Effect of Loading Conditions, Contractile State, and Heart Rate on Early Diastolic Left Ventricular Filling in Conscious Dogs

Che-Ping Cheng, Gregory L. Freeman, William P. Santamore, Martin S. Constantinescu, and William C. Little

We investigated left ventricular (LV) early diastolic filling in 10 normal conscious dogs that had been previously instrumented to measure LV and left atrial (LA) pressures and three orthogonal LV internal dimensions. LV volume was calculated as a general ellipsoid. The pressure within a passive structure increases as it is filled. If myocardial relaxation is rapid enough to substantially aid LV diastolic filling, it may overcome this effect and cause LV pressure to fall despite an increase in volume. Thus, we defined the amount of LV filling that occurred while LV pressure was falling as relaxation filling, which is a measure of the importance of LV relaxation during early diastolic filling. The time constant of relaxation (T) was derived from the exponential fall of LV pressure during isovolumic relaxation. While LV pressure was falling early in diastole (the relaxation filling period), all three LV diameters increased. Autonomic blockade with hexamethonium (5 mg/kg) and atropine (0.1 mg/kg) reduced relaxation filling from 21±6% (mean±SD) to 12±3% of the stroke volume (p<0.01). The mean LA pressure also was significantly decreased (from 12±2 to 10±5 mm Hg, p<0.05), while the duration of the relaxation filling period and T were unchanged. Positive inotropic stimulation with dobutamine (10 μg/kg/min) shortened T without changing LA pressure. The maximum LA-LV pressure gradient, dV/dt_{max}, and relaxation filling all increased. Augmented preload produced by dextran infusion (500 ml/10 min) caused an increase in LA pressure (from 11±3 to 21±8 mm Hg, p<0.05) without altering T. This also increased the maximum LA-LV pressure gradient, dV/dt_{max}, and relaxation filling. Augmented afterload produced by methoxamine (10 mg/3 min i.v.) significantly increased LA pressure (from 9±4 to 15±10 mm Hg, p<0.05) and lengthened T (from 35±4 to 50±7 msec, p<0.05) and the duration of relaxation filling (from 36±5 to 44±9 msec, p<0.01) without altering the maximum LA-LV pressure gradient, dV/dt_{max}, or LV relaxation filling. Incremental changes in heart rate induced by atrial pacing (from 100–180 beats/min) resulted in progressive decreases in the time constant of LV relaxation and the duration of relaxation filling. The LA pressure was also decreased. There was no corresponding increase in the amount of active LV filling until the heart rate reached 180 beats/min. During all these interventions, T correlated with the duration of LV relaxation filling (r=0.99, p<0.05). The amount of relaxation filling and dV/dt_{max} both correlated with the maximum LA-LV pressure gradient. We conclude that early in diastole, LV relaxation promotes LV filling, causing LV pressure to fall despite increasing chamber volume. Early diastolic filling is influenced by interventions that alter rate of LV relaxation or the LA pressure. (Circulation Research 1990;66:814–823)

Recent advances in cardiac nuclear imaging and Doppler flow measurements have focused clinical interest on the dynamics of left ventricular (LV) diastolic filling and shown that disease states may alter the pattern of diastolic filling. Since the mechanism of diastolic filling in normal hearts has not been fully defined, the importance of these alterations is not known.

LV relaxation is an active energy-dependent process that begins after end systole. What role myo-
cardial relaxation plays in aiding normal LV diastolic filling has been debated for the past century. Much evidence suggests that LV relaxation aids LV filling. For example, the mammalian ventricle is capable of sucking blood into its own cavity after exsanguination or severe hypovolemia. As well, the isolated LV can produce diastolic suction. However, the existence, amount, and physiological significance of such force in the normal LV of a conscious animal with an undisturbed mitral anulus has not been previously evaluated.

As a purely passive structure fills, its pressure increases. In the intact heart, early in diastole, myocardial relaxation and recoil of elastic elements decrease LV pressure, while filling tends to increase LV pressure. If LV relaxation is rapid enough to substantially aid early diastolic filling, then LV pressure should continue to decrease while LV volume increases. Thus, the amount of filling that occurs while LV pressure is falling provides a lower limit on the importance of LV relaxation to diastolic LV filling. A previous study of open-chest anesthetized dogs demonstrated that a single minor-axis LV diameter increased early in diastole while LV pressure fell. Since the configuration of the LV may change in early diastole, a single minor-axis diameter may not accurately reflect changes in LV volume. Furthermore, that study was performed in an anesthetized open-chest preparation and may not directly apply to the intact circulation of conscious animals.

Ishida et al have shown that the peak rate of flow across the mitral valve is related to the pressure gradient from the left atrium (LA) to the LV. The pressure gradient to produce mitral flow early in diastole is generated as LV pressure falls below LA pressure. LA pressure normally falls after mitral valve opening as mitral flow exceeds pulmonary venous return. Thus, Nikolic et al have concluded that early diastolic filling must occur while LV pressure is falling. If this were not the case, LV pressure could not decrease after mitral valve opening, and rapid early diastolic filling would not occur. It is not known whether these predictions are accurate in the intact conscious animal with an undisturbed mitral valve apparatus under various loading conditions and alterations in contractile state.

This study has undertaken to determine, in conscious animals, the influences of inotropic state, loading conditions, and heart rate on early diastolic LV filling. We were particularly interested in characterizing the effect of these interventions on the amount of LV diastolic filling that occurs during early diastole while LV pressure is falling and the relation of this filling and the peak LV filling rate to the mitral valve pressure gradient.

Materials and Methods

Instrumentation

Ten healthy adult mongrel dogs weighing 23–30 kg were instrumented after induction with Rompun (1 mg/lb) and thiopental sodium (6 mg/kg) as we have previously described. A sterile left lateral thoracotomy was performed under general anesthesia with halothane (1–2%). The pericardium was widely opened. A micromanometer pressure transducer (Konigsberg Instruments, Pasadena, California) and polyvinyl catheters for transducer calibration (1.1 mm i.d.) were inserted into the LV through an apical stab wound and via the LA through the LA appendage. Three pairs of ultrasonic crystals (5 MHz) were implanted in the endocardium of the LV to measure the anterior-posterior, septal-lateral, and base-apex (long-axis) dimensions. The method for insertion of the endocardial ultrasonic crystals has been previously described in detail. Wires and tubing were tunneled subcutaneously and exteriorized through the posterior neck.

Data Collection

Studies were performed after full recovery from instrumentation (7–10 days after surgery) with the dogs lying on their right side in a sling. The LV and LA catheters were connected to pressure transducers (Statham P23Db, Gould, Cleveland, Ohio) and calibrated with a mercury manometer. The signal from the micromanometer was adjusted to match that of the catheter.

The analog signals were recorded on an eight-channel oscillograph (Astro-Med, West Warwick, Rhode Island), digitized with an on-line analog-to-digital converter (Data Translation Devices, Marlboro, Massachusetts) at 200 Hz, and stored on a floppy disk memory system by use of a computer system (PC’s Limited, Austin, Texas). Each data acquisition period lasted for 12 seconds, spanning several respiratory cycles.

Experimental Protocol

Data were initially recorded with the animals lying quietly on their sides without medication. Then, autonomic blockade was produced by administering hexamethonium (5 mg/kg i.v.) and atropine sulfate (0.1 mg/kg i.v.). On subsequent days, similar autonomic blockade was administered, and control data were recorded. On each of these days after autonomic blockade, one of the following interventions was then used to change ventricular contractility, afterload, preload, or heart rate: 1) Contractility was augmented by the infusion of 10 μg/kg/min i.v. dobutamine. 2) Methoxamine (5–10 mg i.v.) or phenylephrine (0.1–0.2 mg i.v.) were infused to increase the LV systolic pressure by at least 40 mm Hg. 3) Dextran (500 ml) was given intravenously over 10 minutes. 4) In a separate group of six similarly instrumented animals who had pacing wires attached to the atrium, data were acquired as the heart rate was varied by atrial pacing from 100 to 180 beats/min, in increments of 20 beats/min. Other data have previously been reported from this group of animals.
Postmortem Evaluation

At the conclusion of the studies, the animals were killed by lethal injection of barbiturates. The hearts were examined to confirm the proper positioning of the instrumentation.

Data Processing and Analysis

The stored digitized data were analyzed by computer algorithm developed in our laboratory. Hemodynamic values in each dog were obtained by averaging the data obtained during the steady-state recording spanning several respiratory cycles.

End diastole was defined as the relative minimum of LV pressure after the A wave. If this was not clearly apparent, the peak of the R wave of the surface electrocardiogram was used to indicate end diastole. End ejection was defined as the time of minimum dP/dt. The time of mitral valve opening was defined to be when LV pressure fell below LA pressure. The relaxation filling period was defined as starting with mitral valve opening and ending when LV pressure reached its minimum.

The LV was modeled as a modified general ellipsoid using the equation: $V_{LV} = \frac{\pi}{6} \cdot D_A \cdot D_{SL} \cdot D_{LA}$, where $V_{LV}$ is LV volume, and $D_A$, $D_{SL}$, and $D_{LA}$ are the LV anterior-posterior, septal-lateral, and long-axis dimensions, respectively. It has been previously demonstrated that this method gives a consistent measure of LV volume ($r<0.97$, SEE<2 ml), despite changes in LV loading conditions, configurations, and heart rate.14,15,17,21 Stroke volume was calculated as end-diastolic LV volume minus end-ejection volume. We defined relaxation filling as LV volume at minimum LV pressure minus volume at mitral valve opening. This was expressed as a percent of stroke volume.

The time constant of the isovolumic fall in LV pressure was determined by fitting the steady-state data from end ejection to the time that LV pressure (P) had fallen below LA pressure to the equation:

$$P = P_A e^{-t/T} + P_B$$

where t is the time from end ejection, T is the exponential time constant of relaxation, and $P_A$ and $P_B$ were constants determined by the data. The time derivatives of LV pressure and volume were calculated using the five-point Gaussian technique.22

The LA pressure was measured with the micromanometer and adjusted to match LV pressure during middiastole of long diastolic cycles. The peak mitral valve gradient was calculated as the maximum value of LA pressure minus LV pressure. Micromanometer LA pressures were not measured in the group of animals in which heart rate was evaluated.

Statistical Analysis

Multiple comparisons were performed by analysis of variance. Paired comparisons were performed using two-tailed paired t tests. The Bonferroni correction was used to account for multiple comparisions. Differences were considered to be statistically significant at $p<0.05$. Values are expressed as mean±SD. Correlation coefficients were determined by linear regression.

Results

Early Diastolic Filling in Autonomically Intact Animals

Figure 1 is an analog recording illustrating the LV pressure and diameters under control conditions before autonomic blockade. After mitral valve opening, LV pressure continued to fall for 37±6 msec while all three LV dimensions increased. For the group, 18±7% of the total diastolic increase in anterior-posterior diameter occurred while LV pressure continued to fall. Similarly, 17±8% of the septal-lateral diameter increase and 16±11% of the long-axis diameter increase occurred while LV pressure was falling. Thus, under control conditions, 21±6% of the stroke volume entered the LV while the pressure was falling early in diastole (Figures 2 and 3).

Autonomic Blockade

After autonomic blockade, relaxation filling was reduced to 11.9±2.7% of stroke volume ($p<0.05$ compared with preblockade). Mean LA pressure was also reduced from 12.2±2.0 to 9.9±4.7 mm Hg ($p<0.05$). The maximum mitral valve gradient decreased from 8.2±5.6 to 6.0±3.8 mm Hg ($p<0.05$), and dV/dtmax decreased from 232±122 to 158±51 ml/sec ($p<0.05$). No significant changes occurred after autonomic blockade in the time constant of LV relaxation, the duration of the relaxation filling period, or the LV end-systolic volume (Table 1).
Similar results were obtained after autonomic blockade on each of four days (Tables 1 and 2).

**Inotropic State**

Positive inotropic stimulation with dobutamine resulted in a decrease in the time constant of relaxation from 36.0±3.7 to 21.0±3.3 msec (p<0.05) without a change in mean LA pressure or LV end-systolic volume (Figure 4, Table 1). The duration of the relaxation filling period decreased from 36.0±8.1 to 30.2±5.3 msec (p<0.05). The maximum mitral valve gradient (from 6.3±3.8 to 13.3±4.1 mm Hg, p<0.05), relaxation filling (from 11.6±4.4% to 23.4±6.2%, p<0.05), and dV/dt\(_{\text{max}}\) (from 159±60 to 261±30 ml/sec, p<0.05) were all increased by inotropic stimulation.

**Effects of Dextran and Methoxamine**

The effects of alterations in load on hemodynamic parameters are shown in Table 2. After dextran infusion (Figure 5) there were the expected substantial increases in LA pressure (from 10.5±3.3 to 21.4±7.7 mm Hg, p<0.05), LV end-diastolic pressure (from 12.9±5.3 to 27.7±7.5 mm Hg, p<0.05), and volume (from 46.2±6.1 to 53.1±5.8 ml, p<0.05). There was also a small increase in LV end-systolic pressure (from 102±14 to 111±16 mm Hg, p<0.05) and volume (from 32.6±5.5 to 33.8±6.4 ml, p<0.05). The maximum mitral valve gradient increased after dextran infusion (from 5.9±1.3 to 14.0±7.3 mm Hg, p<0.05), as did relaxation filling (from 12.4±4.6% to 32.4±6.0%, p<0.05) and dV/dt\(_{\text{max}}\) (from 156±39 to 300±71 ml/sec, p<0.05). The infusion of dextran produced no change in the time constant of relaxation or the duration of the relaxation filling period. The increase in LV systolic pressure (from 100±14 to 149±21 mm Hg, p<0.05) produced by methoxamine (Figure 6) produced an increase in LA pressure (from 9.4±4.2 to 14.8±9.7 mm Hg, p<0.05), LV end-diastolic pressure (from 12.1±4.4 to 20.3±8.8 mm Hg, p<0.05), LV end-systolic volume (from 46.5±6.9 to 54.8±12.6 ml, p<0.05), and LV end-ejection volume (from 32.0±8.9 to 39.3±10.8 ml, p<0.05) and slowed the rate of isovolumic pressure fall (the time constant increased from 35.2±3.5 to 49.6±6.9 msec, p<0.05). The duration of the relax-

**Figure 2.** Graph of left ventricular (LV) pressure and volume under basal conditions before autonomic blockade in a conscious dog. Volume was calculated from the three dimensions displayed in Figure 1. Note that approximately one third of the stroke volume enters the LV early in diastole before the time of minimum LV pressure (P).

**Figure 3.** Recordings of left ventricular (LV) and left atrial (LA) pressure (P), LV volume (V), and the rate of change of volume (dV/dt) recorded in a resting conscious animal before autonomic blockade. The time of minimum LV P is indicated.
Increased Heart filling were filling at relaxation 20.1±3.2% at filling relaxation increased with rate until corresponding increase in maximum mitral valve gradient, relaxation filling, or dV/dtmax.

### Increased Heart Rate

The effects of incremental changes in heart rate on LV relaxation filling are shown in Table 3. As heart rate increased with pacing, both the time constant of LV relaxation and the duration of LV relaxation filling were progressively decreased. There was no corresponding increase in the amount of relaxation filling until the heart rate reached 180 beats/min. Compared with 100 beats/min, the LV relaxation filling at 180 beats/min increased from 10.8±4.1% to 20.1±3.2% of stroke volume. The LV relaxation filling at 120, 140, and 160 beats/min was similar to that at 100 beats/min. During tachycardia, the LA pressure also decreased, reaching a plateau above 180 beats/min.

### Relation of Duration of LV Relaxation Filling Period to the Rate of Isovolumic Relaxation

We examined possible determinants of early diastolic filling by examining the relation between the duration of relaxation filling and the time constant of relaxation and between the amount of relaxation filling and dV/dtmax with the maximum mitral valve gradient. The duration of the LV relaxation filling period and the time constant of LV relaxation during all the interventions were correlated (r=0.99, SEE=0.70 msec, p<0.05). The amount of filling occurring before minimum LV pressure (the percent relaxation filling) was correlated with the maximum mitral valve gradient (r=0.90, SEE 3.9%, p<0.05). Similarly, dV/dtmax was correlated with the maximum

### Table 1. Effect of Autonomic Blockade and Dobutamine

<table>
<thead>
<tr>
<th></th>
<th>Unblocked</th>
<th>Autonomically blocked</th>
<th>Blocked control</th>
<th>Dobutamine</th>
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<tbody>
<tr>
<td>Heart rate (beats/min)</td>
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<td>139±12*</td>
<td>136±18</td>
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<td>Minimum LV pressure (mm Hg)</td>
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<td>LV end-diastolic pressure (mm Hg)</td>
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<td>LV end-systolic pressure (mm Hg)</td>
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<td>101±13</td>
<td>124±17+</td>
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<td>LV end-diastolic volume (ml)</td>
<td>47.6±9.3</td>
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<td>LV end-systolic volume (ml)</td>
<td>33.3±7.9</td>
<td>32.6±16.7</td>
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<td>Mean LA pressure (mm Hg)</td>
<td>12.2±2.0</td>
<td>9.9±4.7*</td>
<td>9.7±4.4</td>
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<td>Time constant of relaxation</td>
<td>35.2±2.9</td>
<td>36.1±4.7</td>
<td>36.0±3.7</td>
<td>21.0±3.3†</td>
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<tr>
<td>Relaxation filling (% of stroke volume)</td>
<td>20.9±6.1</td>
<td>11.9±2.7*</td>
<td>11.6±4.4</td>
<td>23.4±6.2†</td>
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<tr>
<td>Duration of relaxation filling (msec)</td>
<td>36.9±6.0</td>
<td>36.2±7.8</td>
<td>36.0±8.1</td>
<td>30.2±5.3†</td>
</tr>
<tr>
<td>Maximum mitral valve gradient (mm Hg)</td>
<td>8.2±5.6</td>
<td>6.0±3.8*</td>
<td>6.3±3.8</td>
<td>13.3±4.1†</td>
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<tr>
<td>Maximum dV/dt (ml/msec)</td>
<td>232±122</td>
<td>158±51</td>
<td>159±60</td>
<td>261±30†</td>
</tr>
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</table>

Values are mean±SD; n=10. LV, left ventricular; LA, left atrial.
*p<0.05 vs. unblocked.
†p<0.05 vs. blocked control.

### Table 2. Effect of Dextran and Methoxamine

<table>
<thead>
<tr>
<th></th>
<th>Blocked control</th>
<th>Dextran</th>
<th>Blocked control</th>
<th>Methoxamine</th>
</tr>
</thead>
<tbody>
<tr>
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<td>131±16</td>
<td>134±17</td>
<td>130±17</td>
<td>124±18</td>
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<td>Minimum LV pressure (mm Hg)</td>
<td>4.2±1.7</td>
<td>8.9±2.8*</td>
<td>4.1±2.6</td>
<td>9.3±5.5*</td>
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<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>12.9±5.3</td>
<td>27.7±7.5*</td>
<td>12.1±4.4</td>
<td>20.3±8.8*</td>
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<td>LV end-systolic pressure (mm Hg)</td>
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<td>111±16*</td>
<td>100±14</td>
<td>149±21*</td>
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<td>LV end-diastolic volume (ml)</td>
<td>46.2±6.1</td>
<td>53.1±5.8*</td>
<td>46.5±6.9</td>
<td>54.8±12.6*</td>
</tr>
<tr>
<td>LV end-systolic volume (ml)</td>
<td>32.6±5.5</td>
<td>33.8±6.4*</td>
<td>32.0±8.9</td>
<td>39.3±10.8*</td>
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<tr>
<td>Mean LA pressure (mm Hg)</td>
<td>10.5±3.3</td>
<td>21.4±7.7*</td>
<td>9.4±4.2</td>
<td>14.8±9.7*</td>
</tr>
<tr>
<td>Time constant of relaxation</td>
<td>35.3±2.5</td>
<td>35.7±3.7</td>
<td>35.2±3.5</td>
<td>49.6±6.9*</td>
</tr>
<tr>
<td>Relaxation filling (% stroke volume)</td>
<td>12.4±4.6</td>
<td>32.4±6.0*</td>
<td>12.8±5.5</td>
<td>16.6±5.8</td>
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<tr>
<td>Duration of relaxation filling (msec)</td>
<td>36.9±6.0</td>
<td>38.7±5.0</td>
<td>36.1±5.1</td>
<td>43.6±8.8*</td>
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<tr>
<td>Maximum mitral valve gradient (mm Hg)</td>
<td>5.9±1.3</td>
<td>14.0±7.3*</td>
<td>6.0±2.2</td>
<td>7.4±3.2</td>
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<tr>
<td>Maximum dV/dt (ml/msec)</td>
<td>156±39</td>
<td>300±71*</td>
<td>176±54</td>
<td>210±75</td>
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</table>

Values are mean±SD. LV, left ventricular; LA, left atrial.
*p<0.05 vs. appropriate blocked control.
mitral valve gradient \( (r=0.90, \text{ SEE}=24.6 \text{ ml/sec, } p<0.05) \).

**Evaluation of Volume Calculation**

To provide additional assessment of the method of measuring LV volume, we compared LV volume at the beginning (end ejection) and end (mitral valve opening) of isovolumic relaxation. The change in volume was 1.6±0.4%.

**Discussion**

After the mitral valve opens, two opposing factors influence LV pressure. First, the decay of the active state (and, if end-systolic LV volume is less than equilibrium volume, recoil of elastic elements) tends to decrease LV pressure. Second, flow across the mitral valve increases LV volume, tending to raise LV pressure. Our study demonstrates that for the first 30–40 msec after mitral valve opening, relax-
ation of LV wall tension is normally rapid enough in conscious animals to cause LV pressure to fall, despite a substantial increase in LV volume. This fall in LV pressure produces a pressure gradient to move blood from the LA to the LV, resulting in rapid early diastolic filling, so that more than one fifth of the stroke volume enters the LV under basal conditions in conscious animals, before LV pressure reaches its minimum. Thus, a substantial part of LV diastolic filling occurs before the LV behaves as a passive structure, confirming the role of myocardial relaxation in aiding LV filling. If LV relaxation was not rapid enough to produce the early diastolic LA to LV pressure gradient, rapid early diastolic filling could not occur, LA pressure would not fall after mitral valve opening, and LV filling would be almost totally dependent on atrial contraction.13

Our results extend the previous work of Sabbah and Stein,10 who, in a study of open-chest anesthetized dogs, found that the anterior-posterior diameter of the LV increased by about 4 mm while LV pressure was falling. Since we observed a similar increase in all three diameters of the LV in conscious animals during the relaxation filling period, it is clear that these findings do not result from configurational changes of the LV that occur during isovolumic relaxation.11,23 Moreover, since the position of the heart in the cardiac fossa may influence LV shape and filling dynamics,24,25 studies in open-chest animals may not accurately reflect the pattern of LV filling in normal animals. Our use of conscious intact animals overcomes the potential limitations of anesthetized open-chest preparations and demonstrates that a substantial portion of the stroke volume normally enters the LV while pressure is falling.

In a recent study, Ishida et al12 evaluated mitral valve flow in conscious animals using an electromagnetic flow probe inserted into the mitral anulus. They found that the peak mitral valve flow rate (equivalent to \( dV/dt_{\text{max}} \) in our study) was determined by the pressure gradient between the LA and the LV. Our observation that \( dV/dt_{\text{max}} \) and relaxation filling are related to the maximum mitral valve gradient in conscious animals with an undisturbed mitral appa-

![Figure 6. Recordings of left ventricular (LV) and left atrial (LA) pressure (P), LV volume (V), and rate of change of V (dV/dt) before and after infusing methoxamine.](http://circres.ahajournals.org/)

### TABLE 3. Effect of Heart Rate on Left Ventricular Diastolic Filling

<table>
<thead>
<tr>
<th>Heart rate (beats/min)</th>
<th>Relaxation filling (% stroke volume)</th>
<th>Left atrial pressure (mm Hg)</th>
<th>Duration of relaxation filling (msec)</th>
<th>Time constant (msec)</th>
<th>LV end-systolic volume (ml)</th>
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</thead>
<tbody>
<tr>
<td>100</td>
<td>10.8±4.1</td>
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<td>120</td>
<td>11.1±7.3</td>
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<td>28.1±8.1</td>
<td>26.3±2.7</td>
<td>28.6±13.5</td>
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<td>140</td>
<td>10.4±5.1</td>
<td>3.1±3.1</td>
<td>25.7±7.6</td>
<td>25.0±1.9</td>
<td>36.0±12.3</td>
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<td>160</td>
<td>10.2±4.6</td>
<td>0.9±3.2*</td>
<td>18.3±2.4</td>
<td>23.3±2.2*</td>
<td>31.5±12.8</td>
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<tr>
<td>180</td>
<td>20.1±3.2*</td>
<td>−0.7±2.3*</td>
<td>15.2±6.5*</td>
<td>21.1±1.4*</td>
<td>31.8±13.4</td>
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</table>

Values are mean±SD; \( n=6 \). LV, left ventricular.

*Effects of incremental changes in heart rate significantly different (p<0.05) from control at 100 beats/min.
ratus is consistent with the results of Ishida et al.\textsuperscript{12} Furthermore, they observed that about 18\% of the stroke volume enters the LV before the time of peak mitral valve flow, which occurs just after the time of minimum LV pressure. Our observation that 21\% of the stroke volume enters the LV before minimum LV pressure is slightly higher than the findings of Ishida et al, perhaps due to some impairment of mitral flow by the anular flow probe. Similarly, Courtois et al\textsuperscript{26} found that the maximum mitral valve pressure gradient occurred just before the time of minimum LV pressure and that substantial mitral flow velocity occurred before the time of minimum LV pressure. In contrast to our observations, Ishida et al\textsuperscript{12} observed that volume loading did not alter relaxation filling (termed “early filling volume” in their study). This difference in results may be due to the use of autonomic blockade in our studies, which prevented reflex changes in heart rate and contractile state, which may have offset changes produced by volume loading in Ishida’s study. Our studies also differ in that the mitral anulus and mitral valve apparatus were undisturbed in our study, while the mitral anular circumference was fixed in their animals by a flow probe. Since our observations are generally consistent with those of Ishida et al, we do not believe that this methodological difference explains this minor discrepancy.

Our study supports the hypothesis suggested by Ishida et al,\textsuperscript{12} based on their observations in conscious dogs, that two major factors (myocardial relaxation and LA pressure) determine the early diastolic mitral valve pressure gradient and the rate of LV filling (dV/dt\textsubscript{max}) in the absence of mitral stenosis. In addition, these factors also appear to determine the percentage of the stroke volume which enters the LV early in diastole while pressure is falling. The first factor is the rate of LV relaxation. This is most clearly shown by the effects of dobutamine infusion. In this case, the positive inotropic agent dobutamine increased the LV rate of relaxation, apparent as a fall of the time constant of isovolumic relaxation from 36±5 to 21±3 msec. Although there was no change in LA pressure, the more rapid rate of myocardial relaxation caused an increase in the early diastolic mitral valve pressure gradient. This was associated with an increase in dV/dt\textsubscript{max} and the portion of the stroke volume entering the LV before the time of minimum LV pressure. The duration of the relaxation filling period was related to the time constant of relaxation. It appears that faster relaxation not only produces a larger mitral valve pressure gradient but also causes minimum LV pressure to occur sooner.

A second factor important in determining early diastolic filling is the LA pressure. Dextran infusion doubled mean LA pressure in our study but produced no change in the rate of myocardial relaxation as measured by the time constant of isovolumic relaxation. This is consistent with the previous observation that the preload does not influence relaxation rate if not accompanied by changes in afterload.\textsuperscript{27} The end-systolic LV volume, the heart rate, and the duration of the relaxation filling period were all unchanged. Although the time constant of relaxation was unchanged, the early diastolic mitral valve pressure gradient almost doubled. This may be related to the approximately exponential nature of the decline in LV pressure during isovolumic relaxation, in which the rate of pressure fall (dP/dt) is related to the instantaneous pressure. Since, after dextran infusion, the mitral valve opens at a much higher pressure (due to higher LA pressure), dP/dt will be higher, causing LV pressure to rapidly fall below LA pressure. This increased mitral valve pressure gradient after dextran was associated with an increase in dV/dt\textsubscript{max} and relaxation filling. Opposite effects were observed after autonomic blockade. The time constant of relaxation was unchanged, while LA pressure was decreased. The mitral valve pressure gradient, relaxation filling, and dV/dt\textsubscript{max} were all decreased. Recently, Choong et al\textsuperscript{29} and Courtois et al\textsuperscript{29} have also observed in anesthetized animals that a change in LA pressure without a change in the time course of relaxation can alter LV early diastolic filling.

After an increase in systolic load produced by arterial vasoconstriction, the maximum mitral valve gradient, dV/dt\textsubscript{max}, and relaxation filling were unaltered. It appears that the slowing of myocardial relaxation counterbalanced the increase in LA pressure produced by this intervention. As can be seen from Table 3, extreme increases in heart rate may also influence relaxation filling, but this effect can be explained in terms of the rate of relaxation and LA pressure. As heart rate increased from 100 to 160 beats/min, the LA pressure dropped in a monotonic fashion. Also, the time constant of relaxation decreased as heart rate increased. Relaxation filling was unchanged at rates up to 160 beats/min, consistent with the counterbalancing opposite influences of LA pressure and the time constant of relaxation. However, when the rate was increased to 180 beats/min, a further drop in LA pressure did not occur, but the time constant of relaxation continued to decrease where atrial contraction was occurring during relaxation filling. At this rate (180 beats/min), the percent of stroke volume occurring during the relaxation filling period was significantly augmented. However, the total stroke volume was reduced at the rapid rates, and the total time for diastolic filling was abbreviated.

The LV may normally eject below its equilibrium volume.\textsuperscript{6,13} Thus, if mitral flow is prevented, the LV pressure will fall to subatmospheric levels in diastole, presumably due to recoil of elastic elements that were compressed during systolic ejection.\textsuperscript{30} Because of this effect, the isolated LV will fill despite a zero source pressure.\textsuperscript{6} Thus, it would be expected that changes in end-systolic LV volume would influence the rate of relaxation and early diastolic filling. Consistent with this hypothesis, methoxamine increased the end-systolic volume and the time constant of relaxation. The effect of altered elastic recoil
with the interventions we studied appears to have been reflected in changes in the time constant of relaxation. Thus, from our study, we cannot separate influences of changes in end-systolic volume from changes in the rate of relaxation reflected in the time constant of relaxation.

The amount of relaxation filling measured in this study may underestimate the true importance of diastolic filling during relaxation for two reasons. First, we measured pressure at the apex of the LV. Courtois et al. in the anesthetized closed-chest dog, showed that regional pressure gradients exist within the LV during early diastolic filling. LV pressure reaches its minimum anterior to the mitral valve apparatus later than it does at the apex. Thus, if we had measured pressure anterior to the mitral valve, we should have observed an even greater portion of LV filling occurring while LV pressure was falling. Second, relaxation continues after the time of minimum LV pressure and may continue to contribute to LV filling after this time. Thus, our observation that one fifth of LV stroke volume normally enters the LV while pressure is falling should be interpreted as a lower limit on the importance of relaxation in aiding LV diastolic filling.

Our study must be interpreted in light of potential limitations. The first is our use of endocardial diameter gauges to measure LV volume. This technique has been extensively validated in past studies and accurately reflects LV volume under a wide variety of normal and pathological conditions. We further evaluated the effect of shape changes by assessing the constancy of calculated LV volume during isovolumic relaxation when actual LV volume is constant but LV shape changes. Since LV volume changes by only 1.6 ± 0.4% during this period, it appears that our method is insensitive to changes in LV shape. The accurate measurement of \( \frac{dV}{dt} \) requires a higher frequency content than volumes at single points in the cardiac cycle.11 Our use of three LV dimensions should be accurate to detect large changes in \( \frac{dV}{dt_{\text{max}}} \) as performed in this study, but more detailed evaluation of the \( \frac{dV}{dt} \) signal may not be accurate. Second, our data were acquired over a period of several days. Each intervention was performed after autonomic blockade to prevent reflex changes that might confound interpretation of the data. As can be seen from Tables 1 and 2, the postautonomic blockade hemodynamic measurements and relaxation filling were reproducible from day to day; thus, comparison of our intervention results is justified. Autonomic blockade was used in our study to prevent reflex alterations in heart rate, contractility, and relaxation. This may be somewhat different than the clinical setting, where these variables are not subject to strict control. Third, the use of a simple exponent to characterize relaxation is an approximation since isovolumetric LV pressure does not decay exactly exponentially.20,30,31 However, the calculation of the time constant of relaxation based on the assumption of monoexponential decay is a reasonable approxi-

mation to characterize the time course of pressure fall. Finally, since the variables we studied are influenced by mutually interactive factors, isolated alterations in its determinants could not be performed. Although this cannot be avoided in studies of conscious intact animals, the use of a variety of perturbations with different effects on LA pressure and myocardial relaxation suggest that these are the key factors influencing the development of the mitral valve gradient and early diastolic filling.

In conclusion, a substantial portion of LV filling in normal conscious animals occurs while the myocardium is relaxing before the LV behaves as a passive structure. The duration of the relaxation filling period is related to the time constant of relaxation, and both the percent of the stroke volume that enters the LV during this period and \( \frac{dV}{dt_{\text{max}}} \) are influenced by two factors: LA pressure and the rate at which the LV relaxes. These observations demonstrate the importance of LV relaxation in aiding diastolic filling in normal conscious animals.

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