Calcium, Strontium, and Barium Movements during Ischemia and Reperfusion in Rabbit Ventricle

Implications for Myocardial Preservation

KENNETH I. SHINE, ANNIE M. DOUGLAS, AND NICHOLAS V. RICCHIUTI

SUMMARY Global ischemia was induced in isolated blood-perfused rabbit interventricular septa at 37°C beating at 72 beats/min. Uptake of $^{133}$BaCl$_2$ (0.85 μM) or $^{85}$SrCl$_2$ (0.4 μM) during perfusion with solutions containing 2.5 mM CaCl$_2$ was monitored by gamma probe. Since barium is not sequestered by sarcoplasmic reticulum, its uptake was used to monitor sarcolemmal function. The uptake of $^{133}$BaCl$_2$ increased from 21.1 ± 5.5 to 29.5 ± 8.2 pmol/min ($P < 0.05$) upon reperfusion after 30 minutes of ischemia and from 13 ± 2.9 to 29.3 ± 11.4 pmol/min ($P < 0.025$) after 60 minutes of ischemia. There was no change in $^{85}$Sr uptake until reperfusion after 60 minutes of ischemia when it increased from 8.5 ± 1.8 to 29.3 ± 11.4 pmol/min ($P < 0.025$). The effects of calcium upon recovery were assessed. Ten septa made ischemic for 60 minutes and reperfused with 1.5 mM CaCl$_2$ recovered 31.6 ± 9.1% of preischemia developed tension. Ten other septa reperfused with red cell perfusate containing 0.75 mM CaCl$_2$ for 5 minutes, then exposed to red cell perfusate containing 1.5 mM CaCl$_2$ recovered 67.1 ± 7.5% ($P < 0.05$) of preischemia developed tension. Improved recovery was shown after 20 minutes of ischemia, 77.4 ± 6.1% vs. 103.4 ± 7.8% ($P < 0.025$). Five septa reperfused with anoxic Tyrode’s solution containing 50 μM CaCl$_2$ after 15 minutes and 45 minutes of total ischemia showed no decrease in resting tension despite a progressive rise during ischemia. Resting tension upon reperfusion with red cell perfusate depended strongly upon perfusate calcium concentration. Impaired relaxation during ischemia had calcium-dependent and calcium-independent components. A mild sarcolemmal leak of $^{133}$Ba after 30 minutes progressed to severe disruption after 60 minutes of ischemia. Reperfusion with red cell perfusate containing 0.75 mM CaCl$_2$ for 5 minutes enhanced recovery. Ischemic injury can be significantly modified by the calcium content of the blood with which the myocardium is reperfused.

IN ADDITION to its central role in normal myocardial excitation-contraction coupling, calcium ion may have critical effects on the ability of heart muscle to recover from ischemia or hypoxia. Jennings and co-workers$^1$ convincingly demonstrated that reperfusion of ischemic canine ventricle was accompanied by accumulation of calcium and sodium and loss of potassium and magnesium. Defective sarcolemmal membrane function resulting in an increase in permeability to these ions seemed likely. The appearance of amorphous deposits in the mitochondria, with evidence for swelling and disruption of these organelles, suggested that mitochondrial membranes might suffer a similar insult during ischemia or that influx of calcium upon reperfusion might damage these structures. The precise relationship of these changes to mechanical dysfunction, including abnormalities of relaxation after reperfusion, was unclear. Although accumulation of cytoplasmic calcium would be expected to impair relaxation, the same degree of rigor also could result from an amount of adenosinetriphosphate (ATP) inadequate to allow crossbridge detachment.$^3$ Either phenomenon could account for the “stone heart” syndrome observed by cardiac surgeons.$^4$ Moreover, the accumulation of cellular calcium need not represent solely a defect in calcium influx. The magnitude of accumulation described by Shen and Jennings$^2$ also could be explained by a decrease in calcium efflux from the cell.

Our studies$^5,6$ indicated that increases in resting tension and failure to recover developed tension after reperfusion or reoxygenation of rabbit ventricle could occur although sarcolemmal membrane permeability and sodium-potassium pump function were preserved. Sodium-potassium pump function recovered during reperfusion after 40–45 minutes of global ischemia at 37°C. Longer periods of ischemia resulted in poor recovery of mechanical function and injury to sarcolemmal membrane function.$^5$ If an influx of calcium during reperfusion after ischemia produced significant cellular injury, reperfusion which exposed the heart to a reduced calcium concentration might have a protective effect. The
present studies examined the effects of varying extracellular calcium concentration during and after ischemia on isolated blood-perfused rabbit interventricular septa. The movements of $^{133}$Ba and $^{85}$Sr also were monitored during reperfusion after total ischemia. Since barium appears to enter the cell by a mechanism similar to that for calcium, but is not sequestered by the sarcoplasmic reticulum, $^{133}$Ba uptake of this cation primarily reflects changes in sarcolemmal membrane function. Strontium, on the other hand, is sequestered by sarcoplasmic reticulum. Its movements, then, more clearly parallel those of calcium ion.

## Methods

The experimental preparation was the isolated interventricular septum of 1.5- to 2.0-kg white male New Zealand rabbits perfused with blood according to the technique described by Shine et al. The rabbits were given 10 mg heparin intravenously, anesthetized with pentobarbital, 40 mg/kg, iv, and the heart was removed. The septal artery (a branch of the left coronary) was cannulated with a small polyethylene catheter and perfused at a constant rate by means of a Harvard pump. A triangular portion of the septum, with the perfusion cannula at its base, was suspended on a stand with the two lower corners held by clamps. The apex of the triangle was attached by a suture to a Statham UC4 transducer. The transducer recorded only that vector of tension developed along the axis of the transducer, but the proportion of total force represented by this vector remained constant throughout each experiment. A septum was used for this study if it was not sequestered by the sarcoplasmic reticulum, its movements, then, more clearly parallel those of calcium ion.

### Results

#### $^{133}$Ba Uptake after Ischemia

For Figure 1A a septum was labeled for 100 minutes with red cell perfusate containing 0.85 $\mu$M $^{133}$BaCl$_2$ and 2.5 mM CaCl$_2$. This concentration of BaCl$_2$ caused no observable effect on mechanical...
function. The uptake of $^{133}$BaCl$_2$ became linear after 25–30 minutes of perfusion, thereafter increasing with a gradual positive slope as previously described by Sanborn. Ischemia for 5 minutes caused a decline in tissue counts as blood dripped from the muscle but, upon reperfusion, the uptake rate returned to the extrapolated curve within 5 minutes and then continued along that curve. Three other septa which had been ischemic for 5 minutes and four septa which had been ischemic for 15 minutes showed a rate of $^{133}$Ba uptake during reperfusion not significantly different from the rate of preischemic uptake.

In Figure 1B, data from a similar experiment are shown for a muscle made ischemic for 30 minutes. In contrast to shorter periods of ischemia, the rate of increase in tissue counts was significantly more rapid during reperfusion than would have been expected from the extrapolation of the uptake curve obtained prior to ischemia. On reperfusion, eight of nine septa made ischemic for 30 minutes showed an increase in the rate of $^{133}$Ba uptake above the control rate. The average rates of $^{133}$Ba uptake increased by 33% from 21.1 ± 5.5 pmol/min before ischemia to 29.5 ± 8.2 pmol/min ($P < 0.05$) on reperfusion. All five muscles made ischemic for 60 minutes showed, on reperfusion, an increase in the rate of $^{133}$Ba uptake averaging 108%, from 13.7 ± 2.9 to 28.5 ± 5.7 pmol/min ($P < 0.05$) (Table 1).

As shown in Table 1, recovery of mechanical function was much less complete when ischemia was prolonged from 30 to 60 minutes. In a specific muscles, however, severe impairment of mechanical recovery could occur with only a relatively small increase in rate of $^{133}$Ba uptake. However, all muscles which showed a substantial increase in rate of $^{133}$Ba uptake also showed very poor mechanical recovery. These experiments revealed evidence for an increased net uptake of $^{133}$Ba on reperfusion after 30 minutes of global ischemia.

$^{85}$Sr Uptake after Ischemia

A series of experiments similar to those described for $^{133}$Ba was conducted using $^{85}$Sr. SrCl$_2$ was added to the red cell perfusate containing 2.5 mm CaCl$_2$. There was no observable mechanical effect during perfusion with 0.4 µg SrCl$_2$. The $^{85}$Sr uptake curves prior to ischemia were similar to those observed for $^{133}$Ba although the slopes were lower after 40–50 minutes of labeling (Fig. 2A).

In Figure 2A, a septum was labeled with $^{85}$Sr for 60 minutes, and ischemia was produced for 45 minutes. After the decline in radioactivity induced by loss of perfusate, reperfusion promptly restored radioactivity to the expected value and established a new uptake curve with a slope not different from the preischemia value. The new $^{85}$Sr uptake curve was 7% higher than the control. In eight of nine muscles made ischemic for 30 or 45 minutes, the rates of $^{85}$Sr uptake after reperfusion were unchanged or decreased in comparison to the preischemia uptakes.

In contrast to muscles made ischemic for 45 minutes, septa made ischemic for 60–70 minutes prior to reperfusion showed a striking increase in the rate of $^{85}$Sr uptake. In Figure 2B, a septum was made ischemic for 60 minutes after perfusion with $^{85}$Sr for 70 minutes. During ischemia, the tissue radioactivity declined. Upon reperfusion, the muscle showed a progressive rise in resting tension until severe

![Figure 1](https://example.com/fig1.png)

**Figure 1** $^{133}$Ba uptakes. A: 5 minutes of ischemia during $^{133}$Ba perfusion. B: 30 minutes of ischemia during $^{133}$Ba perfusion.

### Table 1 $^{133}$Ba Uptake before and after Ischemia

<table>
<thead>
<tr>
<th>Ischemia duration (min)</th>
<th>Control (pmol/min per kg dry wt)</th>
<th>Reperfusion (pmol/min per kg dry wt)</th>
<th>DT (% recovery)</th>
<th>RT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 (4)</td>
<td>18.4 ± 3.3</td>
<td>18.5 ± 3.3</td>
<td>76.3 ± 3.7</td>
<td>8.0 ± 3.5</td>
</tr>
<tr>
<td>30 (9)</td>
<td>22.1 ± 5.5</td>
<td>29.5 ± 8.2*</td>
<td>55.3 ± 8.7</td>
<td>9.1 ± 1.6</td>
</tr>
<tr>
<td>60 (6)</td>
<td>13.7 ± 2.9*</td>
<td>28.5 ± 5.7*</td>
<td>34.2 ± 11.3</td>
<td>9.6 ± 3.3</td>
</tr>
</tbody>
</table>

Numbers in parentheses = number of muscles. DT = developed tension; RT = resting tension.

The rates of $^{133}$Ba uptake (pmol/min per kg dry weight) prior to ischemia (control values) were compared to the rates of uptake upon reperfusion. No change in uptake was noted upon reperfusion after 15 minutes of ischemia. A significant increase occurred after 30 minutes of ischemia which was considerably greater after 60 minutes of ischemia. Note the absence of an increased rate of $^{133}$Ba uptake after 15 minutes of ischemia despite mechanical recovery averaging only 76.3 ± 3.7% of preischemia values. Resting tension progressively increased during reperfusion after longer periods of ischemia.

* $P < 0.05$. 

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**References**

The uptake curve upon reperfusion in all cases was higher than the preischemic portion of the curve. There was no significant difference between the increases in the levels of the curve between 45 and 60 minutes which averaged 11.1 ± 3.5% higher than the values prior to ischemia. When the septa were digested and the tissue radioactivity of $^{85}$Sr compared to that of the perfusate, the $^{85}$Sr space after ischemia averaged 3433 ± 47 ml/g dry weight.

**Reperfusion with Solution Having a Reduced Calcium Content**

If tissue damage induced during reperfusion was mediated by an increased rate of calcium influx, then a reduction in calcium concentration during reperfusion might improve recovery of mechanical function.

Interventricular septa contracting at 72 beats/min were perfused for 60-90 minutes at 37°C with red cell perfusate containing 1.5 mM CaCl$_2$. Total ischemia was then produced for 60 minutes by stopping the perfusion. The results are shown in Figure 4. Both the abrupt decline in tension and gradual increase in resting tension previously described were noted. At the conclusion of the ischemic period, perfusion was resumed with the same solution used during the preischemia period. There was a further increase in resting tension associated with resumption of flow; then there was a quiescent period before developed tension returned. This quiescent interval may have represented the period during which a suitable transsarcolemmal potassium gradient was reestablished to permit the restoration of excitability. Subsequently, there was a marked rise in resting tension which progressed to full contracture. In all severely injured muscles there was a rapid and progressive rise in resting tension (contracture) until resting tension reached values similar to the peak developed tension recorded prior to ischemia.

A group of muscles was reperfused with 0.75 mM CaCl$_2$ for 5-6 minutes prior to return to control calcium concentration (Fig. 4, B and C). As shown in Figure 4B, reperfusion for 5 minutes with red cell perfusate containing 0.75 mM CaCl$_2$ produced no rise in resting tension. When 1.5 mM CaCl$_2$ was reintroduced, a rise in resting tension did occur, but ultimate recovery was excellent. The lower tension achieved during red cell perfusion with solution containing 0.75 mM CaCl$_2$, compared to the higher tension recorded immediately on exposure to 1.5 mM CaCl$_2$ suggests that sarcoplasmic calcium levels during contraction were physiologically appropriate for this reduced calcium contraction. On return to red cell perfusate containing control CaCl$_2$ concentration, there was a further rise in resting and developed tension. The resting tension showed some decrease after reaching maximal levels, as in Figure 4B. Resting tension did not decline below the levels achieved during the ischemic interval immediately prior to reperfusion. In several muscles...

**Extracellular Space Measurements—$^{51}$CrEDTA**

Since the uptakes of $^{133}$Ba and $^{85}$Sr were determined by tissue probe analysis, some increase in radioactivity could have resulted from an increase in the size of the extracellular space. This was assessed by using $^{51}$CrEDTA as an extracellular marker as developed for the perfused septal preparation by Crevey and Langer (personal communication). The isotope was added to the red cell perfusate in a concentration of 19 $\mu$M. The uptake of $^{51}$CrEDTA reached a plateau within 25 minutes. Thereafter the slope showed an increase of not more than 0.15% per minute. Ischemia produced a decrease in radioactivity as seen previously for $^{85}$Sr and $^{133}$Ba (Fig. 3). On reperfusion a new level of tissue radioactivity was achieved within 25 minutes.

In all eight septa made ischemic for 30-60 minutes, the slope of the uptake curve after 25 minutes of reperfusion became identical to that during the preischemic period. On the other hand, the level of radioactivity as seen previously for $^{85}$Sr became identical to that during the preischemic period. On the other hand, the level of tissue radioactivity was achieved within 25 minutes. Thereafter the slope showed an increase of not more than 0.15% per minute. Ischemia produced a decrease in radioactivity as seen previously for $^{85}$Sr and $^{133}$Ba (Fig. 3). On reperfusion a new level of tissue radioactivity was achieved within 25 minutes. In all eight septa made ischemic for 30-60 minutes, the slope of the uptake curve after 25 minutes of reperfusion became identical to that during the preischemic period. On the other hand, the level of radioactivity as seen previously for $^{85}$Sr became identical to that during the preischemic period. On the other hand, the level of tissue radioactivity was achieved within 25 minutes. Thereafter the slope showed an increase of not more than 0.15% per minute. Ischemia produced a decrease in radioactivity as seen previously for $^{85}$Sr and $^{133}$Ba (Fig. 3). On reperfusion a new level of tissue radioactivity was achieved within 25 minutes. In all eight septa made ischemic for 30-60 minutes, the slope of the uptake curve after 25 minutes of reperfusion became identical to that during the preischemic period. On the other hand, the level of...
no rise in resting tension occurred on exposure to 1.5 mM CaCl₂ after 0.75 mM CaCl₂ reperfusion. The septum for which data are shown in Figure 4C showed no rise in resting tension on reperfusion and represents the optimal response to 0.75 mM CaCl₂ perfusion for 5 minutes. This type of response never was observed for muscles immediately reperfused with solution containing 1.5 mM CaCl₂.

Twenty septa were perfused according to this protocol with control red cell perfusate containing 1.5 mM CaCl₂. Ten muscles which had been totally ischemic for 60 minutes were reperfused with solution containing 0.75 mM CaCl₂ for 5 minutes, then 1.5 mM CaCl₂ (Table 3). Experiments on a control and an experimental septum were conducted on the same day and in alternating order to minimize effects of changes in red cell perfusate and condition of rabbits. Maximal recovery of developed tension was recorded after 60 minutes of reperfusion. There was significantly better recovery of the septa reperfused with solution containing a reduced CaCl₂ concentration which attained 67.1 ± 7.5% of preischemia values compared to 31.6 ± 9.1% for control muscles (P < 0.02). The derivatives of tension, +dT/dt, and −dT/dt consistently recovered to a greater extent than developed tension in both groups. The principal difference produced by solution containing 0.75 mM CaCl₂ for 5 minutes of perfusion was a reduction in the number of muscles in which there was a severe and irreversible increase in resting tension on reperfusion. The average resting tension on reperfusion was 18.4 ± 11.3 g compared to 12.6 ± 5.4 g with 0.75 mM CaCl₂ in the perfusate.

The effects of reperfusion with solution containing 0.75 mM CaCl₂ were assessed in 12 other muscles made totally ischemic for 20 minutes. Although the severity of injury after this shorter period of ischemia was considerably less, better recovery of developed tension could be shown (Table 4).

Four muscles were reperfused with solution containing 0.3 mM CaCl₂ for 5 minutes, after 60 minutes of total ischemia, according to the protocol described for 0.75 mM CaCl₂. Although some improvement of mechanical recovery was noted in comparison to initial reperfusion with solution containing 1.5 mM CaCl₂, the magnitude was small and was statistically significant only for recovery of +dT/dt (DT recovery 48.8 ± 7.4, +dT/dt 75.5 ± 5.5, −dT/dt 78.0 ± 7.8, compared to data for control muscles in Table 1).

Reperfusion with calcium-free solution (Ca < 10⁻⁷ m) after 20 minutes of ischemia was followed by severe contracture and less than 10% recovery of developed tension in all of five septa. Uncontrolled ectopic pacemakers with very rapid rates occurred on reintroduction of perfusate containing 1.5 mM CaCl₂. In four septa, lengthening the period of reperfusion with 0.75 mM CaCl₂ to 15 minutes had no further protective effect beyond that observed for 5-minute reperfusion.

Low Calcium during Ischemia

Since the increase in resting tension upon reperfusion was strongly influenced by the calcium concentration of the reperfusion solution, the effect of a low calcium concentration on resting tension was studied during the period of ischemia.

Five septa were perfused with solution containing 2.5 mM CaCl₂ and made totally ischemic. After 20
minutes of ischemia, each septum was perfused with anoxic perfusate containing 50 μM CaCl₂ which had been equilibrated with a 98% N₂-2% CO₂ mixture. Each muscle (Fig. 5A) showed a rise in tension associated with resumption of perfusion. This was attributed to the increase in intravascular pressure induced by starting the perfusion pump. There was no decline in tension during the 6-minute perfusion with solution containing 50 μM CaCl₂. On cessation of perfusion, tension declined to the previous ischemic levels and thereafter rose progressively. Perfusion a second time, with the same solution containing 50 μM CaCl₂ after 45 minutes of ischemia also had no effect on the increase in resting tension. On reperfusion with solution containing 2.5 mM CaCl₂, there was no striking rise in resting tension and recovery was excellent. In contrast (Fig. 5, B and C) perfusion with anoxic perfusate containing 2.5 mM CaCl₂ resulted in development of a small amount of tension with each electrical stimulation. Resting tension rose at an increased rate following the second period of perfusion with this solution, and there was a marked rise in resting tension on reperfusion with red cell perfusate containing 2.5 mM CaCl₂. Subsequently, recovery improved but resting tension remained high.

Mechanical recovery after 60 minutes of reperfusion with red cell perfusate was assessed in four muscles which previously had been exposed to the anoxic perfusate containing 50 μM CaCl₂ for 5-minute intervals and compared to data for four septa perfused in the same way with the anoxic perfusate containing 2.5 mM CaCl₂. Developed tension recovered to 70.8 ± 10.0% of preischemia levels in the muscles perfused with solution containing 50 μM CaCl₂ compared to 28.9 ± 12.7% for muscles which had been exposed to solution containing 2.5 mM CaCl₂ during ischemia (P < 0.001).

The effects of 50 μM CaCl₂ on developed tension and resting tension after reperfusion and recovery of stable mechanical function were assessed in four septa. Each muscle was made totally ischemic for 60 minutes, reperfused with solution containing 2.5 mM CaCl₂, and allowed to recover developed tension. Introduction of red cell perfusate containing 50 μM CaCl₂ produced a rapid decline in developed tension. Resting tension did not decline in any of these muscles during this perfusion.
Ca$^{2+}$ prior to reintroduction of 1.5 mM Ca$^{2+}$. The results were similar to those after reperfusion following 60 minutes of ischemia.

**TABLE 4**

<table>
<thead>
<tr>
<th>Recovery 1.5 mm Ca$^{2+}$</th>
<th>Recovery 0.75 mm Ca$^{2+}$-1.5 mm Ca$^{2+}$</th>
</tr>
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<tbody>
<tr>
<td>Control (g)</td>
<td>% recovery</td>
</tr>
<tr>
<td>DT</td>
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<td>36.0</td>
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<td>6.7</td>
</tr>
<tr>
<td>±5.1</td>
<td>±3.4</td>
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</tbody>
</table>

Mechanical responses upon reperfusion after 60 minutes of total ischemia are shown. A statistically significant ($P < 0.02$) improvement in developed tension for muscles reperfused for 5 minutes with 0.75 mm Ca$^{2+}$ was shown. The recoveries of +dT/dt and -dT/dt were also significantly improved. Resting tension after reperfusion was lower following 0.75 mM reperfusion, although the difference was not statistically significant. Much of the improvement arose from protection against severe contracture upon reperfusion. Note that the derivatives of tension recovered to a greater extent than tension itself.

* $P < 0.02$; † $P < 0.01$; ‡ $P < 0.05$.

**Discussion**

Abnormalities of relaxation are consistent findings during myocardial ischemia in a variety of experimental and clinical situations. Calcium-loading of cardiac muscle, whether the result of increased influx of the cation or decreased ability to sequester myoplasmic calcium, could impair relaxation. Intrinsic defects in the proteins involved in crossbridge interaction could impair relaxation but thus far have not been observed. ATP availability is also crucial to the relaxation of crossbridges.

In this report, the role of calcium in producing abnormalities of relaxation was assessed by perfusion with an anoxic perfusate containing 50 μM CaCl$_2$. We observed no effect of this perfusion on the progressive rise in resting tension which occurred during ischemia. This argues against calcium loading as the primary mechanism for this abnormality of relaxation—even in early stages of the process. CaCl$_2$ 50 μM was chosen in order to avoid membrane injury produced by a still lower calcium concentration as reported by Frank et al. The results are consistent with the lack of effects of low calcium on the contracture produced in rat heart during anoxia and substrate deprivation reported by Nayler et al. This response contrasts with the decline in resting tension obtained when muscles were perfused with "zero calcium" solutions after contracture had been induced by digitalis.

Reperfusion for 5 minutes with solution containing a reduced calcium concentration protected the septa after 20 minutes and 60 minutes of ischemia. The basis for the protection remains uncertain. If calcium loading of the cell, and particularly of the mitochondria, were responsible for damage on reperfusion, a period of recovery of cellular function prior to resumption of perfusion with control calcium concentration might be beneficial. During this 5-minute period, the ability of the sarcoplasmic

**TABLE 4**

<table>
<thead>
<tr>
<th>Mechanical Responses to 20 Minutes of Ischemia</th>
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<tr>
<td>Control (g)</td>
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<td>DT</td>
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<td>29</td>
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Mechanical responses upon reperfusion after 20 minutes of ischemia are shown for control muscles and muscles reperfused for 5 minutes with 0.75 mm Ca$^{2+}$ prior to reintroduction of 1.5 mm Ca$^{2+}$. The results were similar to those after reperfusion following 60 minutes of ischemia.
reticulum to sequester calcium might gradually have increased so that increased influx of extracellular calcium could be tolerated. Alternatively, reperfusion with solution containing a decreased calcium concentration reduced myocardial work. This could have allowed recovery of critical ATP stores which contributed to reparative processes and crossbridge detachment prior to resumption of a full work load when 1.5 mM CaCl₂ perfusion was resumed. Such repair might have enhanced the ability to sequester calcium and also may have allowed recovery of other crucial cellular functions. The improvement in recovery of mechanical function observed after the 50 µM CaCl₂ anoxic perfusions during ischemia may have resulted from lower intracellular calcium concentration at the time that reperfusion occurred.

Barium and strontium ions move through the sarcolemmal membrane competitively with calcium ion. They can substitute for calcium ion as part of the slow inward current. Both are weak couplers of excitation to contraction and can induce contracture. Strontium can be sequestered by sarcoplasmic reticulum, but barium is not sequestered, as shown by studies on isolated preparations.

In our experiments on reperfusion, an abnormality of ¹³³Ba uptake was identified after only 30 minutes of total ischemia. The change was a 33% increase in the rate of uptake. This result indicates a defect in membrane selectivity for barium, which occurred early after the onset of ischemia. That this was not the result of a nonselective binding at other sites in the tissue was shown by the absence of an increased rate of ⁸⁶Sr uptake until reperfusion had been delayed for 60-70 minutes after the onset of ischemia. The discrepancy between the time course of events for the two cations suggests that the initial sarcolemmal defect in uptake of ¹³³Ba was apparent because this ion was not sequestered by sarcoplasmic reticulum.

Sequestration of calcium and related cations may be required as part of the process by which they are removed from the intracellular environment to the interstitial space. If this is the case, an increased movement of ¹³³Ba into the cell through injured sarcolemmal membranes became apparent on reperfusion after 30 minutes of ischemia. At this point no increase in the rate of ⁸⁶Sr uptake was observed because sequestration and removal of ⁸⁶Sr from the cell compensated for the sarcolemmal defect. After 60-70 minutes of ischemia, reperfusion did result in an increase of the rate of ⁸⁶Sr uptake because of...
failure of these mechanisms in the face of further sarcolemmal injury. The time course was consistent with our previous description of 42K exchange in rabbit septa which showed that ability to maintain cellular potassium content was impaired on reperfusion after 60 minutes of ischemia. 5

After 60 minutes of ischemia, reperfusion resulted in an increase of 51Cr EDTA counts averaging 11.1%. However, within a few minutes after reperfusion, the rate of increase in 51Cr EDTA counts was identical to that observed prior to ischemia. The absence of a persistent increase in the rate of 51Cr EDTA counts indicated that the injury to the sarcolemma which admitted 86Sr and 133Ba at an increased rate after reperfusion did not admit the larger 51Cr EDTA complex at an increased rate. This sequence demonstrates that ischemic injury of the sarcolemma can be quite selective at certain points in time and does not need to produce large anatomical defects in that membrane when marked cation displacement occurs.

The 51Cr EDTA results also showed that the increased rates of 86Sr and 133Ba observed after reperfusion did not result from progressive extracellular edema, since this mechanism would have caused a similar progressive rise in counts/min of 51Cr EDTA.

We previously noted that, in rabbit interventricular septa, ischemia produced an immediate monoexponential decline in tension accompanied by an asymmetrical decline in dT/dt which was calcium dependent only with high (5 mM) extracellular CaCl2. 9 The maximum rate of relaxation declined immediately with the onset of ischemia and had a greater temperature dependence than the maximum rate of tension development. The difference in temperature dependence was consistent with high metabolic requirements for relaxation. In this report we emphasized that resting tension gradually rose during total ischemia, but this rise was not calcium dependent. The increase in resting tension may have resulted from crossbridge attachment induced by ATP lack. 11

The abnormality of relaxation on reperfusion most likely resulted from both ATP deprivation (during ischemia) and calcium accumulation on reperfusion. The reperfusion insult was minimized by limiting the amount of calcium influx during the earlier period of reperfusion. The mechanisms of protective effect of reperfusion with solution containing 0.75 mM Ca2+ for 5 minutes requires additional investigation. In experiments with reperfusion of intact dog ventricles made ischemic during cardiopulmonary bypass, Follette et al. 12 found 0.6 mM CaCl2 to be optimum for mechanical recovery.

As measured by the 51Cr EDTA counts, the edema during reperfusion occurred rapidly and stabilized within 20–25 minutes, although resting tension remained elevated or, after severe injury, continued to rise. Edema probably played a minor role in this aspect of functional recovery.

These studies emphasized the selective nature of effects of myocardial ischemia on cell function and suggest that sarcolemmal defects may not result from nonspecific rupture of the membrane in the early stages of ischemia and reperfusion but from specific and selective injury. The consistent recovery of +dT/dt and −dT/dt to a greater degree than the recovery of developed tension under all conditions studied argues for injury which results from inactivation of a progressively increasing number of crossbridges in irreversible attachment while other crossbridges continue to move with normal or even increased velocity.

References

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