Reversibly Injured, Postischemic Canine Myocardium Retains Normal Contractile Reserve

Bruce R. Ito, Hidesada Tate, Masao Kobayashi, and Wolfgang Schaper

Transient coronary occlusion (15 minutes) does not result in irreversible myocardial injury but is associated with a depression of contractile function sustained for several hours to days ("stunned myocardium"). The defect in the contractile process responsible for this phenomenon has been suggested to be causally related to a reduced energetic state, altered excitation or excitation-contraction coupling, or damaged contractile filaments. The purpose of this study was to attempt to exclude one or more of these hypotheses by evaluating the contractile reserve of reperfused myocardium. Regional subendocardial segment function was measured (sonomicrometry) in a control region and in an area (treatment region) perfused by a carotid artery to anterior descending coronary artery bypass in 13 chloralose-anesthetized dogs. Dose-response curves were constructed from changes in segment shortening (%SS) in response to intracoronary calcium infusion before ischemia and following 5 or 15 minutes of occlusion and reperfusion (30 minutes). Calcium infusion before ischemia resulted in dose-dependent increases in %SS in the treatment area to a maximum value of 36.6% from a preinfusion value of 25.5% (p<0.01), in the absence of changes in control region shortening (23.7%). After 15 minutes of occlusion and reperfusion, treatment area %SS had fallen to a depressed but stable level (46% of preischemic values; p<0.01). Subsequent calcium infusion at the same doses as in the preischemic trial produced increases in treatment segment function with return of shortening to control levels at an intermediate dose. At the highest dose, %SS was 35.4%, which was not different from the maximal value found in the preischemic trial. Alterations in heart rate and left ventricular systolic and diastolic pressures during calcium infusion were minor and similar before and after ischemia. Calcium-induced increases in regional segment shortening above control levels (113% of control) in reperfused myocardium were sustained with continuous infusion (30 minutes) without deleterious effects on subsequent function. These results demonstrate that stunned myocardium in this model retains a normal contractile reserve in response to calcium, suggesting that the mechanism responsible for postischemic contractile dysfunction involves calcium. (Circulation Research 1987;61:834–846)

It is well documented that transient occlusion of a coronary artery does not result in myocardial necrosis if the duration of the ischemic period is less than 15–20 minutes.1,3 Although the ultrastructural changes observed during the ischemic episode are largely reversed after 30 minutes1 to 60 minutes2 of reperfusion, regional contractile abnormalities persist for several hours to many days.3,4 The prolonged contractile dysfunction observed following periods of myocardial ischemia in the absence of irreversible cellular injury has been termed "stunned myocardium."5 Although many hypotheses have been advanced, the mechanism behind this phenomenon remains unclear. A prominent hypothesis is that reperfusion dysfunction is the result of an inadequate or defective energy supply to the contractile process.5,6,10 and is based on observations of sustained decreases in myocardial ATP levels and loss of adenine nucleotides and nucleosides associated with ischemia.3,11–13 Several studies have shown that these decreased ATP levels are very slowly repleted during reperfusion,7,9,10,13–16 at rates that parallel the return of normal contractile function.9,12 Furthermore, a close relation between ventricular function and decreased tissue ATP levels has been demonstrated in isolated hearts subjected to transient ischemia.14

An alternative explanation has been suggested in studies evaluating the effects of ischemia on cellular components of excitation-contraction coupling. Ischemia-induced abnormalities in the calcium transporting and binding characteristics of cardiac sarcoplasmic reticulum and sarcolemmal membranes have been described using in vitro preparations19–22 and may be involved in the contractile dysfunction during reperfusion. It is also possible that the defect in postischemic myocardium is at the level of the contractile filaments themselves. It has been reported that short periods of ischemia can result in damage to the contractile filaments with alterations in myofilament overlap.23

The present study was designed to evaluate these hypotheses by assessing the contractile reserve of stunned myocardium. If the depressed function in

From the Max-Planck-Institute/Kerckhoff Institute, Department of Experimental Cardiology, D-6350 Bad Nauheim, Federal Republic of Germany.
Address for correspondence: Dr. Bruce R. Ito, Department of Medicine (M-013-J), University of California, San Diego, La Jolla, California 92039.
Address for reprints: Max-Planck-Institute, Department of Experimental Cardiology, Benekestrasse 2, D-6350 Bad Nauheim, Federal Republic of Germany.
Received August 11, 1986; accepted June 30, 1987.
reperfused myocardium is the result of a limited or deficient energy supply or there is damage to the contractile machinery, inotropic agents that increase energy consumption would not be expected to result in a substantial or sustained enhancement of function. This should be apparent as a reduction in the contractile reserve of postischemic myocardium. The approach of the following study was to quantitate the contractile reserve of normal and reperfused myocardium using calcium, a potent positive inotropic agent. Calcium was given intracoronarily to minimize large alterations in heart rate, preload, and afterload, known variables influencing contractile function.

Materials and Methods

General Preparation

Adult mongrel dogs (25–30 kg) of either sex were premedicated with piritramide (2.5 mg/kg i.m.) and anesthetized 30-60 minutes later with α-chloralose (50 mg/kg i.v.). Following intubation with a cuffed endotracheal tube, animals were ventilated with a positive pressure respirator (model 7F, Bird Corp., Palm Springs, Calif.) with an end-expiratory pressure of 3–5 cm H₂O. The inspiratory gas composition consisted of oxygen and nitrous oxide in approximately a 2:1 ratio. Arterial blood was routinely sampled for determination of PaO₂, PaCO₂, or pH (model 1602, Instrumentation Lab., Lexington, Mass.). Blood gas values were maintained within the physiologic range by adjustments of the ventilatory rate and inspired gas oxygen concentration.

A continuous infusion of a sodium bicarbonate solution (1.5%) given as an intravenous drip into the right femoral vein was used to keep blood pH within normal values. The anesthetic level was maintained with a continuous infusion of α-chloralose (4 mg/min) and 0.25–0.50 g supplements when necessary. Aortic blood pressure was measured with a strain gauge manometer (model P23Db, Statham, Hato Rey, Puerto Rico) through a polyethylene catheter placed into the aorta via the right femoral artery. Left ventricular pressure was obtained using a catheter-tip manometer (model 7F, Millar, Houston, Tex.) inserted via the left femoral artery and recorded at full scale and at high gain for measurement of left ventricular (LV) diastolic pressure. The first derivative of LV pressure (LV dp/dt) was determined with an electronic differentiating circuit exhibiting a cut-off frequency in excess of 35 Hz. Heart rate was continuously measured with a cardiotachometer triggered from a standard limb ECG.

The heart was exposed via a left thoracotomy in the 6th intercostal space and suspended in a pericardial cradle. A section of the left anterior descending coronary artery (LAD) distal to the first diagonal branch approximately 2–3 cm from the left main artery was prepared for later cannulation. Bipolar silver pacing electrodes were sutured to the epicardium in the basal region of the right ventricle for the introduction of single extrasystolic stimuli (6–7 volts, 7 msec). Output from the stimulator (model S88, Grass Instrument Co., Quincy, Mass.) was triggered from the R wave of the QRS complex of the ECG and delivered following a set interval. This interval was adjusted for each animal to be just outside of the absolute refractory period, producing ventricular depolarization but minimal generation of tension as indicated by LV pressure. This interval varied between 200–250 msec in the various experiments.

Sonomicrometry

Regional ventricular function was measured with segmental ultrasonic crystal pairs fabricated from 5-MHz piezoelectric material (2 mm diameter). The crystals were implanted into the subendocardium approximately 1–1.5 cm apart and 7–9 mm below the epicardial surface. Each crystal pair was oriented in the circumferential axis perpendicular to the coronary ostium-apex chord. One pair of segment length gauges (treatment segment) was implanted in the center of the distal LAD perfused zone as determined by the cyanosis and hyperemic blush following a short (15-second) occlusion of the prepared LAD vessel. A second pair (control segment) was implanted distant from this zone in an area perfused by the left circumflex artery in the basal region of the anterolateral wall. The circumferential position and depth of all crystal pairs were confirmed on postmortem examination.

Coronary Perfusion

Following anticoagulation with sodium heparin (500 IU/kg i.v.), the prepared section of the LAD was ligated and the distal segment perfused from the left common carotid artery via a short polyethylene and silastic shunt. The occlusion duration for the cannulation procedure was less than 30 seconds and was performed while recording segment function to verify complete recovery of shortening upon reperfusion. Coronary perfusion pressure was measured with a strain gauge manometer (Statham P23Db) and a polyethylene catheter close to the site of cannulation via a T connection in the shunt line. The pressure drop across the perfusion circuit was less than 5–10 mm Hg at all experimentally encountered blood flows. An electromagnetic flow probe (Statham, Oxnard, Calif.) in the perfusion line provided a continuous measurement of coronary blood flow to the perfused region.

Mean coronary blood flow was determined with an electronic averaging circuit.

The electrocardiogram, mean and phasic aortic pressures, LV pressure, LV dp/dt, ultrasonic segment lengths, coronary pressure, and coronary blood flow were recorded on a 10-channel polygraph (Graphtec Corp., Japan). All measurements were obtained under steady-state conditions with respiration suspended at end-expiration to avoid respiratory fluctuations in segment length.

Experimental Protocol

In 13 animals, the effects of intracoronary calcium administration (CaCl₂, 20 mM in saline) on regional segment function was determined first under preischemic conditions. The calcium solution was infused with
a syringe pump directly into the LAD perfusion line at set rates of infusion (0.22–14.7 mg/min i.c.). The first 3 levels of calcium infusion (0.22, 0.59, and 1.47 mg/min) were given in a stepwise manner, and the remaining doses (2.94, 5.88, 11.03, and 14.7 mg/min) were administered approximately 3–5 minutes apart. The duration of infusion at each level was between 2 and 3 minutes with measurements obtained under steady-state conditions in the last 30 seconds of infusion. These infusion rates had been previously established in pilot experiments to span a complete range of a dose-response curve. Several determinations of the total calcium concentration in plasma from arterial blood were made in 2 animals using a fluorescence EDTA titration technique (Jookoo Co. Ltd., Japan). The arterial plasma calcium concentration (5.56±0.21 meq/l) at the end of the intracoronary calcium infusion at the highest dose (14.7 mg/min) was not different from control arterial blood samples (5.74±0.16 meq/l).

Following the preischemic calcium infusion protocol, acute regional myocardial ischemia was produced by complete occlusion of the LAD perfusion line with a screw clamp. In one group of animals (n = 3), the duration of occlusion was 5 minutes with measurements obtained at 1, 3, and 5 minutes after the onset of occlusion. In a second group of animals (n = 10), the duration of occlusion was 15 minutes with measurements taken at 1, 3, 5, 10, and 15 minutes after the onset. In both groups of animals, reflow was produced in a gradual manner by slowly opening the clamp to a full open position within 1 minute. Measurements were made at 1, 3, and 5 minutes, and then every 5 minutes for 30 minutes during the reperfusion period. Approximately 30 minutes after the onset of reperfusion, when regional function was again stable, the calcium infusion protocol was repeated using the same infusion rates to generate a postischemic calcium dose-response curve. In the 10 animals receiving the 15-minute period of ischemia, the response in regional function to postextrasystolic potentiation (PESP) was assessed of this group, calcium was infused (5.88 mg/min) for approximately 30 minutes after the onset of reperfusion, when regional function was again stable, the calcium infusion protocol was repeated using the same infusion rates to generate a postischemic calcium dose-response curve. In the 10 animals receiving the 15-minute period of ischemia, the response in regional function to postextrasystolic potentiation (PESP) was assessed

Regional segment shortening data were also expressed as a percent of the control value taken just prior to the start of preischemic calcium infusion. Calcium dose-response curves were constructed by plotting segment function against the log of the calcium infusion rate.

**Statistical Analysis**

Statistical analyses of the difference between the preischemic and postischemic calcium responses at each infusion dose and comparison of the difference between preinfusion values and values obtained during infusion were made using a repeated measures analysis of variance and Tukey's test for comparing mean values. A paired t test was used to compare the difference between the preischemic and postischemic values obtained before the start of the calcium infusion trial. A p value of less than 0.05 was chosen to indicate statistical significance.

**Results**

An experimental record showing hemodynamic and dimension variables before and during calcium infusion in the control trial prior to ischemia is reproduced in Figure 1. Responses in the same animal with calcium given at the end of the 30-minute reperfusion period following a 15-minute occlusion is shown in Figure 2.

**Hemodynamic Responses to Calcium Infusion**

Hemodynamic measurements were obtained just before and during each calcium infusion period in the preischemic and postischemic states. The mean data were similar between the 5- and 15-minute occlusion groups and are shown together in Figure 3. The intracoronary infusion of calcium before ischemia produced no significant changes in heart rate (HR) or left ventricular systolic pressure (LVSP) at all levels compared with preinfusion values. Left ventricular end-diastolic pressure (EDP) decreased slightly but significantly (p<0.05) by less than 1 mm Hg at the higher calcium infusion rates. Peak LV dP/dt changed in a dose-dependent manner, increasing significantly (p<0.05) to a maximal value of 3,200±180 mm Hg/sec.

Occlusion of the LAD bypass resulted in changes in ventricular hemodynamics typical of acute ischemia with significant increases in HR (+21 beats/min) and LVEDP (+2.2 mm Hg) and decreases in LVSP (−8.0 mm Hg) and LV dP/dt (−460 mm Hg/sec) compared with preocclusion values (p<0.05). These variables returned quickly to or below control values with the onset of reperfusion. At the end of the 30-minute reperfusion period, just prior to the start of the post-
**FIGURE 1.** Effects of intracoronary calcium infusion at 2 different rates prior to ischemia (control conditions). Calcium produced dose-dependent increases in segment shortening in the treatment region with minor changes in end-diastolic length and control region function. This was accompanied by slight increases in left ventricular (LV) pressure, dP/dt, and coronary blood flow.

**FIGURE 2.** Effects of calcium infusion on postischemic contractile dysfunction after 15 minutes of coronary occlusion and reperfusion. Before calcium administration, treatment segment shortening was depressed compared with the preocclusion level (Figure 1, same animal). Calcium infusion produced sustained increases in shortening in the treatment area for the duration of the 3-minute infusion period. Response to an extrasystolic stimulus (PESP) is also shown.
Results are mean ± SEM from the 15-minute occlusion group (n = 10). EDL, End-diastolic length; ESL, end-systolic length; ΔSS, segment shortening (EDL - ESL). Statistical comparisons between control values (preinfusion) and values during calcium infusion in both the preischemic and postischemic trials were made using Tukey’s multiple comparisons procedure. *p<0.05; **p<0.01 as compared to control values. Statistical comparisons between preischemic and postischemic control values were made using a two-tailed paired t test. †p<0.05; ††p<0.01.

The administration of calcium following transient ischemia resulted in changes similar to that observed with calcium given prior to ischemia (Figures 2 and 3). LVSP rose in a dose-dependent fashion from 119.3 ± 2.5 mm Hg to 132.7 ± 3.4 mm Hg at the highest infusion level. A similar trend was observed with LV dP/dt, which rose from the depressed postischemic value of 2,040 ± 120 mm Hg/sec to 3,090 ± 180 mm Hg/sec (p<0.01). These maximal values for LV pressure and dP/dt were not statistically different from the levels reached at the same calcium dose given prior to ischemia. EDP fell slightly but significantly (p<0.05) by less than 1 mm Hg at the higher infusion rates. This decrease was not different from that found in the preischemic calcium trial (p>0.10). There were no significant changes in heart rate at all levels of calcium infusion.

**Effects of Calcium on Regional Segment Function**

Myocardial segment lengths were determined for the control and treatment (LAD-perfused) regions before and after the administration of calcium in the preischemic and reperfused conditions. Data are summarized for the 15-minute occlusion group (n = 10) and are shown in Table 1 and Figure 4. The recordings in Figure 1 illustrate the changes in regional segment length resulting from the intracoronary calcium infusion at 2 levels before myocardial ischemia.

**Treatment region.** The intracoronary infusion of calcium resulted in rapid (<5 seconds) dose-dependent decreases in ESL with minor decreases in EDL (Figure 1). ΔSS reached a stable level within approximately 1 minute, remaining elevated for the duration of the 3-minute infusion period. Segment lengths returned to preinfusion levels within 1 minute after the onset of infusion. In the 15-minute occlusion group, mean values for %ΔSS increased significantly from a preinfusion level of approximately 25% to a maximal value.
of 36.6% at the highest infusion level ($p<0.05$) (Table 1, Figure 4). EDL decreased slightly with calcium infusion (less than 2%) at the highest infusion rates compared with the preinfusion value. Similar responses in treatment ΔSS with calcium infusion were observed in the 5-minute occlusion group with Δ%ASS increasing to a maximal value of 44% from the preinfusion level of 29%.

Coronary occlusion resulted in rapid increases in EDL and decreases in segment function in the treatment region ($p<0.01$), with systolic bulging apparent within 1–3 minutes (Figure 5). Reperfusion after 15 minutes of occlusion produced an early transient increase in shortening in the treatment segment at 1 minute to within approximately 75% of the preocclusion value followed by a gradual decline of function to a depressed but stable level between 10 and 30 minutes. At the end of the 30-minute reperfusion period, just before the start of the postischemic calcium trial, ΔSS and Δ%ASS were significantly decreased compared to the preocclusion control values ($p<0.01$) (Table 1). In the 15-minute occlusion group, Δ%ASS had fallen to approximately 12%, representing a reduction to 46% of control values (Figure 4). Reperfusion in the 5-minute group resulted in a slightly greater return of %ASS to 19%, which was approximately 65% of the preocclusion value (29%). EDL in both groups had returned to the preocclusion level (10 mm) by the end of the reperfusion period.

The infusion of calcium in the postischemic condition (Figure 2) produced changes in treatment segment function similar to that observed in the preischemic trial. Although end-systolic segment length was substantially elevated after the ischemic episode, the administration of calcium resulted in a rapid decrease in ESL below the preocclusion level, remaining at this stimulated level for the duration of the infusion period. In the 15-minute group, mean values for Δ%ASS increased in a dose-dependent manner, reaching preocclusion control values at approximately the midpoint of the dose-response curve at an infusion rate of 2.94 mg/min (Table 1, Figure 4). Higher infusion rates resulted in further elevations in segment function with Δ%ASS rising to approximately 35% at the highest dose (14.7 mg/min). This value was not statistically different ($p>0.10$) from the maximal level obtained at the

![Figure 4. Average responses in regional segment end-diastolic length (EDL) and percent segment shortening (Δ%SS) at each of the calcium infusion rates, before and after 15 minutes of coronary occlusion (n = 10). Values for Δ%SS in the treatment region are shown during calcium infusion (during) and just before the start of infusion (before) at the depicted rates. Calcium infusion produced dose-dependent increases in treatment segment shortening to similar maximal levels before and after ischemia induced contractile depression. These increases occurred in the absence of changes in control segment shortening. *$p<0.05$ compared with preinfusion (control) values. **$p<0.01$ compared with preinfusion (control) values, †$p<0.01$ compared with the corresponding value in the preischemic trial.]
FIGURE 5. Effect of 15 minutes of coronary artery occlusion and reperfusion on regional segment shortening (%ΔSS) normalized to preocclusion values (n=10). Negative values for %ΔSS during ischemia indicate systolic bulging. Occlusion and reperfusion produced a stable depression of function in the treatment region in the absence of major changes in function in the control area. *p<0.01 compared with preocclusion (control) values.

same dose in the preischemic calcium trial. Similar results were observed with postischemic calcium infusion in the 5-minute group with %ΔSS increasing to a maximal value of 43% at the highest infusion rate compared with the value of 44% obtained in the preischemic trial. Postischemic calcium infusion was associated with small decreases in EDL (2–2.5%) significant at the higher infusion rates (p<0.05). However, EDL at each dose was not different from the corresponding value obtained in the preischemic trial.

Control region. Calcium infusion into the LAD coronary artery during the preischemic condition was associated with minor changes in EDL and ESL in the control segment located in the region perfused by the circumflex artery. Systolic ΔSS and %ΔSS were not different from preinfusion values at all calcium doses (Table 1, Figure 4). Acute LAD coronary artery occlusion resulted in minor but significant increases in EDL (+2%) accompanied by insignificant increases in ASS and %ASS compared to preischemic control levels. All control segment dimensions had returned to preocclusion levels by the end of the 30-minute reperfusion period. EDL was insignificantly lower (1%) compared with preischemic levels. Calcium infusion during reperfusion was associated with no significant changes in EDL, ΔSS, or %ΔSS compared with the values before infusion (Table 1, Figure 4). In addition, there were no significant differences between these values at each infusion level compared with the corresponding values obtained in the preischemic trial.

Effect of PESP on Regional Segment Function

The effects of post-extrasystolic stimulation (PESP) on segment function were assessed before and during calcium infusion in both the preischemic and postischemic states and is depicted in Figure 6. Before calcium infusion in the preischemic condition, PESP resulted in increases in shortening in the treatment region with %ΔSS rising to approximately 30% from the prestimulation value of 25% (p<0.01). With calcium infusion, the magnitude of the increase in shortening induced by PESP declined in a dose-dependent manner. At the highest dose, PESP no longer resulted in a further increase in %ΔSS above the calcium stimulated level. The effects of PESP on contractile function in the postischemic state were similar to that observed before coronary occlusion, but with increases in %ΔSS above a depressed level of contractile function (Figure 6). In the absence of calcium infusion, PESP resulted in a rise in %ΔSS in the treatment region to approximately 19% from the depressed value of 12%. The increase in shortening induced by PESP declined with calcium infusion, with no further rise of shortening above the calcium stimulated level produced at the highest dose. In contrast to the treatment region, the effects of PESP on segment function in the control region were similar before and after ischemia and not greatly altered by calcium infusion. In the presence of PESP stimulation, %ΔSS was 29.8 ± 1.0% in the preischemic state and 29.0 ± 1.2% in the postischemic condition, significantly above the control values.
value of approximately 24% (p<0.01). At the highest dose of calcium infusion, these values were slightly but not significantly decreased to 28.0±1.8% and 28.6±1.8%, respectively.

Effects of Continuous Calcium Infusion

Continuous calcium infusion (5.88 mg/min) for 30 minutes following the postischemic dose-response trial (n=3) resulted in sustained increases in treatment segment function for the duration of the infusion period (Figure 7). In these animals, %ΔSS fell from a preischemic value of approximately 33% to a depressed level of 17% following 15 minutes of ischemia and reperfusion. With calcium infusion, shortening rose to approximately 37% and remained at this level for 30 minutes, representing an increase to 113% of preocclusion control values. Segment function in the control region was not greatly influenced by the calcium infusion, with %ΔSS remaining stable (25–27%) for the duration of the infusion period.

Effect of Postischemic Dysfunction on Response to Calcium

To better evaluate the influence of the stunning phenomenon on the inotropic response to calcium infusion, the apparent calcium sensitivity of normal and postischemic myocardium was assessed by plotting the response in %ΔSS expressed as a percent of the maximal response in each trial against the calcium dose (Figure 8). The calcium dose was calculated as the amount of calcium added per milliliter blood to compensate for the small difference in blood flow between the preischemic and postischemic trials. The curves for the calcium response for the two trials were virtually superimposable with the half-maximal response at an added calcium amount of approximately 150 μg/ml blood. This value corresponded to a calcium infusion rate of between 1.47 and 2.94 mg/min.

Relation of Coronary Blood Flow to Contractile Function

A comparison of the response in coronary blood flow and contractile function in the treatment region before and after ischemia was made by plotting %ΔSS expressed as percent of the preinfusion control value against coronary blood flow also expressed as percent of control. Figure 9 illustrates the similarity in the relation of myocardial function to blood flow, before and after 15 minutes of transient ischemia, over the range of calcium infusions. Coronary blood flow and segment function were both significantly depressed following ischemia (p<0.01). The return of segment function to preischemic levels with calcium infusion

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**Figure 7.** Average responses to continuous calcium infusion for 30 minutes on regional segment function after 15 minutes of ischemia (n=3). Percent segment shortening (%ΔSS) is shown before ischemia (Cont 1), after ischemia (Cont 2), and just before the onset of the calcium infusion (t=0) for comparison.

Continuous calcium infusion produced a sustained increase in treatment segment shortening followed by a rapid return to preinfusion levels upon cessation.

**Figure 8.** Effect of transient ischemia on the calcium dose-response curve. Average segment shortening responses (%ΔSS) in the 15-minute occlusion group (n=10) are expressed as a percent of the maximal value above the preinfusion level for both the preischemic and postischemic trials. The calcium dose is expressed as the added calcium amount (μg) per milliliter blood to correct for the small difference in coronary blood flow in the preischemic and postischemic trials.
flow was not different between the preischemic and postischemic trials (p>0.10).

Discussion

This study demonstrates that stunned myocardium (myocardium subjected to a short ischemic period followed by reperfusion resulting in severely depressed but stable contractile function) retains normal maximal contractile capability during inotropic stimulation. The intracoronary infusion of calcium following 5 or 15 minutes of complete coronary occlusion and reperfusion resulted in dose-dependent increases in subendocardial %ΔSS to maximal levels not different from that obtained during calcium infusion prior to ischemia. These increases in shortening occurred in the absence of major alterations in ventricular hemodynamics.

Role of Heart Rate and Loading Conditions

The extent of myocardial segment shortening is primarily determined by the frequency of contraction, loading conditions (preload and afterload), and the inotropic level. The interpretation of experiments addressing the effects of inotropic interventions on shortening is complicated by changes occurring secondary to alterations in heart rate, filling pressure, and intraventricular systolic pressure. In this study, the experimental aim was to assess the ability of reperfused myocardium to increase its level of contractile function during inotropic stimulation without major alterations in heart rate and loading conditions. The infusion of calcium in the present experiments was not associated with changes in heart rate, in either the preischemic or postischemic trials, making it unlikely that this variable played a significant role in the results. In the intact heart, the effects of preload or afterload on regional ventricular function are difficult to assess, being dependent on factors such as wall thickness, radius of curvature, and the orientation of muscle fibers. However, directional changes in preload or afterload can be estimated from alterations in LVEDP and LVSP, respectively. Calcium infusion in the present study was associated with small and similar decreases in LVEDP (less than 1 mm Hg) and end-diastolic segment length (approximately 2%) in both the preischemic and postischemic trials. These decreases in EDL were slightly larger in the treatment region compared to the control area (Table 1). Regional myocardial differences in the dependence of EDL on end-diastolic pressure in the midventricular region as opposed to the basal regions of the heart may have been contributing factors. Since these changes in EDP and EDL are in the opposite direction required for the augmentation of wall function by the Frank-Starling relation, it is unlikely that this mechanism played a major role in the increases in shortening observed here. Calcium infusion was also associated with small increases in LVSP (6–13 mm Hg) at the higher infusion rates, reaching similar levels in the preischemic and postischemic trials. Both the magnitude and the direction of these changes make it improbable that afterload was a major factor in this study. Segment shortening in the control region was not greatly influenced by the calcium infusions, in either the preischemic or postischemic trials (Figure 4). This would suggest that the increased shortening in the treatment area was due to a regional effect of calcium and that major changes in the ventricular loading conditions had not occurred. For these reasons, the increases in segment function in response to calcium infusion demonstrated in this study were probably not secondary to changes in heart rate, preload, or afterload.

Previous Studies

Several studies have reported the effects of inotropic agents on contractile function in reperfused myocardium following ischemic periods of various duration. The administration of calcium after 45 minutes...
of ischemia or dopamine following 2 hours of LAD artery occlusion has been shown to produce improvements in contractile function during reperfusion. Direct comparison of the results from the present study with these previous works is difficult since it is likely that the measured contractile function in these preparations was from a mixture of reversibly injured or stunned myocardium and irreversibly injured or necrotic tissue usually present with ischemic periods of these durations. Smith reported that the intracoronary infusion of isoproterenol returned midwall myocardial segment shortening velocity and oxygen consumption toward preischemic levels following a 10-minute period of LAD occlusion. Similar results were found by Mercier and coworkers measuring epicardial shortening with silastic gauges when dopamine was infused intravenously following a 30-minute or 3-hour period of coronary occlusion. Dopamine returned segment shortening to control levels provided the area was not necrotic. Recently, Bolli and coworkers demonstrated that the postischemic contractile depression following 15 minutes of LAD occlusion could be reversed for 30 minutes with intravenous isoproterenol with no deleterious effects on subsequent function measured as regional wall thickening. Also, Becker and coworkers have demonstrated that intravenous epinephrine or extrasystolic potentiation could reverse the contractile depression resulting from repetitive 5-minute occlusions. In this study, %ΔSS increased to 31% from a control value of 22% in response to epinephrine and PESP. After ischemia, epinephrine and PESP increased %ΔSS to 25% from the depressed level of 8%. However, the interpretation is somewhat complicated by the observation of a reduced inotropic response in the nonischemic regions following the occlusion protocol.

The results presented here are in basic agreement with these previous studies in the finding that reperfused myocardium does respond to inotropic stimulation. The contractile depression in reperfused myocardium following 5 or 15 minutes of coronary occlusion and 30 minutes of reperfusion was effectively reversed with inotropic stimulation with calcium (Figure 4) and could be maintained for 30 minutes without further functional depression (Figure 7). However, the experiments shown here demonstrate that this represents only part of the contractile capabilities of stunned myocardium. The postischemic calcium dose that resulted in a contractile function equal to the preocclusion level was approximately in the middle of the infusion range. Further increases in infusion rate beyond this level produced even further increments in segment shortening. At the highest infusion rate %ΔSS was equal in both preischemic and postischemic myocardium, in the presence of similar levels of heart rate, LVSP, and left ventricular diastolic pressure. This contractile capability of reperfused myocardium was not observed in these previous studies. The reason for this discrepancy may lie with the difference in the inotropic agent used and the method of administration, as well as the type of anesthesia. Many positive inotropic drugs, such as catecholamines, also have prominent effects on heart rate and peripheral vascular resistance, especially when given intravenously. Large alterations in heart rate, preload, and afterload would limit the degree of inotropic stimulation attainable when evaluating the maximal contractile response. An additional problem associated with the use of catecholamines in assessing contractile reserve is the apparent reduction in the sensitivity of reperfused myocardium to catecholamine stimulation.

Effect of the Stunning Process on the Inotropic Response

The objective of these experiments was to assess the calcium-induced inotropic response of stunned myocardium in comparison with normal myocardium over a wide stimulation range approaching maximal levels of shortening. Calcium infusion produced reversible increases in segment shortening to approximately 142% of control levels. The addition of a second inotropic stimulus, post-extrasystolic potentiation, during the highest level of calcium infusion did not result in further increases in %ΔSS (Figure 6). Postextrasystolic potentiation has been shown to be a potent form of inotropic stimulation. Thus, the lack of further response with PESP suggests that for the loading conditions of these experiments, a near maximal degree of shortening was reached with calcium infusion before and after ischemia.

The maximal response to inotropic stimulation was not significantly reduced by a period of ischemia and reperfusion sufficient to result in severe contractile dysfunction (Figure 4). Furthermore, there was no clear relation between the degree of the postischemic dysfunction and the ability of the depressed myocardium to respond to calcium stimulation. The maximal calcium-induced shortening levels were similar before and after ischemia in both the 5- and 15-minute occlusion groups even though the degree of reperfusion dysfunction was different (65% versus 46% of control values). This result was also apparent within a group of animals receiving the same duration of ischemia. An animal of the 15-minute occlusion group exhibited systolic bulging (−9% ΔSS) throughout the reperfusion period. However, with calcium infusion, shortening increased to within 98% of the maximal level obtained before ischemia, similar to animals with much less injury. These data demonstrate that in the 15-minute ischemia model the stunning process results in a lowering of the contractile state without a reduction in the contractile reserve.

It can be argued that inotropic-induced increases in regional function of depressed, reperfused myocardium is achieved only at a much higher energy cost. In the present study, calcium infusion resulted in increases in regional ventricular function and increases in coronary blood flow. It has been shown in a variety of preparations that a near linear relation exists between coronary blood flow and myocardial oxygen consumption. This relation has also been demonstrated during increases in ventricular function and oxygen consump-
tion in response to hypercalcemia.40 Decreases in contractile function in postischemic myocardium has been shown to be associated with reductions in coronary flow and regional oxygen consumption,41 suggesting that these two variables remain coupled in reperfused myocardium. If increases in contractile function in reperfused myocardium occur at a higher energy cost, one would expect an elevated coronary flow for a given level of function (assuming an unchanged oxygen extraction). In the present study, the relation of coronary flow to segment shortening was similar before and after transient ischemia (Figure 9). An increase in segment shortening to preischemic levels was associated with a return of coronary blood flow toward control from a reduced postischemic value. In a similar manner, at the highest calcium dose when shortening was equal in the preischemic and postischemic trial, coronary flow was also not different. It is possible that the coronary flow responses were influenced by a direct effect of calcium on coronary vascular resistance. Although it is presently controversial, calcium has been shown to produce a small vasoconstriction in isolated coronary vessels, in isolated hearts, and in the intact dog.42 A major role of this mechanism in the interpretation of the present study is unlikely since a differential effect of calcium on the vasculature of normal and postischemic myocardium would have to be postulated. Thus, the data presented here suggest that the increases in the contractile function in reperfused myocardium during calcium stimulation were probably not achieved at a higher energy cost compared to normal myocardium.

Possible Mechanisms of Stunning

The present hypotheses proposed to explain the mechanism responsible for the low level of contractile function in reperfused but not irreversibly damaged myocardium can be conveniently divided into 3 categories: 1) those related to the energetic state or energy supply to the contractile components, 2) those related to excitation or excitation-contraction coupling, and 3) those related to damage of the contractile elements.

Numerous studies have demonstrated that ischemia is associated with a decrease in myocardial tissue levels of adenine nucleotides and creatine phosphate.21,43-45 If reperfusion is instituted within a short period of time, irreversible cellular injury does not result.1 Although creatine phosphate levels quickly return to or above control levels with reperfusion, levels of ATP and total adenine nucleotides remain depressed for hours to days.10,13,15,17 This slow rate of repletion is believed to be a result of the cellular loss of nucleotide precursors during ischemia15,18 and the relatively slow process of de novo synthesis.16,43,44 It has been reported that a close correlation exists between myocardial ATP levels and ventricular function in isolated hearts subjected to hypoxia or ischemia.19,45 Furthermore, the repletion of the adenine nucleotide pool has been shown to parallel the return of normal contractile function46 in postischemic hearts. These data have suggested a causal relation between the energy status and myocardial function in postischemic hearts. Thus, it has been advanced that postischemic dysfunction is the result of an inadequate or defective energy supply to the contractile process.9,10 The results of the present experiments argue against this concept. Postischemic myocardium with depressed function due to an energy deficiency could respond to inotropic stimulation to some degree ("whipping a tired horse" analogy). However, it is unlikely that myocardium limited by its energy supply could reach the same maximal level of contraction exhibited by normal myocardium independent of the severity of the ischemia-induced contractile damage and maintain an enhanced level for a prolonged period without further functional damage. For similar reasons, it is unlikely that postischemic dysfunction is the result of cellular injury at the level of the contractile filaments. The same maximal contractile capability before and after ischemia necessarily implies an intact contractile apparatus.

There is accumulating evidence that myocardial ischemia is associated with abnormalities in excitation or excitation-contraction coupling with damage to cellular homeostatic mechanisms for the control of intracellular calcium levels.19,46 This loss of calcium regulation and subsequent intracellular calcium accumulation is associated with the transition from reversible injury to irreversible cellular damage.47 Although the precise mechanisms responsible for this phenomenon is not clear, recent in vitro studies have suggested that defects in the calcium transport mechanisms of the sarcolemmal membrane or sarcoplasmic reticulum may be involved. Reductions in the rate of ATP-dependent calcium transport and adenylate cyclase activity have been demonstrated in sarcolemmal membrane vesicles isolated from postischemic hearts.22 Also, ischemia-induced abnormalities in the ability of sarcoplasmic reticulum to transport calcium in preparations of whole heart homogenates and isolated cardiac sarcoplasmic reticulum47-51 have been reported and attributed to the effects of acidosis and oxygen free radical generation.49 Recent in vivo studies have also indicated that oxygen free radicals may play a role in the generation of postischemic dysfunction.48-50 Residual abnormalities in myocardial cell excitation may also contribute to the stunning phenomenon. Decreases in action potential amplitude, dV/dt, and conduction velocity in postischemic myocardium have been recently reported.51

The data from the present experiments are consistent with a hypothesis that postischemic dysfunction in the absence of irreversible injury has as its basis a defect in some component of excitation or excitation-contraction coupling resulting in a reduction in the amount of calcium available for myocardial activation during systole. Elevation of the extracellular levels of calcium by exogenous administration was capable of returning contractile function to normal and preischemic maximal levels. Thus, in contrast to the conditions during ischemia where calcium overload becomes a problem, these data would indicate that a relative calcium deficiency exists in reperfused, stunned myo-
Cardiac. Possible mechanisms responsible for this effect include a depression in the transsarcolemmal calcium flux (channel or pump mediated) or a reduction in the amount of calcium stored in the sarcoplasmic reticulum and released during systole. It is also possible that the calcium sensitivity of the contractile proteins has been altered. However, the observation of an unchanged apparent calcium sensitivity in postischemic myocardium (Figure 8) would argue against this mechanism playing a major role. The results from this study cannot clearly differentiate between these possibilities. Nonetheless, the data indicate that the mechanism responsible for postischemic dysfunction should act to reduce contractile function without alterations in the contractile reserve, apparent sensitivity to calcium, or coupling of myocardial function to coronary flow.

In conclusion, these data demonstrate that stunned myocardium exhibiting depressed contractility following 5 or 15 minutes of coronary occlusion still retains a normal contractile reserve in response to inotropic stimulation with calcium. These calcium-induced increases in function occur in the absence of major changes in preload, afterload, and heart rate and are independent of the severity of the postischemic dysfunction. The apparent calcium sensitivity and relation of function to coronary flow of reperfused myocardium is similar to that in normal myocardium. These results are consistent with a hypothesis that the mechanism behind postischemic dysfunction involves calcium, possibly due to a defect in excitation or excitation-contraction coupling in stunned myocardium.

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Key Words • calcium • postischemic dysfunction • stunned myocardium
Reversibly injured, postischemic canine myocardium retains normal contractile reserve.
B R Ito, H Tate, M Kobayashi and W Schaper

Circ Res. 1987;61:834-846
doi: 10.1161/01.RES.61.6.834

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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