Load-dependent relaxation with late systolic volume steps: servo-pump studies in the intact canine heart

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ABSTRACT In isolated heart muscle preparations an abrupt increase in load during the latter portion of contraction (at a time when there is little if any potential to develop additional force) causes a premature and more rapid relaxation; this load-dependent relaxation characterizes relaxation in myocardium with normal sarcoplasmic reticulum. The purpose of our study was to assess whether or not the phenomenon of load-dependent relaxation is present in the intact heart and to describe the left ventricular response to abrupt load increments (volume steps) throughout the cardiac cycle. Using a microcomputer-controlled servo-pump attached to the apex of an intact canine heart, we studied the effects of 6 ml steps on left ventricular pressure transients during relaxation. Each volume step was carried out in a single beat with 20 stabilization beats separating the intervention beats; thus, at a heart rate of 120 beats/min, a sequence of 10 intervention beats could be carried out in less than 2 min. By performing the experiments on a single-beat basis (control vs intervention beat), we were able to minimize reflex and other physiologic feedback mechanisms that might alter the results. Studies were performed in five anesthetized dogs. In ejecting beats, an early step (immediately after aortic valve opening) caused an increase (3%) in the duration of systole (the time from the onset of pressure rise to the instant at which left ventricular pressure had declined to one-half its maximal value); in contrast, a late step (just before aortic valve closure) caused a decrease (7%) in the duration of systole. In non-ejecting beats (single-beat aortic occlusion plus volume step), early steps (during the first third of systole) caused an increase in the duration of systole (245 ± 18 to 257 ± 19 msec; p < .05); when the volume step was placed later (near the time of peak pressure), the duration of systole fell (245 ± 18 to 231 ± 17 msec; p < .05). These results indicate the presence of load-dependent relaxation in the intact canine heart. Although these data were obtained during abrupt load changes in single cardiac cycles, it is possible that alterations in the mechanisms underlying the phenomenon of load-dependent relaxation can be responsible for disordered relaxation in clinical and experimental studies of the diastolic properties of the left ventricle. Circulation 75, No. 6, 1287–1294, 1987.

RELAXATION, which refers to the process by which myocardium returns to its initial length and tension, is influenced by the interaction of deactivation (the decay of active force-generating capacity) and loading conditions (forces affecting myocardial fiber length). These forces or loads may be categorized as those that are applied early in the cardiac cycle and those that are abruptly applied late in the cycle. The application of an early systolic load (when activating calcium is available to the contractile proteins) results in the recruitment of more cross-bridges and a prolonged systole. By contrast, late in the cardiac cycle cytosolic calcium is below the threshold necessary for activating new cross-bridges and consequently late systolic load increments result in a premature onset of relaxation; existing bridges cannot support the additional load, the muscle yields, relaxation is premature, and the duration of systole is abbreviated. This phenomenon is called "load-dependent relaxation." In using the term "load-dependent relaxation," Brutsaert referred to a series of observations made on isolated feline papillary muscles in which he and his collaborators observed that isotonic contractions against progressively lighter afterloads manifest progressively shorter overall durations. He attributed the reduction in duration of these isotonic contractions to an abbreviation of the relaxation phase rather than to changes in...
earlier phases of the contractile cycle. Moreover, he noted that an additional load increment applied during the relaxation phase resulted in a premature onset of relaxation. This load-dependent relaxation was demonstrated in mammalian ventricular muscle, but it did not appear to be a property of muscle from frog ventricles (in which there is only a sparse sarcoplasmic reticulum), nor was it present in mammalian myocardium that had been made hypoxic or treated with caffeine. Brutsaert used this and other evidence to propose that calcium-sequestrating membranes are required for the process that gives rise to the "load-dependence" of relaxation. It is important to recognize, however, that the concept of load-dependent relaxation, as defined by Brutsaert, should be distinguished from the idea that simple alterations in peak systolic pressure or afterload may influence the rate of relaxation.2-4

Others have attempted to arrive at a quantitative understanding of relaxation in the intact heart by examining the effects of short-term hemodynamic interventions on the rate of left ventricular tension or pressure decline during the isovolumic relaxation period2-4 or the rate of fiber lengthening during the early diastolic filling phase.5,6 These and other studies of relaxation and filling have been reviewed by others7 and will not be discussed here. In anesthetized dogs, Goethals et al.8 used a balloon occluder to narrow the aorta at various times after the onset of ejection. They observed that the resulting sharp rise of pressure in the ventricle caused ventricular ejection to be prolonged (by comparison with control nonocclusion beats), but only when the pressure rise was imposed during the first third of the ejection period. When the pressure increment was applied during the last third of ejection, relaxation began prematurely. A similar result showing that a sudden aortic occlusion during the last third of the ejection period gave rise to an abbreviated ejection was reported earlier by Noble.9 In the present study we did not attempt to manipulate systolic pressure by occluding the aorta, but rather we used left ventricular volume increments as a more controlled experimental approach to the study of load-dependent relaxation. The purpose of the study was to assess the presence of load-dependent relaxation in the intact heart; studies were performed in ejecting and nonejecting beats.

Methods

A schematic diagram of our experimental apparatus is shown in figure 1; the details are presented elsewhere.10 The major components of the system are (1) a microcomputer-controlled servo-pump connected to the apex of a dog's heart with a half-inch inner-diameter cannula and (2) a pneumatically activated aortic occluder, also controlled by the microcomputer. The microcomputer was also responsible for pacing the heart and for performing other miscellaneous chores (display, printing the log of the experiment, turning a data recorder on and off, etc.). Left ventricular pressure was measured with a high-fidelity pressure transducer (Miller PC-360) and recorded on a cassette data recorder (TEAC R-81) and a photographic (Electronics for Medicine) recorder.

We studied the hearts of five open-chest, anesthetized, mongrel dogs weighing 25 to 29 kg. The dogs were anesthetized preoperatively with 1 mg/kg morphine sulfate and 30 mg/kg pentobarbital. Supplementary anesthetic doses of morphine were given approximately every hour. The animals were ventilated to maintain physiologic arterial blood gases. After a median sternotomy, the pericardium was opened, a bipolar electrode was affixed to the right atrium, and the sinus node was crushed. The pacing signal (120 beats/min) was generated by the microcomputer.

Purse-string sutures were then sewn around the apex of the left ventricle. With temporary occlusion of the venous return to the heart, a half-inch stab wound was made in the left ventricular apex; the cannula was quickly inserted into the left ventricle, the purse-string suture was tightened, and venous return was reestablished. This procedure could be accomplished within 30 sec with only trivial blood loss. Finally, the aortic occluder was placed (in the "open" position) just above the aortic valve. The heart and the circulation were otherwise intact during the experiments.

An example of our experimental method for the nonejecting beat protocol is shown in figure 2. Shortly after the pacing signal (100 msec), the aortic occluder was triggered (arrow marked "on"), taking 30 msec to close, thus preventing left ventricular ejection during the subsequent systole. Next, at a time prescribed by the microcomputer, the servo-pump infused 6 ml of blood directly into the ventricle. The volume infusion
FIGURE 2. Example of left ventricular pressure (top) and servo-pump volume displacement (bottom) in dog 1. Shortly after an atrial pacing stimulus, the aortic occluder was activated, thus preventing ejection of ventricular blood into the aorta. Next, at a time prescribed by the microcomputer, the servo-pump infused 6 ml of blood directly into the left ventricle; the pump then remained stationary. The aortic occluder was released (arrow indicates off) during the subsequent diastole. Later the servo-pump was commanded to return slowly to its baseline position and to remain stationary until commanded again to move. A stabilization period of 20 cardiac cycles intervened between each single-beat experiment.

Pattern was a rapid ramp taking 15 msec to complete, after which the servo-pump remained stationary for at least the rest of systole. Release of the aortic occluder (arrow marked "off") took place during the subsequent diastole. The servo-pump was then commanded to return slowly to its original position and to hold this position until commanded again to move. A stabilization period of 20 cardiac cycles intervened between these single-beat experiments. By performing an experiment within a single cardiac cycle, we were able to minimize reflex or other physiologic feedback mechanisms that might alter cardiac function.

The effect of each volume step was quantified by measuring changes in an index of the duration of systole; this index, measured as the interval between the onset of left ventricular pressure rise and the instant at which it had declined to one-half its maximum value (time to Pmax/2), has been used to study relaxation in isolated muscle preparations and in the intact heart. We reasoned that if the contraction side of the left ventricular pressure is unchanged and a quick stretch shortens the duration, then relaxation must have been premature and/or more rapid.

We could not use the isovolumic relaxation time constant in these studies because the time course of isovolumic pressure decline is distorted by the quick stretch intervention and the time course of pressure decline is no longer an exponential function. As will be seen, changes in the magnitude and timing of peak (− dP/dt) are likewise difficult to assess during the quick stretch interventions. For these reasons we were restricted to the use of a duration index and visual inspection of the pressure curves. All five dogs were studied with the nonejecting beat protocol; three of the five were studied in the ejecting mode (i.e., the aortic occluder was not activated). In the ejecting beat experiments, each quarter of the ejection period was studied; in the nonejecting experiments an entire series of steps (from late diastole to late systole) was studied.

A series of 10 such single-beat experiments constituted a full sequence. During a sequence, the time at which the volume infusion was given was advanced by 30 msec intervals from one experimental beat to the next. Each sequence was repeated five to 10 times; extrasystolic and postextrasystolic beats were excluded from the analysis, but at least 5 beats were always available for averaging. Left ventricular pressures of the experimental beats were recorded, along with a digitally coded beat number for each, on the data recorder. The beat numbers and volume-infusion times (relative to the pacing stimuli) were printed by the computer, along with other information, on the experiment log. The left ventricular pressure data were digitized and averaged at 2 msec intervals; at least 5 beats with the same volume-infusion time were averaged (for these beats the standard deviation of peak pressure was less than 4%). At a heart rate of 120 beats/min it was possible to complete a sequence consisting of 10 single-beat experiments in less than 2 min, a time considered short enough for the preparation to remain stable. Furthermore, since every event (i.e., pacing, aortic occluder triggering, pump motion, etc.) was programmed and precisely timed by the microcomputer, the same sequence could be repeated several times for later averaging of the results.

Student’s paired t test was used to determine the significance of differences in the effect of early and late volume increments on the duration of systole. A probability of less than .05 was considered to be significant. Data are presented as the mean ± SD.

Results

The effects of early and late volume increments in each of the five dogs are shown in figures 3 to 8. The ejecting beat protocol was followed in three dogs (Nos. 1 to 3) and the nonejecting beat protocol was used in all five dogs. Examples from ejecting beat experiments are shown in figures 3 and 4 (dogs 1 and 2) while data from the nonejecting beat experiments are shown in figures 5 to 8 (dogs 2 to 5).

Ejecting beats. In these experiments the aortic occluder did not operate and the heart was allowed to eject normally. We began with this protocol, believing that it would be useful to disturb the heart and circulation as little as possible. A representative example of left ventricular pressure as a function of time (with volume increments during early and late ejection) is shown in figure 3 (dog 1). Timing of the volume injection (6 ml of blood) was adjusted so that the early injection occurred before peak pressure but after aortic valve opening (near the onset of ejection); the late injection was given just before aortic valve closure (near the end of ejection). In this experiment, the early volume increment caused an increase in the duration of systole (time to Pmax/2 increased 10 msec), whereas the late increment shortened the duration of systole (time to Pmax/2 fell 28 msec). This effect was reproducible in all ejecting beat experiments; volume steps during the first quarter of the ejection period caused a
small (average 3%) increase in the time to Pmax/2, while volume steps in the last quarter of the ejection period caused a decrease (average 7%) in the time to Pmax/2.

As is shown in figure 4 (dog 2), the timing of each of the four volume steps was set so that each quarter of the ejection period could be assessed independently. Volume steps during the first quarter of the ejection period caused an increase in the time to Pmax/2, whereas steps in the second half caused an abbreviation in time to Pmax/2. The transition from one effect (an increase in duration) to the opposite effect (a decrease in duration) occurred in the second quarter of ejection. The abbreviation was most marked when the step was given in the third quarter of ejection (time to Pmax/2 fell 23 msec). These results produced by volume steps are qualitatively similar to those produced by abrupt pressure increments.  

In the ejecting heart one could not be certain how much of the volume step remained in the ventricle (causing the wall to stretch) and how much escaped into the aorta. To ensure that the ventricular volume was increased by a known increment at a specific time, we went on to single-beat studies in which outflow was prevented by the aortic occluder.

Nonejecting beats. A typical result form one of these experiments is shown in figure 5 (dog 2). When a 6 ml infusion was begun early in systole (265 msec after the atrial pacing stimulus), the duration of systole increased (12 msec) relative to the isovolumic contraction. The same volume infusion given later in systole (340 msec after the pacing stimulus) produced a ventricular pressure trace with an earlier and more rapid initial decline in pressure; the decrease in time to Pmax/2 was 30 msec. The solid line in each panel shows the average of 5 isovolumic (control) beats, and the broken line represents the average of 5 infusion beats (each preceded and followed by a stabilization period of 20 beats in which neither the servo-pump nor the aortic occluder operated, as explained earlier).

It might be argued that the solid and broken lines of figure 5 are not strictly comparable because the final volume of the ventricle after the infusion steps was larger than the volume during the isovolumic beats. The example shown in figure 6 (dog 3) avoids this
problem by directly comparing infusion beats only; the volume of the ventricle after the infusion is identical for both the solid curve (early infusion beats) and the broken curve (late infusion beats). Thus, when the comparison is made at equivalent left ventricular volumes, the duration is shortest when the infusion is late; the difference in the time to \( P_{\text{max}}/2 \) between the two experiments was 9 msec.

A series of four volume steps and a control isovolumic beat is shown in figure 7 (dog 4). This example illustrates the transition from one result (increased duration with an early step) to a quite different result (shorter duration with a late step). The abbreviation of systole was most marked and the peak \((-\Delta P/\Delta t\) was greatest when the volume step was given near the instant of peak pressure (volume step 4); compared with control, the duration fell by 13 msec. When the comparison was made at equal left ventricular volumes (step 1 vs step 4) the duration fell by 23 msec.

This result is expressed quantitatively in figure 8 (dog 5). The vertical axis gives the change in duration (time to \( P_{\text{max}}/2 \)) when 10 equally spaced (30 msec intervals) volume steps were given during the interval between late diastole and late systole. Zero on this scale corresponds to the isovolumic (no-infusion) set of beats. Each ordinate (mean ± SD) represents the average of seven single-beat interventions. The mean ± SD describing the duration of 40 control beats is plotted at zero on the horizontal axis; a curve showing ventricular pressure (the average of 40 isovolumic beats) is also displayed for reference.

This example clearly shows that volume steps in late diastole and very early systole cause an increase in the duration of systole. The prolongation is substantial; it averaged 18 msec, which is approximately 11% of the time required for the pressure to fall from its peak to 10 mm Hg in a control isovolumic beat. As the volume step is placed later, the prolongation decreases and eventually becomes negative. As is shown in figure 8, the maximum negative value (the maximum abbreviation of duration) occurs very near the instant that peak isovolumic pressure is achieved in an isovolumic beat;
time to Pmax/2 averaged 13 msec less than that seen in the control isovolumic beat. In all five dogs the peak negative value was achieved when the volume was injected near the time of peak isovolumic pressure.

All five dogs demonstrated an increase in the duration of systole with an early volume step; when the volume step was placed in the first one-third of systole the duration index increased by an average of 12 msec. In contrast, all five dogs demonstrated a decrease in the duration index when the step was placed at or near the instant of peak pressure; the average decrease was 14 msec (see table 1).

**Discussion**

Our data confirm the presence of load-dependent relaxation in the intact heart. A quick stretch (volume increment) of the left ventricular chamber during the very early portion of systole prolongs the duration of systole whereas a similar stretch given late in the cycle causes a premature onset and a more rapid rate of pressure decay (figures 4 and 5). The observation that relaxation is exquisitely sensitive to late systolic loads is therefore not merely a phenomenon limited to isolated-muscle preparations. In ejecting hearts, the transition for one effect (prolonged time to Pmax/2) to the other (abbreviated time to Pmax/2) occurs near the end of the first third of the ejection period. In the nonejecting beats the transition also occurs near the end of the first third of systole, well before the instant of peak pressure. However, this latter interpretation (of a transition zone before the instant of peak pressure) depends on our use of the control isovolumic beat for comparison with the beat subjected to the volume increment.

**TABLE 1**

Effects of an early or late volume increment (quick stretch) on the duration of nonejecting beats

<table>
<thead>
<tr>
<th>Dog</th>
<th>Control</th>
<th>Early stretch</th>
<th>Late stretch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>263</td>
<td>278</td>
<td>254</td>
</tr>
<tr>
<td>2</td>
<td>265</td>
<td>277</td>
<td>235</td>
</tr>
<tr>
<td>3</td>
<td>240</td>
<td>243</td>
<td>234</td>
</tr>
<tr>
<td>4</td>
<td>238</td>
<td>248</td>
<td>225</td>
</tr>
<tr>
<td>5</td>
<td>221</td>
<td>239</td>
<td>208</td>
</tr>
<tr>
<td>Mean</td>
<td>245</td>
<td>257*</td>
<td>231*</td>
</tr>
<tr>
<td>SD</td>
<td>18</td>
<td>19</td>
<td>17</td>
</tr>
</tbody>
</table>

\*p < .05 vs control.
There is an alternative interpretation of these results. We have previously suggested that it may be desirable to compare only the interventions with equal left ventricular volumes. The rationale for this is that left ventricular volume, among other factors, determines peak pressure and other pressure transients. As shown in figure 8, the early volume increments cause an increase in the time for pressure to rise and fall to one-half the peak value (relative to the control isovolumic beat). Late, the duration index declines (again relative to the control isovolumic beat). However, if we compare only those points corresponding to volume infusions (i.e., disregard the "control" isovolumic data shown by the broken line), the data indicate that the time to Pmax/2 only becomes shorter as the volume step is given later. This effect continues until the instant of peak isovolumic pressure (approximately 290 msec in figure 8) and thereafter the effect diminishes. Thus, when the effects of volume step interventions are compared at equal chamber volumes, the duration index remains unchanged for early steps; as the interventions occur later the duration declines, reaches a minimum near the instant of peak pressure, and thereafter tends to return toward control.

It seems reasonable to propose that this effect is due to muscle yielding. Any muscle, whether skeletal, cardiac, or smooth, will begin lengthening once the load or stress (force per unit of cross-sectional area) applied to the muscle exceeds its capacity to bear the load. Skeletal muscle, for example, begins yielding when the applied stress exceeds a level about twice the maximum isometric value for any particular length and level of activation. The yielding phenomenon is thought to involve the mechanical interruption of crossbridges. As isovolumic systole progresses, the stiffness of the left ventricular wall increases, reaches a peak, and then falls. Thus the increase in pressure within the ventricle, which would accompany a given increase in volume, also rises, reaches a peak, and then falls. Our observation that the "change in time" plot (figure 8) reaches a trough at the same moment that isovolumic pressure reaches a peak is compatible with the idea that yielding within the ventricular wall is most extensive at the instant of maximum isometric force. Seen this way, the ventricular muscle appears to be most vulnerable to yielding under a particular volume increment at a time when it is bearing its greatest stress. It may be, however, that yielding is primarily related to cytosolic calcium levels. Indeed, in ejecting beats, the maximum effect of a volume step (our data) or a pressure increment (ref. 8) occurs in the second half of the ejection period, well after the instant at which peak stress is thought to occur. The reason for these differences (between ejecting and nonejecting beats) is not apparent from our current studies, but it is possible that differences in deactivation and free calcium levels can be responsible for differences in the time at which a load increment causes its maximum effect.

Some discussion of the final portion of the pressure curve (after 320 msec) is also in order. These late volume steps are difficult to interpret, in part because the volume step itself produces an initial rise in pressure that artificially influences the time to Pmax/2. Although these pressure records are prolonged by comparison with the control isovolumic beat, we have consistently observed that a volume increment given very late in contraction (i.e., 380 msec) produces a pressure record that is still abbreviated with respect to records for steps given in late diastole and early systole (110 through 200 msec in figure 8). In the current studies, data from the final portion of the pressure tracing (i.e., after 380 msec) were not used because the intervention produces its effect after the measurement of duration is made. Isolated muscle preparations are probably better suited to study this portion of the cardiac cycle.

Clearly, more research is required to understand completely the phenomenon discussed in this article. Brutsaert has concluded that the load dependence of relaxation requires the presence of a functional sarcoplasmic reticulum. Indeed, the declining portion of the "change in time" plot (200 to 300 msec in figure 8) may be the mechanical expression of a biochemical function (i.e., function of the sarcoplasmic reticulum); thus relaxation disorders that originate in the sarcoplasmic reticulum might be accompanied by changes in the load dependency of relaxation. It will be important to characterize the effects of inotropic drugs, ischemia, caffeine, and other interventions as part of a program investigating the coupling between mechanical and activation-deactivation determinants of relaxation.

Another potential avenue for future research includes the role of stretch nonuniformities in the heart. Suppose, for example, an early contraction in one region of an isovolumic ventricle (region 1) gave rise to a stretch in a later-contracting area (region 2). The fact that region 1 stretches region 2 during its early period of rising tension would be expected to cause region 2 to relax later than it would have otherwise. This in turn would result in greater tension in region 2 (compared with region 1) during relaxation; region 1 would yield and relax prematurely. Thus there exists the possibility...
of a feedback mechanism based on the phenomenon discussed in this article; this mechanism would tend to exacerbate the spatial and temporal nonuniformities of ventricular relaxation found in several pathologic condition, including ischemic heart disease.17,18

Finally, the phenomenon discussed herein might affect late systolic indexes of left ventricular chamber contractility derived from measurements of end-systolic pressure and volume.19 As is shown in figure 4, elastance (ΔP/ΔV) at 400 msec in beat 4 is considerably different from that in beat 3; at nearly equal ventricular volume, the pressure in beat 4 is approximately twice that in beat 3. Reflected pressure waves or other late systolic events might contribute to such change in patients with or without heart disease. Under some circumstances, therefore, the load dependency of relaxation (the inability of heart muscle to bear a late systolic load increment) might limit the use of late systolic pressure-volume data in the assessment of left ventricular contractility.

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