 and 0.39±0.14 \(\mu\text{mol/L}\), respectively,\(^{15}\) whereas those during reperfusion with unmodified perfusate were 1.67±0.11

![Graph showing the relation between the maximum diastolic \([\text{Ca}^{2+}]_{\text{i}}\) and change in LV dev P.](http://circres.ahajournals.org/)

\[ Y = -9.344X - 4.020 \]
\[ r = 0.728 \]
\[ p < 0.01 \]

**TABLE 2. Aequorin Signal Intensity and Estimated \([\text{Ca}^{2+}]_{\text{i}}\).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L_{\text{max}}), (\mu\text{A})</td>
<td>84.4±12.1</td>
</tr>
<tr>
<td>Peak (\text{Ca}^{2+})</td>
<td></td>
</tr>
<tr>
<td>(L), (nA)</td>
<td>3.6±0.3</td>
</tr>
<tr>
<td>(L/L_{\text{max}}) ((10^{-9}))</td>
<td>5.1±2.3</td>
</tr>
<tr>
<td>([\text{Ca}^{2+}]_{\text{i}}) ((10^{-9}))</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Diastolic (\text{Ca}^{2+})</td>
<td></td>
</tr>
<tr>
<td>(L), (nA)</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>(L/L_{\text{max}}) ((10^{-9}))</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>([\text{Ca}^{2+}]_{\text{i}}) ((10^{-9}))</td>
<td>0.3±0.1</td>
</tr>
</tbody>
</table>

\(L_{\text{max}}\) indicates maximal luminescence (L). Values are mean±SD. In each experiment, peak \([\text{Ca}^{2+}]_{\text{i}}\) and resting \([\text{Ca}^{2+}]_{\text{i}}\) values were estimated by averaging 10 serial beats at steady state, while hearts were paced at 120±5 beats per minute at 34°C. Experiments were terminated by perfusion with 4% Triton X-100 in 50 mmol/L CaCl₂. See text for details.

and 1.37±0.11 \(\mu\text{mol/L}\), respectively (Fig 1). Tani and Neely\(^{14}\) suggested that \(\text{Ca}^{2+}\) accumulation during early reperfusion may contribute to failure of functional recovery. Our data also clearly show that mechanical dysfunction at 20 minutes of reperfusion after ischemia was well correlated with the increase of diastolic \([\text{Ca}^{2+}]_{\text{i}}\) (but to a lesser extent, systolic \([\text{Ca}^{2+}]_{\text{i}}\)) during early reperfusion. The short duration of ischemia may explain the limited \([\text{Ca}^{2+}]_{\text{i}}\) increase during ischemia and its poor correlation with postischemic dysfunction.

We propose that the degree of elevation in \([\text{Ca}^{2+}]_{\text{i}}\) during early reperfusion, like that during prolonged ischemia, could have an effect on ultimate recovery by influencing the extent of activation of various \(\text{Ca}^{2+}\)-dependent proteases and phospholipases, which may alter the responsiveness of the contractile elements to \(\text{Ca}^{2+}\).\(^{34-36}\) This enzymatic activation may be caused primarily by the rise in diastolic \([\text{Ca}^{2+}]_{\text{i}}\) and to a less extent by the rise in systolic \([\text{Ca}^{2+}]_{\text{i}}\). The rise in diastolic \([\text{Ca}^{2+}]_{\text{i}}\) is thought to be potentially important, since it may indicate saturation of the sarcoplasmic reticulum with \(\text{Ca}^{2+}\).

**Role of \(\text{Na}^+\)-\(\text{H}^+\) Exchange and \(\text{Na}^+\)-\(\text{Ca}^{2+}\) Exchange in \(\text{Ca}^{2+}\) Overload**

Tani and Neely\(^{14}\) referred to the hypothesis of Ladzunski et al.,\(^{26}\) who described the mechanism of \(\text{Ca}^{2+}\) overload during reperfusion, and showed consistent results by radioisotope studies. Namely, during ischemia accumulated \(\text{H}^+\) is slowly exchanged for extracellular \(\text{Na}^+\) because of extracellular acidosis, but during the first few minutes of reperfusion,\(^{37-39}\) intracellular \(\text{H}^+\) is rapidly exchanged for extracellular \(\text{Na}^+\) with normal \(\text{pH}_{\text{i}}\). Increased cytosolic \(\text{Na}^+\) therefore causes excessive \(\text{Ca}^{2+}\) uptake. Our data concerning acidemic and hypernatrexic prevention of the increase in intracellular \(\text{Ca}^{2+}\) also support this hypothesis. There is a species difference in the accumulation of \(\text{Na}^+\) and \(\text{H}^+\) during ischemia based on \(\text{Na}^+,\text{K}^+\)-ATPase activity\(^{40}\) and in the \(\text{Na}^+\)-\(\text{H}^+\) exchange activity.\(^{41}\) \(\text{Na}^+\)-NMR studies show a small increase of \([\text{Na}^+]_{\text{i}}\), within 5 minutes of global ischemia in ferret hearts.\(^{39}\) To the contrary, intracellular \(\text{H}^+\) accumulation occurs even within 2 minutes in rat hearts.\(^{29}\) With unmodified reperfusion, \(\text{pH}_{\text{i}}\) rapidly returns to 7.4; then an intracellular-extracellular \(\text{H}^+\) gradient is produced, which highly activates \(\text{Na}^+\)-\(\text{H}^+\) exchange\(^{24}\) during the first minutes of reperfusion. As shown by Wallert and Frohlich,\(^{24}\) at \(\text{pH} = 6.55\), lowering the \(\text{pH}_{\text{i}}\) from 7.4 to 6.6 reduces the activity of \(\text{Na}^+\)-\(\text{H}^+\) exchange by \(>50\%\). Therefore, extracellular acidemia during this period may ameliorate the intracellular \(\text{Na}^+\) accumulation, which may subsequently cause intracellular \(\text{Ca}^{2+}\) accumulation (\(\text{Ca}^{2+}\) overload) via \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchange. Acidosis also modulates \(\text{Ca}^{2+}\) overload by several mechanisms: it inhibits \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchange directly,\(^{42,43}\) and via blockade of the \(\text{L}\)-type \(\text{Ca}^{2+}\) channel,\(^{44-46}\) it inhibits \(\text{Ca}^{2+}\) release from the sarcoplasmic reticulum,\(^{47,48}\) and inhibits binding of \(\text{Ca}^{2+}\) to troponin C\(^{48-50}\) and to sarcoslemmal phospholipids.\(^{51}\) Acidosis is also known to increase \([\text{Ca}^{2+}]_{\text{i}}\), when \(\text{pH}_{\text{i}}\) is close to 6.6, primarily by reducing the buffering capacity of \([\text{Ca}^{2+}]_{\text{i}}\), by intracellular organelles.\(^{52-55}\) To avoid the additional effects caused by this increase in \([\text{Ca}^{2+}]_{\text{i}}\), we limited the duration and extent of acidosis in our experiments. Judging from the method of producing acidemia, \(\text{pH}_{\text{i}}\),
Fig 8. Representative recording showing averaged intracellular \( \text{Ca}^{2+} \) transient and left ventricular (LV) developed pressure before ischemia (solid line) and at 0 to 1.5 minutes (dashed line) of global ischemia in the blood-perfused dog heart (A) and in the Krebs’ buffer-perfused dog heart (B).

and its duration, we estimate that pH, is 6.8 to 7.0.\(^{55}\) In this range of pH, the change in intracellular \( \text{Ca}^{2+} \) handling (ie, \( \text{Ca}^{2+} \) release from the sarcoplasmic reticulum or uptake by the sarcoplasmic reticulum) may not be so significant.\(^{55}\)

Recently Vandenberg et al\(^{56}\) showed that Na\(^+\)-coupled acid extrusion contributes just 35% of total H\(^+\) efflux to the recovery of pH, after myocardial ischemia and that lactate-coupled acid extrusion contributes more. They suggested that Na\(^+\)-H\(^+\) exchange may not be adequate to cause a substantial rise in intracellular Ca\(^{2+} \). Moreover, Holmberg et al\(^{37}\) showed inappropriate opening of the Ca\(^{2+} \) channels after oxidant stress. From these findings, we suggest the following modification of the hypothesis of Ladzunski et al\(^{26}\): Instead of assuming that activation of Na\(^+\)-H\(^+\) exchange and Na\(^+\)-Ca\(^{2+} \) exchange may cause excessive Ca\(^{2+} \) accumulation, we hypothesize that Ca\(^{2+} \) release (probably caused by inappropriate opening of the sarcoplasmic reticulum Ca\(^{2+} \)-release channel with a subsequent increase in trigger Ca\(^{2+} \) entry) and therefore a rise in [Ca\(^{2+} \)], may be maintained by an inability of the cell to expel excessive intracellular Ca\(^{2+} \) via Na\(^+\)-Ca\(^{2+} \) exchange, because a higher intracellular Na\(^+\) activity caused by the activation of Na\(^+\)-H\(^+\) exchange may inhibit the “orthodox mode” of Na\(^+\)-Ca\(^{2+} \) exchange (Ca\(^{2+} \) efflux). The findings of Vandenberg et al\(^{56}\) also support a relative insensitivity of Na\(^+\)-H\(^+\) exchange to an increased extracellular Na\(^+\) in our hypernatremic protocol.

**Kinetics of Na\(^+\)-Ca\(^{2+} \) Exchange**

Blaustein and Hodgkin\(^{58}\) demonstrated that in cyanide-poisoned squid axons the energy source for Na\(^+\)-Ca\(^{2+} \) exchange is the electrochemical potential gradient of both Na\(^+\) and Ca\(^{2+} \) across the membrane. The accumulated results indicated that the stoichiometry is most probably 3Na\(^+\):1Ca\(^{2+} \) and, consequently, electrogenic. Recently, Kimura et al\(^{27}\) isolated the membrane currents (\( I_{\text{NaCa}} \)) generated by Na\(^+\)-Ca\(^{2+} \) exchange in single cardiac myocytes using patch-clamp techniques. As described in the study of Kimura et al, we estimated the effect of the change in the [Na\(^+\)], on \( I_{\text{NaCa}} \) by the following equation:

\[
I_{\text{NaCa}} = k[(N_a)^3 \cdot \text{Ca}_{\text{exp}}((n-2)K E F / R T) - (N_a)^3 \cdot \text{Ca}_{\text{exp}}(-(n-2)(1-r)K E F / R T)]
\]

where \( k \) is a scaling factor that determines the magnitude of the current, \( N_a \) is intracellular Na\(^+\), \( \text{Ca}_o \) is extracellular Ca\(^{2+} \), \( n \) is the stoichiometry for Na\(^+\) and Ca\(^{2+} \), and \( r \) is a partition parameter used in the rate theory and represents the position of the energy barrier in the electrical field, which indicates the steepness of the voltage dependence of the current, E is voltage difference, F is Faraday’s constant, R is the gas constant, and \( T \) is absolute temperature. Extracellular Ca\(^{2+} \) was 1.5 mmol/L. If we use 3, 5.94 \( \mu \text{A} \) (mmol/L)\(^4 \) (\( \mu \text{F} \))\(^{-1} \), and 0.36 for \( n \), \( k \), and \( r \), respectively, we obtain the following equation:

\[
I_{\text{NaCa}} = 5.94 \times 10^{-5} \cdot [(N_a)^3 \cdot 1.5 \exp(0.36E F / R T) - (N_a)^3 \cdot \text{Ca}_{\text{exp}}(-0.6E F / R T)]
\]

If we incorporate 0.5 and 1\( \times \)10\(^{-3} \) mmol/L for the intracellular Ca\(^{2+} \) values from our experiment and 5 mmol/L for the intracellular Na\(^+\) values,\(^{59}\) we obtain (\( \mu \text{A}/\mu \text{F} \)) = -0.07 (-0.10 and -0.13) and -0.16 (-0.21 and -0.28) of the Na\(^+\)-Ca\(^{2+} \) exchange current at 0 mV of membrane potential with 141 mmol/L external Na\(^+\).
(155 and 169 mmol/L external Na+), where negative current means inward current. These values show =30% and 70% augmentation of Na+-Ca2+ exchange in the orthodox mode by our procedure of increasing the [Na+]o at the peak [Na+]t.

**Limitations of the Study**

There are several potential problems that could complicate the interpretation of our experimental results. First, in the present study, we assumed that the ionized 

[Mg2+]i = 1 mmol/L. During prolonged hypoxia or ischemia, [Mg2+]i would rise as ATP was consumed and interfere with the ability to make quantitative [Ca2+]i measurements during this period. To minimize these problems, we limited our experimental ischemia to a short period, during which time a significant decline in [ATP] would not be expected to occur. Second, our quantitative [Ca2+]i values during ischemia are somewhat smaller than those previously reported from our laboratory in ferret63,66 or rabbit15 hearts. This difference may be dependent on species or blood perfusion versus buffer reperfusion. Allen et al61 reported 210 to 220 milliseconds of APD90 (time to the 50% decline of the action potential) for ferrets paced at 1 Hz and 30°C; Krishnan et al62 reported 133.5 ± 9.7 milliseconds (epicardium) and 172.5 ± 4.8 milliseconds (endocardium) of APD90 for dogs paced at 2 Hz at 34°C. Action potential in ferrets may be to some extent longer than those in dogs. If this is the case, a longer action potential duration may facilitate Ca2+ influx by the L-type Ca2+ channel and Na+-Ca2+ exchange and reduce Ca2+ extrusion by Na+-Ca2+ exchange in diastole, which may cause more Ca2+ accumulation (Ca2+ overload). Moreover, sarcoplasmic reticulum Ca2+-loading conditions may be different in dogs and ferrets, which may be exacerbated by the aequorin-loading procedure (low Ca2+ method) in ferrets. Another possibility is that species difference in the activity of the ATP-sensitive K+ channel may explain the difference in the [Ca2+], modulation during ischemia.63 The ATP-sensitive K+ channel is known to be affected by intracellular ATP, ADP levels, and pH. Depending on the difference in the metabolic conditions, each species may show a different time course of the activation of the ATP-sensitive K+ channel and, thereby, different time courses in [Ca2+], during ischemia. To test the difference between blood perfusion and buffer perfusion, we replaced blood with Krebs’ solution in dog hearts. The diastolic [Ca2+]t rises during ischemia with buffer perfusion was greater than that with blood perfusion (Fig 8), and this result was qualitatively similar to the result from the ferret heart.16 Moreover, this result suggests that blood may protect hearts from ischemic Ca2+ overload. Third, the modulation of coronary flow during reperfusion by interventions may affect recovery. However, reactive hyperemia showed no significant differences among the three protocols. Fourth, the aequorin signal recorded in these experiments reflects [Ca2+], in a small region of myocytes located at the LV apex. It is reasonable to assume that performance of these cells is reflective of the heart as a whole, but this hypothesis will require further testing with localized loading in different regions of the heart, especially under conditions such as ischemia and postischemic recovery when endocardial versus epicardial oxygenation gradients are present. Fifth, our data showed a little variability of the systolic Ca2+ transient and Ca2+ oscillatory phenomena in diastole, partly because of the filtering conditions and partly because of the design of the light-collecting setups of the blood perfusion system, which requires placement of a photomultiplier tube in a light-tight box with consequent exposure to higher humidity and temperature than is standard for our small animal experiments. Because of higher temperature and humidity, the shot noise of the tube is increased. In the study shown in Fig 8, we used, with individual light signals, a chopping filter to eliminate spiky noise combined with a 50-Hz low-pass filter, which cannot completely eliminate diastolic fluctuation of intracellular Ca2+. We assume that this diastolic noise may be either a random background noise (which is eliminated by averaging 50 to 100 signals) or a spontaneous subthreshold Ca2+ release from the sarcoplasmic reticulum. For example, in Fig 3, top, the peak systolic intracellular Ca2+ transients represented by the peak of the gray tracings increase in amplitude during ischemia, and the diastolic noises (or Ca2+ oscillation) represented by the most dense part of tracings in [Ca2+]t also increase. The latter may imply an augmented Ca2+ release from the sarcoplasmic reticulum caused by Ca2+ overload. (An averaged or filtered diastolic [Ca2+]t, also slightly increases.) Sixth, our method for determining the responsiveness of the myofilaments to Ca2+ has a possible complication, eg, whether the population of cells from which light is measured will be a good representative of the population of cells from which tension is measured. To test the viability of myocytes loaded with aequorin by macroinjection, we isolated myocytes from ferret heart after loading, which showed no contractile differences from unloaded myocytes (J.X. Wang, personal communication). These data directly address the ability of our approach to produce indicator-loaded cells with preserved mechanical function. Seventh, the exact mechanism for aequorin-loading is not yet known; however, similar qualitative and quantitative intracellular Ca2+ transients were obtained in papillary muscles loaded by macroinjection17 and myocytes isolated from hearts loaded by the macroinjection approach.18 These, in turn, are the same as those obtained after direct microinjection into myocytes.19 The rationale behind our loading approach has been based on the assumption that we are able to produce reversible membrane hyperpermeability through exposure to solutions containing a low [Ca2+].64 However, hyperpermeability can be demonstrated in cardiac cells after a variety of mechanical and chemical treatments (see Reference 65 for review), and it is possible that the process of macroinjection itself may in some way contribute to loading. It is of interest to note that molecular biologists have recently been loading large DNA molecules into myocytes for expression by using a similar macroinjection approach. As with our macroinjection technique for aequorin, the exact mechanism behind gene loading remains to be delineated, but its success suggests the possible existence of specialized muscle cell transport systems for large molecules.65 Finally, the degree to which internal shortening may contribute to our observed results cannot be determined in a multicellular whole-heart preparation.
Clinical Implications

The present study may have unique clinical relevance because of the physiological nature of this large animal model perfused with blood, which includes leukocytes. First, even after a brief period of interruption of the coronary flow (2 to 3 minutes), Ca\(^{2+}\) overload may occur and affect mechanical function, probably without significant cell death. Second, this mechanical dysfunction can be attenuated by interventions during reperfusion: mildly acidic (pH 6.6) and/or hypernatrempic perfusate (149 or 157 mmol/L Na\(^+\)) and lower heart rates (<2 Hz). Although these interventions decrease myocardial function temporarily, they may be applied, for example, during perfusion after percutaneous transluminal coronary recanulation to attenuate mechanical dysfunction and arrhythmia.

In conclusion, the present study confirms and extends previous observations that there is a transient early rise in both systolic and diastolic [Ca\(^{2+}\)] during reperfusion after global ischemia, which may cause late postischemic desensitization of myofilaments to intracellular Ca\(^{2+}\) and postischemic dysfunction without changing intracellular Ca\(^{2+}\) handling. We also have presented data supporting the hypothesis that Ca\(^{2+}\) overload during reperfusion is caused by activation of Na\(^+\)-H\(^+\) and Na\(^+\)-Ca\(^{2+}\) exchange.

Acknowledgments

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