Effects of nifedipine on diastolic function during brief periods of flow-limiting ischemia in the conscious dog

ROBERT J. APPELEGATE, M.D., RICHARD A. WALSH, M.D., AND ROBERT A. O’ROURKE, M.D.

ABSTRACT To determine the contribution of transsarcolemmal calcium flux to abnormal diastolic function produced by brief periods of flow-limiting ischemia and reperfusion, we evaluated early and late diastolic function during transient coronary occlusion and reperfusion before and during administration of intravenous nifedipine (NIF) (10 ± 1 μg/kg/min) in nine preinstrumented conscious dogs. We also assessed the effects of nitropressure (NTP) (2 ± 0.2 μg/kg/min) during an identical period of ischemia and reperfusion to independently assess the consequences of altered loading alone on diastolic function. To minimize the effects of temporal dysynchrony and altered ventricular loading conditions on isovolumetric relaxation, we developed a conscious dog preparation of reversible transient (30 to 60 sec) bilateral coronary occlusion (BCO). BCO was characterized by significant systolic depression: maximum (+)dP/dt decreased (from 2617 ± 600 to 1981 ± 565 mm Hg/sec, p < .05), left ventricular transverse dimension shortening diminished (from 20 ± 5 to 9 ± 5 %, p < .05), and the left ventricle dilated (42.4 ± 6.4 to 43.8 ± 6.3 mm, p < .05). Concomitantly the time constants of isovolumetric relaxation prolonged (from 22 ± 3 to 28 ± 4 msec, p < .05) and minimal diastolic left ventricular pressure increased (from -3 ± 6 to 6 ± 6 mm Hg, p < .05). The passive diastolic pressure-dimension relationship shifted upward and to the right and was associated with increased chamber stiffness (from 0.50 ± 0.26 to 1.03 ± 0.53 mm Hg/mm, p < .05) and increased left ventricular end-diastolic pressure (from 7 ± 7 to 19 ± 7 mm Hg, p < .05). Reperfusion immediately after BCO was characterized by prompt restoration of systolic contractile performance [maximum (+)dP/dt 3220 ± 530 mm Hg/sec] but persistently abnormal early and late diastolic function (time constant of isovolumetric relaxation 30 ± 6 msec, left ventricular end-diastolic pressure 20 ± 7 mm Hg). The effects of drug administration on ventricular function during BCO were then evaluated under matched loading conditions. NTP improved time constant of isovolumetric relaxation (20 ± 8 vs 28 ± 4 msec, p < .05) and minimal diastolic left ventricular pressure (2 ± 5 vs 6 ± 6 mm Hg, p < .05) during BCO, but NIF did not (time constant of isovolumetric relaxation 27 ± 6 msec, minimal diastolic left ventricular pressure 7 ± 5 mm Hg). NIF also failed to improve passive chamber stiffness during BCO (1.24 ± 0.4 vs 1.03 ± 0.53 mm Hg/mm, p = NS), while NTP reduced heart size during BCO and reduced chamber stiffness (0.84 ± 0.39 mm Hg/mm). During reperfusion NIF similarly failed to improve indexes of diastolic function. We conclude that during transient flow-limiting ischemia and reperfusion (1) abnormal active and passive diastolic properties are not related to excess calcium-channel transcellular calcium flux, and (2) preload reduction improves isovolumetric relaxation and ameliorates early diastolic dysfunction.


ACUTE ALTERATIONS in left ventricular diastolic function have been reported during pacing- and exercise-induced ischemia in man and animals.1-4 Potential mechanisms for these changes include: impaired ven-

tricular relaxation, altered ventricular geometry, coronary turgor, passive myocardial elastic properties, and ventricular interdependence.5-7 Ischemia-mediated increased cytosolic calcium concentration produced by enhanced transcellular calcium influx or diminished calcium sequestration by the sarcoplasmic reticulum may lead to prolonged actin-myosin crossbridge interaction. This reduced inactivation of contractile proteins may be responsible for the impaired isovolumetric relaxation and apparent increase in diastolic chamber stiffness observed in man and animals during pacing-induced ischemia.1, 2, 8, 9 Clinical stud-
ties have demonstrated improvement of abnormal diastolic function with nifedipine during brief periods of pacing-induced angina. A direct myocardial effect through prevention of calcium overload associated with myocardial ischemia has been postulated as a potential mechanism for this beneficial action of calcium-entry blockade. Direct and indirect evidence exists for increased transcellular calcium flux during prolonged periods of hypoxia, flow-limiting ischemia, and during the reperfusion after prolonged ischemia. However, the existence of abnormal calcium flux during brief periods of ischemia is controversial and its contribution to diastolic dysfunction remains unclear. If increased transcellular calcium flux is responsible for part or all of the diastolic abnormalities seen during ischemia then prior calcium-entry blockade should restore function or ameliorate the severity of the diastolic dysfunction. However, we have previously demonstrated that calcium-entry blockers improve ventricular relaxation by altered loading conditions and reflex sympathetic activation. These indirect effects of calcium-entry blockade may influence subsequent analysis of relaxation and diastolic left ventricular function independent of any direct effects on myocardial calcium influx. Accordingly, this investigation was designed to dissociate the direct effects of calcium-entry blockade on left ventricular performance during brief periods of flow-limiting ischemia from indirect effects secondary to systemic arterial vasodilation and sympathetic reflex stimulation. To address these issues, a conscious dog preparation of reversible global left ventricular flow-limiting ischemia was developed and the effects of prior calcium-entry blockade with nifedipine were evaluated before and after β-adrenergic blockade. Nifedipine was chosen as the representative calcium-entry blocker because of its potent arterial vasodilating property and because of its frequent use in animal and clinical studies that have evaluated the effectiveness of calcium-entry blockade during various forms of hypoxia and ischemia. The contribution of nifedipine-induced systemic vasodilation to altered diastolic function during ischemia was assessed by experiments with equihypotensive infusions of nitroprusside.

Methods

Animal preparation. Nine healthy mongrel dogs (20 to 30 kg) were surgically instrumented for long-term physiologic monitoring by methods previously described for this laboratory. Briefly, after premedication with xylazine and pentobarbital, endotracheal intubation was accomplished and anesthesia was maintained with 1.5% halothane. Polyvinyl 16-gauge catheters were implanted in the descending aorta, the apex of the left ventricle, and left atrium via a left thoracotomy with use of sterile technique. A solid-state micromanometer pressure transducer (P-18 Konigsberg Instruments, Inc., Pasadena, CA) was implanted in the apex of the left ventricle. Two 5 MHz piezoelectric crystals (4 mm in diameter) were placed on the endocardium across the left ventricular anteroposterior minor diameter for continuous measurement of left ventricular internal dimension and global percent shortening. Miniature 2 mm subendocardial ultrasonic segment gauge pairs for measurement of percentage of regional myocardial shortening were implanted in areas perfused by the left anterior descending (LAD) and left circumflex (LCX) coronary arteries. The regional ultrasonic crystal pairs were aligned perpendicular to the long axis and parallel to circumferential hoop fibers of the left ventricular wall close to the endocardium according to the method of Franklin et al.

The proximal portions of the LAD and LCX coronary arteries were exposed, and hydraulic occluders were fitted around the coronary arteries and sutured in place. These polyvinyl circumferential occluders (5 to 7 mm) made in our laboratory allowed reversible complete occlusion of the coronary artery distal to the occluder. Care was taken to prevent obstruction of the coronary arteries in the deflated state. An electromagnetic flow probe was positioned on the LCX coronary artery proximal to the hydraulic occluder on the first three animals to monitor phasic and mean coronary flow and to document completeness of occlusion. Thereafter animals were instrumented without coronary flow probes. The pericardium of each animal was widely opened and left unapossed in each animal. A diagrammatic representation of the study preparation is shown in figure 1. All wires and tubes were tunneled subcutaneously to the back of the neck, and the animals were allowed a minimum of 2 weeks of recovery before experimentation. This protocol received prior approval of our American Association for Accreditation of Laboratory Animal Care–approved Lab Animal Research Committee.

Data collection. Each study was performed with the dog lying on its right side unrestrained in a hammock. The solid-state micromanometers were checked and calibrated before implantation with a standard mercury manometer. Before each study, the left ventricular and aortic catheters were connected to Statham P23dB external pressure transducers precalibrated with a mercury manometer with the vertebral column at atmospheric pressure as the zero reference point. Each day zero drift of the system was corrected by matching the left ventricular end-diastolic pressure signal obtained by the solid-state system to the left ventricular end-diastolic pressure measured simultaneously through the left ventricular catheter. The first derivative of left ventricular pressure was obtained by on-line analog-to-digital conversion and differentiation of the high-fidelity analog left ventricular pressure signal with an IBM PC AT microprocessor. Mean aortic pressure was derived electronically. Coronary phasic flow was recorded with a Zepeda EDP 2 square-wave flowmeter (Zepeda Instruments, Seattle). Zero coronary flow was determined by complete inflation of the hydraulic occluder. The transit time of ultrasound between the 5 MHz ultrasonic crystals and the 2 mm regional segment crystal pairs was measured with a multichannel sonomicrometer (Schuessler and Associates, San Diego) and was converted to distance, assuming a constant velocity of sound in blood of 1.55 m/sec. The resolution of this system is 0.08 mm with 5 MHz crystals. High-fidelity left ventricular pressure, the first derivative of high-fidelity left ventricular pressure (dp/dt), aortic pressure, coronary flow, the left ventricular minor axis dimension, apical and basal regional segment lengths, and a surface electrocardiogram were recorded on an eight-channel forced-ink pen oscillograph (Beckman Instruments, Inc., Fullerton, CA) at a paper speed of 25 mm/sec, with an average of 15 to 25 consecutive cardiac cycles recorded.
The analog signals were also digitized with an on-line analog-to-digital convertor (Dual Control Systems, Los Angeles) at 5 msec intervals and stored on floppy disks with the use of a microcomputer system (DEC PDP or IBM PC AT).

**Data analysis.** Data were analyzed by means of computer algorithm. End-diastolic transverse dimension and segment length were defined as the distance between ultrasonic crystal pairs at the Z point of the high-fidelity left ventricular pressure signal or at the left ventricular pressure at which (+)dP/dt increased by 150 mm Hg/sec for at least 50 msec when no A wave was present at higher heart rates. End-systolic lengths were measured at the maximum intercrystal distance before maximum (−)dP/dt, which corresponds to maximal segmental shortening during left ventricular ejection in the normal left ventricle. Percentage of shortening of the left ventricular transverse and regional segment lengths were calculated as the end-diastolic length minus the end-systolic length divided by the end-diastolic length times 100.

Signals for 15 to 25 sinus beats were averaged for each data point, except during occlusion and reperfusion when single beats were analyzed to permit matching of loading conditions. Post-predrain beats and beats that varied more than 10% in cycle length from the average heart rate were excluded from analyses.

The decay of left ventricular pressure during isovolumic relaxation can be described by a monoexponential fit of the form P(t) = P₀×e⁻⁵ᵗ. The isovolumetric relaxation period was defined as the time from maximum (−)dP/dt to 5 mm Hg above end-diastolic pressure of the same beat to provide an isovolumetric period beginning after aortic valve closure and ending before mitral valve opening. The time constant of isovolumetric relaxation (T) was computed by the methods of both Weiss et al. and Raff and Glantz. T was calculated from the linear regression of the natural log (ln) of pressure vs time for the points obtained every 5 msec during the isovolumetric period. The line described has a slope of (−)⁻¹/T. The correlation coefficient for the plots of ln pressure vs time was 0.97 (mean = 0.97 ± 0.01 SD) in all cases. However, this method has been shown to be sensitive to errors in the absolute value of pressure due to pericardial or pleural changes. Therefore, T was also derived by the method of Raff and Glantz, who demonstrated that dP/dt is a linear function of pressure, with a slope of (−)⁻¹/T, regardless of the magnitude of the baseline shift. T was defined as the negative reciprocal of the slope of the linear regression of dP/dt vs pressure for the points obtained during the isovolumetric period. The correlation coefficient for the plots of dP/dt vs pressure was 0.91 in all cases (mean = 0.97 ± 0.02 SD). The linear regression of pressure and dP/dt was also characterized by its pressure-axis intercept. This extrapolated baseline pressure calculated at dP/dt = 0 theoretically represents the asymptote to which pressure would fall if dissipation of force continued infinitely, and has been reported as a measure of the extent of relaxation.

Left ventricular dynamic elastic chamber stiffness was evaluated by examining left ventricular pressure-volume (P-V) relationships during the period from minimum diastolic pressure to the peak of the A wave. Data obtained at 5 msec intervals during this period were fit to a monoexponential function P = be⁻⁵⁺C, where P = left ventricular pressure; b = left ventricular pressure intercept; k = chamber stiffness; D = chamber dimension; and C = empirically derived constant. In three animals the left ventricular P-V relationship during ischemia approached a linear relationship and their data were excluded from analysis of chamber stiffness.

**Protocols.** Baseline hemodynamic recordings were made at rest before drug administration or occlusion of the coronary arteries. Simultaneous inflation of the hydraulic occluders on both the LAD artery and LCX coronary artery was then performed with a Y connector and sustained for 30 to 60 sec to obtain a left coronary end-diastolic pressure of 15 to 20 mm Hg. Bilateral coronary occlusion (BCO) rather than single-vessel coronary occlusion was used to elicit a severe ischemic response similar to that seen during prolonged periods of ischemia, and to minimize the effects of dysynchronous segmental relaxation on global variables of isovolumic relaxation seen with single-vessel occlusion or hypoxic and normal muscles contracting in series. Additionally, since it has been shown that the load-
Table 1
Left ventricular minor-axis dimensions and regional segmental lengths at rest and during brief periods of ischemia (n = 9)

<table>
<thead>
<tr>
<th></th>
<th>End-diastole (mm)</th>
<th>End-systole (mm)</th>
<th>%S</th>
<th>%Δ%S (Rest vs Isch)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Isch</td>
<td>Rest</td>
<td>Isch</td>
</tr>
<tr>
<td>LV minor axis</td>
<td>42.2 ± 6.4</td>
<td>43.8 ± 6.3^a</td>
<td>33.9 ± 6.5</td>
<td>39.7 ± 6.2^a</td>
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<tr>
<td>Regional segment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>12.1 ± 2.2</td>
<td>12.7 ± 2.4^a</td>
<td>10.6 ± 2.1</td>
<td>12.1 ± 2.2^a</td>
</tr>
<tr>
<td>Apical</td>
<td>12.9 ± 4.3</td>
<td>13.5 ± 5.2^a</td>
<td>9.9 ± 3.0</td>
<td>11.8 ± 4.0^a</td>
</tr>
</tbody>
</table>

Values are the mean ± SD.

Isch = ischemia during BCO; LV = left ventricular; %S = percent shortening; %Δ%S = percent change in percentage shortening.

*p < .05 vs rest.

Results

Ischemia. A representative analog recording obtained at control, during a coronary occlusion, and during occlusion after pretreatment with nifedipine is shown in figure 2. Within seconds of the onset of occlusion peak left ventricular pressure and (+)dP/dt fell, left ventricular end-diastolic pressure rose, and heart rate increased. Both left ventricular minor-axis end-systolic dimension and basal and apical end-systolic segment length in the ischemic zones increased significantly. This was accompanied by an increase in left ventricular minor-axis end-diastolic dimension and basal and apical end-diastolic segment lengths. Left ventricular minor-axis dimension and both the apical and basal ischemic segments showed a corresponding equivalent reduction in shortening. Qualitatively similar changes were seen after nifedipine.

Hemodynamic variables at rest and during BCO are shown in table 2. By experimental design nifedipine and nitroprusside caused equivalent reductions in mean arterial pressure (96 ± 19 to 83 ± 17 vs 96 ± 19 to 81 ± 15 mm Hg, respectively) and in left ventricular end-systolic pressures (59 ± 15 to 49 ± 16 vs 58 ± 15 to 49 ± 17 mm Hg, respectively). Heart rate was also similar after nifedipine and nitroprusside (128 ± 27 vs 129 ± 26 beats/min). Drug administration resulted in a significant but small reduction in left ventricular end-diastolic pressure after nifedipine (7 ± 7 to 4 ± 6 mm Hg) and a greater fall after nitroprusside (8 ± 7 to 1 ± 4 mm Hg). Left ventricular minor-axis dimension declined at rest after nifedipine and nitroprusside. Neither left ventricular minor-axis shortening (AP%$S$) nor maximum (+)dP/dt were significantly altered by administration of either drug. Thus, in dogs...
at rest the two drugs had similar effects on afterload and contractile function and both reduced preload, although nitroprusside was associated with a more substantial reduction in end-diastolic pressure.

Ischemia resulted in a significant reduction in mean arterial pressure from control (96 ± 17 to 85 ± 22 mm Hg), and during infusion of nifedipine (83 ± 17 to 72 ± 18 mm Hg), but not during infusion of nitroprusside (81 ± 15 to 76 ± 15 mm Hg). However, left ventricular end-systolic pressure was equivalent after nifedipine and nitroprusside during ischemia (38 ± 15 vs 40 ± 15 mm Hg), as was heart rate (166 ± 25 vs 165 ± 16 beats/min). Left ventricular end-diastolic pressure increased significantly during BCO at control (7 ± 7 to 19 ± 7 mm Hg, p < .05). Left ventricular end-diastolic pressure was similar during the control BCO and after BCO plus nifedipine, but was significantly lower after nitroprusside plus BCO (13 ± 7 vs 18 ± 6 mm Hg after nifedipine). Heart size increased and segmental shortening decreased during ischemia in each dog. Left ventricular end-diastolic dimension increased during ischemia at control (42.4 ± 6.4 to 43.8 ± 6.3 mm Hg) and after nifedipine (41.2 ± 7.5 to 43.8 ± 6.1 mm Hg) and nitroprusside (39.6 ± 6.6 to 42.1 ± 6.8 mm Hg). Depressed global contractile performance during ischemia was evidenced by a significant fall in maximum (+)dP/dt at control (2617 ± 600 to 1981 ± 565 mm Hg/sec) and during infusion of nifedipine (2638 ± 529 to 1698 ± 525 mm Hg/sec) and nitroprusside (2620 ± 782 to 1983 ± 517 mm Hg/sec). Shortening in both the apical and basal ischemic segments were equivalently reduced under all conditions and paralleled changes in global shortening (table 1). These data indicate that brief periods of occlusion in this model of flow-limiting ischemia result in severe extensive systolic dysfunction, as manifested by an approximately 50% reduction in global and regional shortening, a 25% fall in maximum (+)dP/dt, and an increase in heart size. Neither nifedipine nor nitroprusside significantly improved systolic left ventricular function during ischemia, although heart size was smaller after nitroprusside. In fact, nifedipine further impaired systolic performance during ischemia compared with control or nitroprusside, as evidenced by a significant reduction in maximum (+)dP/dt during BCO (table 2).

The effects of drug administration on left ventricular early diastolic function during ischemia appear in table 3. Peak (−)dP/dt fell during BCO at control (2233 ± 516 to 1454 ± 597 mm Hg/sec) and during infusion of nifedipine (2148 ± 724 to 1240 ± 549 mm Hg/sec) and nitroprusside (2495 ± 469 to 1487 ± 371 mm Hg/sec). Both nifedipine and nitroprusside accelerated the rate of isovolumetric relaxation at rest. T₁ shortened

![FIGURE 2. Representative analog recording depicting pressure, dimension, and segmental length variables at control, during BCO, and during BCO after pretreatment with nifedipine. Occlusion promptly caused significant systolic impairment, as evidenced by decreased left ventricular pressure, dP/dt, and dimension shortening, and was accompanied by increases in end-diastolic chamber size, regional segment lengths, and pressure. Similar qualitative changes were evident after pretreatment with nifedipine.](image-url)
TABLE 2
Hemodynamic variables at rest and during brief periods of ischemia at control and during equihypotensive infusions of nifedipine and nitroprusside (n = 9)

<table>
<thead>
<tr>
<th></th>
<th>HR (bpm)</th>
<th>MAP (mm Hg)</th>
<th>LVESP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>EDD (mm)</th>
<th>AP%S</th>
<th>Maximum (+ dP/dt) (mm Hg/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>90 ± 12</td>
<td>96 ± 19</td>
<td>59 ± 15</td>
<td>7 ± 7</td>
<td>42.4 ± 6.4</td>
<td>20 ± 5</td>
<td>2617 ± 600</td>
</tr>
<tr>
<td>Isch</td>
<td>148 ± 26A</td>
<td>85 ± 22A</td>
<td>45 ± 19A</td>
<td>19 ± 7A</td>
<td>43.8 ± 6.3A</td>
<td>9 ± 5A</td>
<td>1981 ± 565A</td>
</tr>
<tr>
<td>Nifedipine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>128 ± 27B</td>
<td>83 ± 17B</td>
<td>49 ± 16B</td>
<td>4 ± 6B</td>
<td>41.2 ± 7.5B</td>
<td>18 ± 4</td>
<td>2638 ± 529</td>
</tr>
<tr>
<td>Isch</td>
<td>166 ± 25A</td>
<td>72 ± 18A</td>
<td>38 ± 15A,8</td>
<td>18 ± 6A</td>
<td>43.8 ± 6.1A</td>
<td>7 ± 4A</td>
<td>1698 ± 525A,8B</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>129 ± 26B</td>
<td>81 ± 15B</td>
<td>49 ± 17B</td>
<td>1 ± 4B</td>
<td>39.6 ± 6.6B,8C</td>
<td>18 ± 5</td>
<td>2620 ± 782</td>
</tr>
<tr>
<td>Isch</td>
<td>165 ± 16A</td>
<td>76 ± 15B</td>
<td>40 ± 15A</td>
<td>13 ± 7A,8,8C</td>
<td>42.1 ± 6.8A</td>
<td>8 ± 5A</td>
<td>1983 ± 517A</td>
</tr>
</tbody>
</table>

Values are the mean ± SD.

AP%S = anteroposterior left ventricular percentage shortening; EDD = end-diastolic dimension; HR = heart rate; Isch = ischemia during bilateral coronary occlusion; LVESP = left ventricular end-systolic pressure; MAP = mean arterial pressure.

*p < .05 vs rest; †p < .05 vs control; ‡p < .05 vs nifedipine.

from a control value of 22 ± 3 msec to 17 ± 5 msec after nifedipine and to 16 ± 6 msec after nitroprusside. T2 at rest, however, was not significantly altered by nifedipine or nitroprusside. Ischemia alone caused a significant prolongation of T1 at control (22 ± 3 to 28 ± 4 msec), during nifedipine (17 ± 5 to 27 ± 6 msec), and during nitroprusside (16 ± 6 to 20 ± 8 msec). T1 during BCO plus nitroprusside was significantly shorter than at either control or after nifedipine. T2 was prolonged during ischemia at control (35 ± 8 to 68 ± 50 msec) and during nifedipine (33 ± 8 to 69 ± 35 msec) and nitroprusside (33 ± 8 to 53 ± 27 msec).

The extrapolated baseline pressure at rest tended to be lower after nifedipine and nitroprusside than at control, but the differences were not significant. During BCO extrapolated baseline pressure became more negative under all the experimental conditions. Left ventricular minimum diastolic pressure at rest tended to be lower after nifedipine (−5 ± 5 mm Hg) and nitroprusside (−5 ± 5 mm Hg) compared with control (−3 ± 6 mm Hg), but the differences were not significant. Ischemia resulted in a significant increase in minimum left ventricular pressure at control (6 ± 6 mm Hg) and after nifedipine (7 ± 5 mm Hg) and nitroprusside (2 ± 5 mm Hg).

To evaluate late diastolic function the left ventricular P-D relationship from minimum diastolic pressure to the peak of the A wave was plotted at control, during ischemia, and during ischemia after nifedipine and nitroprusside for all nine animals in the study (figure 3). The P-D relationship was shifted upward and to the right during the control BCO in all of the animals and the slope of the P-D relationship during ischemia was steeper than at rest. Similar changes were seen during ischemia after nifedipine. Dynamic chamber stiffness constants (k) were calculated with use of the points from LVP min to the peak of the A wave as depicted in figure 3 and are displayed in table 4. Ischemia resulted in a significant increase in chamber stiffness (0.50 ± 0.26 to 1.03 ± 0.53 mm Hg/mm) that was unaltered by nifedipine (1.24 ± 0.41 mm Hg/mm). The P-D relationships during the BCO plus nitroprusside were shifted upward to the right but less than for
either ischemia alone or ischemia and nifedipine. Similarly, chamber stiffness after nitroprusside during BCO was intermediate between that of control and ischemia.

The occlusion protocol was repeated after β-blockade with propranolol to eliminate the effects of reflex sympathetic stimulation elicited by the vasodilation produced by both nifedipine and nitroprusside. Variables of early diastolic left ventricular function after β-blockade at rest and during ischemia are depicted in Table 5. The impairment in left ventricular relaxation during ischemia at control and after nifedipine and nitroprusside are qualitatively similar to what was observed before β-blockade. Minimum left ventricular pressure, $T_1$, and $T_2$ all increased during ischemia and remained elevated after nifedipine. Nitroprusside resulted in a lower value for all of these variables during ischemia compared with ischemia at control or after nifedipine, indicating that altered loading and not reflex sympathetic stimulation was responsible for the apparent improvement in diastolic function during BCO plus nitroprusside.

**Reperfusion.** To evaluate the effect of nifedipine on reperfusion after transient flow-limiting ischemia data were collected early in the reperfusion period after occlusion in five of the nine animals. A representative analog recording of reperfusion after a control occlusion and reperfusion after nifedipine and nitroprusside is shown in Figure 4. At control the depression in contractile function and blood pressure accompanying BCO was promptly reversed after deflation of the hydraulic occluders and subsequent reperfusion. Of note, in the early phases of reperfusion in this prepa-

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**TABLE 4**
Left ventricular diastolic elastic chamber stiffness constants at rest and during brief periods of ischemia at control and after equihypotensive infusions of nifedipine and nitroprusside

<table>
<thead>
<tr>
<th></th>
<th>$k$ (mm Hg/mm)</th>
<th>$b$ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REST</td>
<td>0.50±0.26</td>
<td>1.18±0.52</td>
</tr>
<tr>
<td>BCO</td>
<td>1.03±0.53$^A$</td>
<td>2.57±1.47</td>
</tr>
<tr>
<td>BCO + NIF</td>
<td>1.24±0.41$^A$</td>
<td>2.89±1.23$^A$</td>
</tr>
<tr>
<td>BCO + NTP</td>
<td>0.84±0.39</td>
<td>1.64±0.77</td>
</tr>
</tbody>
</table>

Values are the mean ± SD.
$k =$ chamber stiffness; $b =$ diastolic left ventricular pressure intercept; NIF = nifedipine; NTP = nitroprusside.

$^A p < .05$ vs rest.
TABLE 5
Left ventricular early diastolic function at rest and during brief periods of ischemia after \( \beta \)-blockade alone and after \( \beta \)-blockade plus equihypotensive infusions of nifedipine and nitroprusside (n = 9)

<table>
<thead>
<tr>
<th></th>
<th>LVP min ( (\text{mm Hg}) )</th>
<th>Maximum ( (\text{mm Hg/sec}) )</th>
<th>( T_1 ) (msec)</th>
<th>( T_2 ) (msec)</th>
<th>( P_e ) (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BB alone</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Rest</td>
<td>(-1.7 \pm 5.1)</td>
<td>(2176 \pm 503)</td>
<td>(23 \pm 4)</td>
<td>(38 \pm 8)</td>
<td>(-21 \pm 10)</td>
</tr>
<tr>
<td>Isch</td>
<td>(9.6 \pm 5.9^A)</td>
<td>(1271 \pm 575^A)</td>
<td>(34 \pm 8^A)</td>
<td>(101 \pm 93)</td>
<td>(-59 \pm 80)</td>
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<td>BB + NIF</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>(-2.2 \pm 4.5)</td>
<td>(2084 \pm 444)</td>
<td>(20 \pm 5)</td>
<td>(35 \pm 10)</td>
<td>(-19 \pm 14)</td>
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<tr>
<td>Isch</td>
<td>(6.6 \pm 5.6^A)</td>
<td>(1085 \pm 467^A,B,D)</td>
<td>(33 \pm 9^A)</td>
<td>(110 \pm 84^A)</td>
<td>(-64 \pm 47^A)</td>
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<tr>
<td>BB + NTP</td>
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<td></td>
</tr>
<tr>
<td>Rest</td>
<td>(-3.1 \pm 5.0)</td>
<td>(2186 \pm 446)</td>
<td>(19 \pm 4^B)</td>
<td>(32 \pm 6)</td>
<td>(-18 \pm 11)</td>
</tr>
<tr>
<td>Isch</td>
<td>(2.4 \pm 6.1^A,B)</td>
<td>(1330 \pm 514^A)</td>
<td>(26 \pm 7^A,B,C)</td>
<td>(64 \pm 31^A)</td>
<td>(-36 \pm 33)</td>
</tr>
</tbody>
</table>

Values are the mean \( \pm \) SD.

\( ^A \)p < .05 vs rest; \( ^B \)p < .05 vs BB alone; \( ^C \)p < .05 vs BB + NIF; \( ^D \)p < .05 vs BB + NTP.

Additionally, left ventricular end-diastolic pressure and heart size remained elevated at the same time contractile function had returned to or exceeded resting levels.

Indexes of left ventricular systolic and diastolic function during ischemia and reperfusion are listed in table 6. These data were collected at the same time early in reperfusion and after the same period of ischemia in each animal. Left ventricular end-systolic pressure rose considerably after reperfusion at control (\(51 \pm 25\) to \(72 \pm 17\) mm Hg) and after nifedipine (\(37 \pm 25\) to \(52 \pm 26\) mm Hg) and nitroprusside (\(45 \pm 19\) to \(54 \pm 24\) mm Hg). Coincident with the increase in left ventricular pressure, heart rate fell during reperfusion. However, the fall in heart rate after nifedipine was significantly less than that seen at control or after nitroprusside. By study design left ventricular end-diastolic pressure during reperfusion was similar to that seen during ischemia. End-diastolic pressure on reperfusion was lower after nitroprusside (\(8 \pm 8\) mm Hg) than at control (\(20 \pm 7\) mm Hg) or after nifedipine (\(20 \pm 8\) mm Hg). Both left ventricular minor-axis and segmental shortening improved during reperfusion, but left ventricular end-diastolic dimension and end-diastolic segment lengths remained unchanged from those seen during ischemia.

Peak \( (\text{dP/dt}) \) improved during reperfusion at control and after nifedipine and nitroprusside compared with that during ischemia. Isovolumetric relaxation as assessed by \( T_1 \) was further impaired after reperfusion at control (\(30 \pm 6\) vs \(26 \pm 5\) msec) and after nifedipine (\(29 \pm 4\) msec), but was improved slightly after nitroprusside (\(19 \pm 6\) vs \(21 \pm 5\) msec). \( T_2 \) tended to improve during reperfusion at control (\(36 \pm 7\) vs \(78 \pm 67\) msec), after nifedipine (\(46 \pm 17\) msec), and after nitroprusside (\(31 \pm 8\) msec), but the differences were not significant. Extrapolated baseline pressure became less negative during reperfusion and tended to be more negative after nifedipine and nitroprusside, but the differences were not significant when compared with values during ischemia. Minimum left ventricular pressure remained unchanged during reperfusion at control (\(20 \pm 6\) vs \(5 \pm 8\) mm Hg), but it was increased significantly after nifedipine (\(9 \pm 5\) mm Hg) and was significantly lower after nitroprusside (\(-2 \pm 5\) mm Hg). Thus, the relative effects of drug administration on reperfusion hemodynamics were the same as those observed during ischemia.

**Discussion**

This study demonstrates that calcium-entry blockade with nifedipine improves isovolumetric relaxation at rest by favorably altering loading conditions but fails to improve impaired relaxation or the increased chamber stiffness produced by brief periods of flow-limiting ischemia. Moreover, nifedipine failed to improve diastolic dysfunction during the early reperfusion period immediately after ischemia at a time when systolic performance had returned to resting levels. By contrast, equihypotensive nitroprusside administered before ischemia and reperfusion of the same severity and duration as used during infusion of nifedipine reduced left ventricular size, improved isovolumetric relaxation, and reduced chamber stiffness. These data indicate that increased calcium channel-mediated calcium influx is not the mechanism responsible for the abnormal early and late diastolic function in this preparation of brief flow-limiting ischemia and reperfusion.

Calcium overload has been implicated or demon-
strated during prolonged periods of hypoxia and ischemia \(^{11-15}\) in isolated papillary muscle and perfused heart preparations. Pretreatment with calcium-entry blockers appears to prevent the net increase in cellular calcium, improve ventricular performance, and minimize depletion of high-energy phosphates. \(^{11, 29}\) Some investigators have argued that the gain in tissue calcium occurs independent of calcium channel flux and that calcium-entry blockers act indirectly as cardioplegic agents by minimizing energy utilization during ischemia \(^{15}\); however, this issue remains unresolved.

Data on calcium transients during less prolonged periods of ischemia are scant. Hypoxia of brief duration in isolated papillary muscles does not appear to be associated with a net mean increase in free intracytoplasmic calcium at a time when tension is significantly depressed as assessed with aequorin bioluminescence. \(^{30}\) However, the aequorin light response was highly variable, with increases in the aequorin signal up to 153% of control seen in some muscles so that the precise meaning of the group data remains unclear.

Indirect evidence suggesting a role for increased net calcium influx during brief periods of ischemia has been reported. \(^{31}\) During ischemia, a depolarizing injury current presumed to be triggered by calcium and capable of provoking arrhythmias can be demonstrated and is specifically blocked by calcium-entry blockers. Whether this current is of sufficient magnitude to change net calcium influx and influence mechanical events is unresolved. Studies that have evaluated calcium-entry blockers during pacing-induced angina in patients with coronary artery disease have suggested a direct myocardial effect of these agents as a potential mechanism for their salutary actions. \(^{10}\) Typically an upward shift in the diastolic pressure-volume relationship occurs during pacing-induced angina and is attenuated or completely eliminated after pretreatment with nifedipine. However, this benefit could not be demonstrated in an animal preparation of pacing-induced ischemia with verapamil. \(^{32}\) The reason for the difference in results is not obviously apparent, but suggests that the benefit in human studies may have been secondary to favorable effects on the supply and demand relationships during ischemia and not to direct effects on myocardial transcellular calcium flux.

Our data do not show any benefit from calcium-entry
blockade on multiple variables of systolic and diastolic function during flow-limiting ischemia and reperfusion. Moreover, our data indicate that systolic contractile depression may be further impaired by a calcium antagonist during brief flow-limiting ischemia. The apparent discordance in results between this study and others that evaluated the effects of nifedipine during demand-induced ischemia may reflect the differences in myocardial pH and high-energy phosphate depletion occurring as a result of these different forms of ischemia.33 However, it may also represent the beneficial effects of calcium-entry blockade on determinants of myocardial oxygen supply and demand independent of direct effects on myocardial calcium flux. In this study nitroprusside was chosen as a short-acting vasodilator to provide loading conditions during ischemia comparable to those seen with nifedipine but without myocardial calcium-entry blocking activity. Although we did not show a beneficial effect of nifedipine on left ventricular systolic or diastolic function during ischemia, we did observe a favorable effect with nitroprusside, indicating that indexes of left ventricular diastolic function remain sensitive to alterations in loading conditions. Thus, in studies demonstrating a beneficial effect of nifedipine on left ventricular function during ischemia accompanied by alterations in loading conditions it is important to dissociate the direct effect of myocardial calcium-entry blockade from indirect effects of loading conditions by providing an appropriate hemodynamic control.

The reperfusion period evaluated at the same end-diastolic pressure as during ischemia was characterized by prompt restoration of systolic performance but persistently abnormal early and late diastolic function. Persistent diastolic function abnormalities during reperfusion may be a consequence of increased transcellular calcium flux, impaired sarcoplasmic reticulum uptake of calcium, altered chamber loading, free radical production, or a coronary erectile effect related to reactive hyperemia. Several studies have documented a significant increase in net calcium influx promptly on reperfusion after prolonged periods of hypoxia and ischemia but not during the hypoxic or ischemic period itself.15,34 Few data are available regarding calcium flux during reperfusion after brief periods of flow-limiting ischemia. Nifedipine failed to improve relaxation and other indexes of diastolic function during reperfusion, and as seen in figure 4, in some animals prolonged restoration of contractile function by virtue of its negative inotropic effects. If calcium overload during reperfusion occurred in this preparation of flow-limiting ischemia, it did so independent of the calcium channel. These results indicate that abnormalities of calcium influx during reperfusion are critically dependent on the duration and severity of the preceding ischemia, and at least early in the course of flow-limiting ischemia and reperfusion do not contribute to the accompanying mechanical abnormalities.

The preparation of bilateral coronary occlusion used in this study was chosen to provide severe ischemia of brief duration to allow multiple occlusions in a single animal and to minimize the effects of dysynchronous relaxation on subsequent evaluation of relaxation.26,35 While we cannot completely exclude an effect of temporal dysynchrony on relaxation during ischemia, global and regional wall motion were similar during drug administration with both nifedipine and nitroprusside, making it unlikely that this factor was responsible for differences in the rates of isovolumetric relaxation. The occlusions were very reproducible and readily revers-
TABLE 6
(Continued)

<table>
<thead>
<tr>
<th>Maximum (-dP/dt) (mm Hg/sec)</th>
<th>T₁ (msec)</th>
<th>T₂ (msec)</th>
<th>Pₑ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1586 ± 796</td>
<td>26 ± 5</td>
<td>78 ± 67</td>
<td>−47 ± 59</td>
</tr>
<tr>
<td>2143 ± 508*</td>
<td>30 ± 6</td>
<td>36 ± 7</td>
<td>−8 ± 10</td>
</tr>
<tr>
<td>1261 ± 751</td>
<td>26 ± 6</td>
<td>86 ± 40</td>
<td>−54 ± 21</td>
</tr>
<tr>
<td>1760 ± 815*</td>
<td>29 ± 4</td>
<td>46 ± 17</td>
<td>−19 ± 19</td>
</tr>
<tr>
<td>1520 ± 476</td>
<td>21 ± 5</td>
<td>58 ± 37</td>
<td>−38 ± 26</td>
</tr>
<tr>
<td>2625 ± 1579</td>
<td>19 ± 6⁶,c</td>
<td>31 ± 8</td>
<td>−18 ± 13</td>
</tr>
</tbody>
</table>

Possible, with restoration of baseline left ventricular function within 2 to 3 min of reperfusion. Although depletion of ATP and other high-energy phosphates may have occurred after the first occlusion, previous data obtained with the use of more prolonged occlusions (5 min) followed by 20 min of reperfusion failed to demonstrate cumulative ischemic depletion of high-energy phosphates. Nevertheless, drug administration was randomized during this study so that metabolic changes would have occurred equally in either the nifedipine or nitroprusside group during ischemia.

Both flow-limiting and demand-induced ischemia have been shown to impair early diastolic function, including the rate of isovolumetric relaxation. Several mechanisms have been proposed to explain these abnormalities, including both mechanical and metabolic processes. Since ischemia is accompanied by complex hemodynamic derangements, including significant increases in end-diastolic pressure, it is possible that prolongation of relaxation is a consequence of altered loading. However, it has been shown, at least in the nonischemic dog ventricle, that isolated increases in preload do not influence the time course of isovolumetric relaxation, so that during ischemia the contribution of altered loading to relaxation remains unclear. Nonetheless, we have demonstrated load-dependent improvement in the rate of isovolumetric relaxation after nitroprusside during flow-limiting ischemia but not after nifedipine, suggesting that mechanical factors may contribute significantly to changes in early diastolic function. Thus, putative effects of calcium-entry blockers on early diastolic function should be interpreted cautiously in the absence of appropriately matched hemodynamic controls.

Isovolumetric relaxation may be characterized by the extent of relaxation in addition to the rate of relaxation, but the precise physiologic meaning of this value remains unclear. In this study, extrapolated baseline pressure became more negative during ischemia, suggesting that more complete relaxation had occurred, but this is noted at the same time minimum diastolic left ventricular pressure was significantly elevated (table 3). Because extrapolated baseline pressure is a derived pressure and not a measured pressure it is subject to errors in the method of its estimation. All other variables of early and late diastolic function in this study indicate that the left ventricle was less compliant during ischemia, so that it is difficult to interpret changes in this variable in terms of observable physiologic events.

Previous descriptions of the passive diastolic pressure-volume and pressure-segment relationship during flow-limiting ischemia have been varied. Isolated heart studies have shown either an increase or no change in chamber compliance during prolonged periods of global ischemia, but decreases in compliance have also been demonstrated. Similarly, some studies evaluating pressure-segment length relationships during ligation of a single coronary artery have reported a decrease in segment stiffness, while others have demonstrated an increase in stiffness. In the present study the dynamic P-D relationship typically shifted upward and to the right during ischemia. Dynamic chamber stiffness is influenced by several factors and it is likely that the cause of the shift in this study is multifactorial. Decreased coronary turgor contributes to the rightward shift of the curve, but cannot explain the increase in slope during global ischemia. Similarly, pericardial restraint does not play a role in the shift of the relationship since the pericardium is left open in this preparation. Altered right ventricular compliance due to ischemia with subsequent alterations in the left ventricular P-D relationship as a result of the change in ventricular interdependence may have occurred since the dog typically has a left dominant coronary circulation. Previous data obtained with the use of LCX artery occlusion for 2 min supports a significant role for right ventricular contribution to the shift in the left ventricular pressure-volume relationship during ischemia, even in the absence of an intact pericardium. The increased chamber stiffness is also a likely consequence of the significant increase in ventricular volume alone, and may not reflect altered material properties of the myocardium. Finally, the impaired rate of relaxation during ischemia may have altered the pressure-dimension relationship. The fact that minimal left ventricular diastolic pressure was elevated during ischemia suggests that relaxation was
not complete during the early rapid filling phase and may have impaired filling during later stages of diastole. However, because an acute increase in elastic wall stiffness and enhanced right ventricular contribution to left ventricular pressure may have occurred during ischemia in addition to a significant increase in ventricular size it is difficult to clearly dissociate the contribution of impaired relaxation throughout diastole from these other factors.

Clinical implications. Calcium-entry blockers have been extensively used to treat patients with ischemic heart disease. Their effectiveness results from a favorable influence on the supply-demand relationship governing coronary flow during rest and exercise. While they may prevent or interrupt ischemia in the setting of maintained coronary perfusion (so-called demand ischemia), this study fails to demonstrate any benefit on ventricular performance early in the course of flow-limiting ischemia, a situation analogous to the earliest phase of myocardial infarction and coronary angioplasty, or to reperfusion such as that immediately after restoration of blood flow in these states. Reduction in preload, however, appeared to improve both systolic and diastolic function during ischemia, possibly because of decreased wall stress, and supports a beneficial role for this form of therapeutic intervention during flow-limiting ischemia and reperfusion.

In conclusion, abnormalities in diastolic function can be detected early after the onset of various forms of ischemia but few data are available characterizing the mechanisms responsible for these changes. In addition to the demonstration of depletion of high-energy phosphates, several studies have indicated or demonstrated increased net calcium influx during pacing-induced ischemia, during prolonged periods of hypoxemia and ischemia, and during the reperfusion period after ischemia, and have reported salutary effects of calcium-entry blockade with nifedipine on abnormal diastolic function. During transient flow-limiting ischemia accompanied by systolic contractile depression and diastolic dysfunction including an impaired rate of isovolumetric relaxation this study failed to demonstrate any beneficial effects from pretreatment with nifedipine. Additionally, during the early reperfusion period immediately after ischemia nifedipine failed to improve diastolic dysfunction, which persisted despite the return of systolic function to near resting levels. Thus, impaired diastolic function early in the course of flow-limiting ischemia and reperfusion is not related to excess calcium-channel transcellular calcium flux. Pretreatment with nitroprusside, however, ameliorated early and late diastolic function abnormalities during transient flow-limiting ischemia, primarily as a consequence of a reduction in preload, indicating that mechanical abnormalities significantly contribute to the diastolic dysfunction early in the course of this form of ischemia. Because alteration of loading conditions alone can improve diastolic function during ischemia independent of direct effects on myocardial calcium flux, forms of ischemia dependent on increases in the demand determinants of oxygen consumption may be especially sensitive to load alteration. We therefore caution interpretation of hemodynamic results that evaluate calcium-entry blockade and ascribe effects to direct myocardial actions in the absence of appropriate load-matched hemodynamic controls.

We greatly appreciate the assistance of Ms. Deborah Palmer during the preparation of this manuscript and the technical assistance of Donald Watkins, Danny Escobedo, and David Cragg.

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