Metabolic Protection by Verapamil During Graded Coronary Flow Reduction Independent of Effect on Baseline Systolic Function

Separation of Mechanical and Ionic Markers of Ischemia

Mark G. Jenkins, MD, Timothy A. Johnson, PhD, Connie Engle, AAS, and Leonard S. Gettes, MD

Pretreatment with the calcium channel-blocking agent verapamil lowers the coronary flow associated with the first rise in myocardial extracellular potassium ([K+]e). The mechanisms underlying this effect are unclear. It is not known whether this effect is a manifestation of verapamil-induced reduction in baseline cardiac work before the reduction in coronary flow, is dependent on a selective depression of contractility within the low-flow region, or is independent of an effect on myocardial work. This study was performed to determine the relations between changes in regional contractility and [K+]e, before and after verapamil (0.2 mg/kg followed by 6.5 μg/kg/min) when left anterior descending (LAD) coronary flow is progressively reduced and when verapamil-induced alterations in baseline myocardial work are prevented by atrial pacing and by dobutamine (4.3±2.2 μg/kg/min) to maintain systemic arterial blood pressure and contractility. Before verapamil-dobutamine, myocardial [K+]e rose and regional contractility fell when LAD coronary flow was reduced to 87.7±9.6% and 83.4±7.4%, respectively, of the unrestricted control value (p=NS). After verapamil-dobutamine, the threshold flow for rise in [K+]e, decreased to 56.4±13.5% of the unrestricted control flow (p=0.003), but the threshold flow for regional contractility fall was unchanged (84.8±11.3%). Our results indicate that the protective effect of verapamil on preventing ischemia-induced [K+]e release is not dependent on a reduction in baseline myocardial work. In this setting, calcium channel blockade by verapamil results in a dissociation between the ionic and mechanical events that occur when coronary flow is reduced. (Circulation 1989;80:1870–1877)

Calcium channel antagonists are widely used to treat patients with ischemic heart disease.1 In experimental myocardial ischemia, these agents have been shown to preserve the metabolic state of myocardium,2–12 reduce the degree of ultrastructural cellular damage,12–14 and reduce infarct size.11,14–20 The mechanisms thought responsible for these effects include improved myocardial perfusion secondary to coronary vasodilation21–23; reduced myocardial work due to effects on afterload, heart rate, and regional contractility22–24; and metabolic protection independent of effects on myocardial perfusion and myocardial work.25,26

Previous work in our laboratory27 has shown that an increase in myocardial extracellular potassium [K+]e is a sensitive marker of the onset of myocardial ischemia induced by the graded reduction of coronary flow. This increase was coincident with a fall in myocardial extracellular pH and occurs either with or slightly before a decrease in regional contractility. We have recently observed28 that the flow in the left anterior descending (LAD) coronary artery at which the first rise in [K+]e occurs is reduced by verapamil when heart rate is held constant, indicating a shift in the ischemia threshold as defined by the first rise in [K+]e. We also noted that after verapamil, contractility within the ischemic zone declined before the rise in [K+]e, implying that the verapamil-induced depression of regional contractility may have protected the myocardium within

From the Division of Cardiology, School of Medicine, University of North Carolina at Chapel Hill, North Carolina.

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Address for correspondence: Leonard S. Gettes, MD, Division of Cardiology, CB# 7075, Burnett-Womack Building, University of North Carolina, Chapel Hill, NC 27599.

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the LAD distribution from the metabolic effects of the decreased coronary flow. However, in these experiments, the administration of verapamil followed an initial graded reduction in LAD flow when contractility in the reperfused myocardium had returned to only 91% of its original value. In addition, the intravenous administration of verapamil before the second reduction of LAD flow lowered systemic arterial blood pressure and caused a depression of contractility in the reperfused area that exceeded the depression of contractility in the normal, nonischemic area. Thus, we could not exclude the possibility that the initial reduction in LAD flow “stunned” the myocardium, making it more susceptible to the negative inotropic effects of the drug and that the reduction in contractility prior to the rise in [K⁺], was an artifact of the method rather than a mechanism underlying the apparent shift in the ischemia threshold. For that reason, we have performed experiments designed to assess the effects of verapamil on the ionic and mechanical markers of low-flow ischemia when blood pressure, contractility, and heart rate, the major determinants of cardiac work, were controlled.

Methods

Twelve domestic pigs (weight, 27–39 kg) were immobilized with ketamine (10 mg/kg) and anesthetized with thiamylal sodium (25 mg/kg). Anesthesia was maintained with α-chloralose (30–50 mg/kg) as previously described.27–29 Respiration was maintained with room air supplemented with 100% O₂ through a cuffed endotracheal tube by a Harvard pump adjusted to maintain a pH of 7.4±0.05 and PCO₂ of 35–45 mm Hg. Catheters were placed in the femoral artery and vein for blood pressure monitoring, blood sampling, and drug infusion. Left ventricular pressure and its first derivative (dP/dt) were monitored by a Millar pressure transducer inserted through the left ventricular apex.

After midsternal thoracotomy, the heart was suspended in the pericardium and the right atrium paced at 120 beats/min. The left anterior descending coronary artery was dissected just below its origin, and a 20-MHz pulsed Doppler flow probe was placed around the vessel to record LAD flow velocity. An inflatable balloon occluder (In Vivo Metric Systems) was placed distal to the flow probe and connected to a saline-filled 1-ml syringe in a Harvard infusion apparatus (Figure 1). This permitted the controlled gradual reduction of LAD flow. Regional contractility was determined by measuring systolic thickening with a 10-MHz pulsed Doppler crystal attached to the epicardium in the center of the ischemic zone by cyanoacrylate adhesive. Epicardial temperature was monitored by a Yellow Springs Instruments telemeter probe and maintained at 35–38°C with a heating pad and covering the experimental preparation with a plastic sheet. Heparin (initial bolus of 150 units/kg fol-

allowed by 1,000 units/hr) was administered to prevent thrombosis during coronary flow reduction.

Miniature potassium-selective plunge electrodes were constructed and calibrated by the method described previously from our laboratory.29 Twelve to 18 electrodes were placed in the center of the ischemic zone, and the precise position was determined after each experiment by dissection from surrounding muscle. All electrodes included in this study were located in the midmyocardium (≥3 mm from the epicardial and endocardial surfaces). The amplified signals from the potassium-selective electrodes were sampled every 30 seconds by a DEC PDP-11/03 minicomputer during the TO segment and millivolt readings converted to milliequivalents per liter with the Nernst equation as previously described.29 The integrity of the electrodes in vivo was determined by the rapid intravenous injection of 3 meq KCl before each flow reduction and by the gradual infusion of a 10% KCl solution after the final flow reduction. Data were accepted only from electrodes that demonstrated reproducible responses to each potassium chloride bolus and consistent Nernstian response to potassium chloride infusion. Three to nine (average, 5.9) electrodes per experiment satisfied these requirements; this represented 45% of the total number of electrodes inserted. Blood pressure, left ventricular pressure, dP/dt, systolic thickening, coronary flow velocity, and the output of the potassium-selective electrodes were monitored continuously on a Graphtec Linearcorder and sampled every 30 seconds during balloon inflation at paper speeds of 25 and 50 mm/sec.

Experimental Protocol

The preparation was allowed to stabilize for at least 60 minutes after electrode placement. Reductions in coronary flow were then produced by gradual inflation of the balloon occluder adjusted to reduce flow by approximately 5% a minute. The duration of flow reduction was limited to 5 minutes.
after the onset of potassium rise. Sequential reductions in flow were separated by at least 50 minutes of reperfusion.

Three experiments (group A) were performed to assess the reproducibility of the method. In these animals, three sequential flow reductions were performed. We then performed six experiments (group B) in which verapamil (0.2 mg/kg over 20 minutes followed by 0.0065 mg/kg/min) was administered intravenously before the third flow reduction. Dobutamine was then infused at a rate appropriate to restore contractility in the reperfused myocardium and to maintain systemic arterial blood pressure at the baseline level observed before the verapamil infusion. An additional three experiments (group C) were performed in which verapamil and dobutamine were administered before the initial flow reduction to determine whether the results obtained in the group B experiments could have been influenced by the preceding drug-free flow reductions. The effect of dobutamine infusion alone was examined in two experiments in which no verapamil was given. At the end of the experiment in six animals, flow through the Doppler cuff was calibrated by a timed collection of blood. The weight of the ischemic zone was determined in each experiment by injection of monastral blue after ligating the LAD.

Data Analysis

Systolic thickening was measured according to the method of Hartley et al.20 The systolic interval was defined as beginning with the upstroke of dP/dt and terminating 20 msec before peak negative dP/dt. Recordings were made every 30 seconds and corresponded to the sampling of the potassium-selective electrodes. At each 30-second interval, the mean systolic thickening from four consecutive beats was determined. Baseline systolic thickening was defined as the mean of four such samples taken before flow reduction. Flow measurements are reported as the percentage of unrestricted flow just before flow reduction. Our criteria for threshold changes in potassium and regional contractility were as previously described27 (i.e., an increase in extracellular potassium of ≥0.3 mM and a 10% reduction in systolic thickening). The flow for the threshold rise in [K+] was determined for each electrode. The threshold flow for the electrode showing the earliest change in [K+] and the mean threshold flow of all the electrodes in each experiment (referred to as EK and MK, respectively) were determined separately and compared in the preverapamil and postverapamil flow reductions. The flow associated with the threshold change in systolic thickening (referred to as regional contractility [RC]) was also compared for the preverapamil and postverapamil flow reductions. Unless otherwise noted, data are presented as mean±SD. Statistical evaluation was performed with Student's t test for paired data. A p value of 0.05 or less was considered statistically significant. Statistical analysis is not reported for experiments in groups A and C because of the small number of experiments (n=3 in each group).

Results

Effects of Serial Flow Reductions on Threshold Measurements

The reproducibility of the threshold flow for changes in [K+] and regional contractility were examined in the three experiments of group A. In each of these experiments, three sequential flow reductions were performed without drug administration. Table 1 shows the mean values of serum potassium, heart rate, systolic blood pressure, left ventricular end-diastolic pressure, dP/dt, and regional contractility before each flow reduction. A slight reduction in dP/dt was observed with successive flow reductions, and a slight decline in systolic blood pressure occurred after the initial flow reduction. Figure 2 shows the threshold flows for the

<table>
<thead>
<tr>
<th>CFR</th>
<th>Serum K (meq/L)</th>
<th>HR (beats/min)</th>
<th>SBP (mm Hg)</th>
<th>EDP (mm Hg)</th>
<th>dP/dt (mm Hg/sec)</th>
<th>RC (%) baseline</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3.9±0.4</td>
<td>120</td>
<td>119±10</td>
<td>10.7±1.2</td>
<td>1,333±189</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>3.8±0.5</td>
<td>120</td>
<td>109±7</td>
<td>10.7±1.2</td>
<td>1,267±76</td>
<td>100±14</td>
</tr>
<tr>
<td>3</td>
<td>3.9±0.5</td>
<td>120</td>
<td>107±16</td>
<td>10.7±1.2</td>
<td>1,233±175</td>
<td>109±5</td>
</tr>
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</table>

Values are given as mean±SD.

CFR, coronary flow reduction; SBP, systolic blood pressure; EDP, left ventricular end-diastolic pressure; RC, regional contractility.

FIGURE 2. Bar graphs of group A experiments (n=3): Threshold flow expressed as percent of baseline flow for initial change in regional contractility (RC threshold), earliest changing potassium electrode (EK threshold), and mean of all potassium electrodes (MK threshold). CFR, coronary flow reduction.
Effects of Verapamil During Serial Flow Reductions

Table 2 shows the mean values of serum potassium, heart rate, systolic blood pressure, left ventricular end-diastolic pressure, dP/dt, and regional contractility before the control and verapamil-dobutamine flow reductions, as determined in the six group B experiments. There were no differences in serum potassium or heart rate during successive flow reductions. A 6.7 mm Hg decline in systolic blood pressure was apparent from the first and second control flow reductions (p=0.003), but systolic blood pressure was the same in the second control flow reduction and the third (verapamil-dobutamine) flow reduction. Left ventricular end-diastolic pressure was the same in the two control flow reductions and increased by 3.8 mm Hg after infusion of verapamil and dobutamine (p=0.02). There was an insignificant decline in dP/dt between the first and second control flow reduction, but the values were similar before the second control flow reduction and the third (verapamil-dobutamine) flow reduction. Regional contractility was similar before each flow reduction. Also, baseline unrestricted flow before each flow reduction was not significantly different. Restoration of regional contractility after administration of verapamil required 4.3±2.2 μg/Kg/min dobutamine.

The threshold flows for the initial decline in regional contractility and the initial rise in [K⁺]ₑ in these experiments are shown in Figure 3. The threshold flow for decline in regional contractility was similar during the two control flow reductions (84.1±6.1% vs. 83.4±7.4%, p=NS). The threshold flow for the rise in [K⁺]ₑ as determined from all the electrodes was also similar during the two control flow reductions. In addition, there were no significant differences between the threshold flow for either the earliest or mean rise in [K⁺]ₑ and fall in regional contractility during each control flow reduction.

During the verapamil-dobutamine flow reduction, the threshold flow for the decline in regional contractility occurred at 84.8±11.3% of unrestricted flow, not significantly different from the control flow reduction. However, the threshold flow for the initial rise in [K⁺]ₑ was reduced to 56.4±13.5% of unrestricted flow as determined from the earliest changing electrode (p=0.003) and to a mean of 51.5±11.5% of unrestricted flow as determined from all [K⁺]ₑ electrodes (p=0.002). The threshold flow for the rise in [K⁺]ₑ was significantly lower than the threshold flow for decline in regional contractility during the verapamil-dobutamine flow reduction.

Figure 4A shows the average change in extracellular potassium recorded from the earliest changing electrode for all six group B experiments during the second control flow reduction and the third (verapamil-dobutamine) flow reduction. Flow was...
FIGURE 4. Top panel: Mean [K\(^+\)]\(_e\), rise (±SEM) recorded from the earliest changing electrodes during the second (control) and third (verapamil-dobutamine) flow reductions in group B. Bottom panel: Mean (±SEM) changes in regional contractility during the second (control) and third (verapamil-dobutamine) flow reductions in group B. CFR, coronary flow reduction.

Reduced to only 65% of unrestricted flow during the second control flow reduction to limit the duration of ischemia to 5 minutes after the initial rise in [K\(^+\)]\(_e\) occurred. At each level of flow below 95% of baseline, the rise in extracellular potassium was significantly lower after verapamil-dobutamine. Thus, although systolic blood pressure, heart rate, and regional contractility, the major determinants of cardiac work, were the same before the second and third flow reductions, verapamil shifted to lower levels the coronary flow associated with a given rise in myocardial extracellular potassium.

Figure 4B shows the average change in regional contractility at each level of coronary flow during the second (control) and third (verapamil-dobutamine) flow reduction in the group B experiments. The progressive reduction in coronary flow was associated with a progressive decline in regional contractility during both flow reductions. There were no significant differences in systolic thickening between the control and verapamil-dobutamine flow reductions at each level of flow.

Figure 5 shows the relation between the change in [K\(^+\)]\(_e\), recorded from the earliest changing electrode and the decline in regional contractility during the Group B experiments. During the second control flow reduction, the rise in [K\(^+\)]\(_e\) and the decline in regional contractility are related such that each 0.5 meq/L rise in [K\(^+\)]\(_e\) is associated with a decline in regional contractility of approximately 15%. During the verapamil-dobutamine flow reduction, regional contractility decreased by 65% before [K\(^+\)]\(_e\) rose by 0.5 meq/L. Thereafter, [K\(^+\)]\(_e\) rises more rapidly such that a 1.5 meq/L rise is associated with each 15% further decline in regional contractility.

Effects of Verapamil During the Initial Flow Reduction

The possibility that the effects of verapamil-dobutamine were influenced by the preceding control flow reductions was tested in the three group C experiments. In these experiments, verapamil and dobutamine were given before the initial flow reduction. Figure 6 shows that, as in the group B experiments when verapamil and dobutamine were administered after the two control flow reductions, the threshold flow for the earliest change in regional contractility (87.1±4.3%) was higher than the threshold flows for the electrodes showing the earliest rise in [K\(^+\)]\(_e\) (55.2±9.2%) or the mean value for all electrodes (43.2±17.5%). These values were similar to those obtained during the third (verapamil-dobutamine) flow reduction in the group B experiments and indicate that the previous periods of

FIGURE 5. Plot of relation of change in regional contractility to change in [K\(^+\)]\(_e\), (ΔSK) during the second (control) and third (verapamil-dobutamine) flow reduction in group B. CFR, coronary flow reduction.

FIGURE 6. Bar graph of group C experiments (n=3). Threshold flow for initial change in regional contractility (RC threshold), earliest changing potassium electrode (EK threshold), and mean of all potassium electrodes (MK threshold) during the initial (verapamil-dobutamine) flow reduction.
ischemia did not influence the effects of verapamil in the group B experiments.

**Effect of Dobutamine on Threshold Measurements**

The effect of dobutamine alone was examined in two of the group A experiments by administering dobutamine after the third control flow reduction. The infusion rate was adjusted to produce a doubling of dP/dt. This required a dobutamine infusion of 2.3 μg/kg/min and resulted in a rise in systolic blood pressure from 103 to 118 mm Hg, an increase in dP/dt from 1,150 to 2,380 mm Hg/sec, and a 20% increase in LAD flow. During the subsequent (fourth) flow reduction, the initial rise in [K+]e and decline in regional contractility occurred at similar degrees of flow reduction (contractility threshold flow, 87.8%; earliest changing potassium electrode threshold flow, 87.8%; mean potassium threshold flow of all electrodes, 81.2% of unrestricted flow).

**Flow Calibration**

The ischemic zone perfused by the LAD distal to the occluder represented 45% of the total left ventricular mass. The estimated flow to this zone as determined by timed collection was 1.02 ml/min/g.

**Discussion**

The purpose of the present study was to examine the effects of verapamil on the mechanical and ionic events associated with coronary flow reduction when its effects on reducing baseline myocardial work were prevented by pacing to control the heart rate and by infusing dobutamine to restore blood pressure and regional contractility to their preverapamil levels. It is unlikely that verapamil influenced myocardial perfusion through collateral channels because the porcine heart has negligible collateral circulation.31,32 Furthermore, studies during acute ischemia in the dog, which has extensive coronary collaterals, have shown no effect of verapamil on ischemic zone perfusion.19,20,23,33–35 After verapamil and dobutamine, the 3.8 mm Hg rise in end-diastolic pressure indicates that pressure development equivalent to control occurred at a higher preload; this suggests that some residual negative inotropic effect of verapamil persisted. Although we cannot exclude the possibility that this contributed to our results, it does not alter our conclusions regarding the effect of verapamil when the major determinants of myocardial work (i.e., blood pressure, heart rate, and regional contractility) are restored to baseline.

We observed that in the absence of verapamil, the rise in [K+]e and fall in regional contractility occurred at similar levels of reduced flow. This suggests an association between the mechanical and the ionic events that occur during coronary flow reduction. These events are believed to result when anaerobic metabolism leads to the hydrolysis of high energy phosphates and the development of intracellular acidosis.36–38

After verapamil in the presence of dobutamine, the threshold flow for a rise in [K+]e was significantly reduced. This effect cannot be explained on the basis of a reduction in baseline myocardial work. The threshold flow for a reduction in regional contractility was not altered and occurred at a significantly higher flow than the initial rise in [K+]e. Thus, treatment with verapamil resulted in a dissociation between the mechanical and ionic markers of myocardial ischemia.

The presence of a protective effect of calcium channel blockade that is independent of tension development is supported by recent experiments with noncontracting heart preparations. Watts et al25 showed that administration of diltiazem to isolated perfused noncontracting rat hearts reduced the lactate production and the reduction in ATP associated with induced ischemia. Henry and Wahl26 showed that diltiazem and nitrendipine administered to noncontracting rabbit hearts significantly delayed the development of hypoxic contracture, a finding consistent with the preservation of high energy phosphate required to relax actomyosin cross-bridges. Our results with an in situ porcine heart preparation are consistent with these studies.

Whether enhancement of ischemia-induced contractile dysfunction is a means by which verapamil protects against ischemic injury is controversial. Smith et al39 lowered LAD flow in dogs to a level causing reduced contractility and then administered verapamil. They found that verapamil depressed contractility in the ischemic myocardium at doses that had no significant effect on contractility in the nonischemic region. They postulated that the selective depression of ischemic zone contractility would reduce myocardial oxygen consumption and thereby lessen ischemic injury. Romson et al40 pretreated dogs with verapamil before the graded reduction of left circumflex coronary artery flow and found that despite its negative inotropic effect during unrestricted flow, pretreatment with verapamil did not exaggerate contractile dysfunction as flow was lowered. They argued that enhancement of ischemia-induced mechanical dysfunction was not a mechanism by which verapamil protected against the effects of ischemia. In both of these studies, the hemodynamic effects of verapamil were not controlled and an index of ischemia other than contractility was not monitored. If enhancement of ischemia-induced mechanical dysfunction had been a major mechanism underlying verapamil’s effect on [K+]e rise in our experiments, we would have expected an exaggerated reduction in regional contractility compared with the control flow reduction. Our results (Figure 4, bottom panel) indicate that this was not the case. The fact that regional contractility was similar at each level of reduced flow in the control and verapamil-dobutamine flow reductions suggests that a mechanism unrelated to enhancement of ischemia-induced mechanical dysfunction was active.
The dissociation between the mechanical and ionic markers of ischemia after verapamil was an unexpected finding. It is possible that this represents a differential sensitivity of the contractile apparatus and the ionic homeostatic mechanisms to reduced coronary flow that were uncovered by verapamil. Recent studies by Marban et al.41 with gated nuclear magnetic resonance spectroscopy suggest that this may occur. These investigators measured changes in contractile force, intracellular calcium concentration, and inorganic phosphate concentration during the cardiac cycle in isolated ferret hearts. Their results showed that a moderate reduction in coronary flow caused a decline in the excitation-induced intracellular calcium transient and tension development without a significant buildup of inorganic phosphate. It is possible that the verapamil-induced block of the voltage-dependent calcium channel augments the reduction in the excitation-induced calcium transient that occurs when coronary flow is reduced causing contractile dysfunction to occur with less reduction in high-energy phosphate than in the control setting. This would explain our observation that regional contractility fell without a concomitant rise in [K+]e during the early phase of flow reduction but would not explain the finding that the decrease in regional contractility occurred at the same flow reduction before and after verapamil. An alternative explanation is that the decline in contractility is a mechanical effect related to the collapse of the microvasculature induced by the reduction in coronary perfusion pressure and not a consequence of ischemia. This decline in contractility would, thus, be a manifestation of the “garden hose effect” as described by Lochner et al.42 It is possible that even in the absence of drug, the decline in contractility during the early phase of flow reduction is beneficial, lessening the energy requirements for tension development and allowing more energy for other vital metabolic processes. Verapamil, when administered in association with dobutamine, does not appear to influence the contractile change associated with the reduction of flow but permits a greater degree of flow reduction before the appearance of the metabolic abnormalities that characterize the ischemic process. Thus, verapamil appears to separate a flow-dependent reduction in regional contraction from a flow-dependent effect on membrane function. Further experiments to determine the effect of verapamil on high-energy phosphates, intracellular calcium, and intracellular hydrogen during the progressive reduction in coronary flow are needed to test the validity of these hypotheses and to determine if these effects are peculiar to verapamil or are characteristic of other calcium channel antagonists.

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