Afterloading Increases the Left Ventricular End-Systolic Force-Length Relation Slope in Dogs

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SUMMARY

We evaluated the effects of pressure loading produced by gradual aortic occlusion on left ventricular (LV) myocardial contractility by assessing changes in the slope of the LV end-systolic force-diameter (Fes-Des) relation. Eleven adult mongrel dogs were prepared with ultrasonic crystals for measuring LV diameter and a micromanometer for measuring LV pressure. Preload was decreased by vena caval occlusion (VCO), and afterload was increased by aortic occlusion (AOO). The slopes (Ec) and extrapolated diameter intercepts (Do) of the LV Fes-Des relation were determined for each dog from the end-systolic data obtained during VCO and AOO. During VCO and also AOO, the heart rate showed little change (134±18 vs. 134±16 bpm in VCO, 135±16 vs. 132±17 bpm in AOO). The values of Ec and Do during VCO were 62.8±15.6 g/cm and 1.20±0.36 cm, respectively, while during AOO the respective values were 122.6±18.6 g/cm and 1.66±0.38 cm. Ec and Do were both significantly increased during AOO in comparison with VCO (p<0.001). These results suggest that gradual aortic occlusion increases LV myocardial contractility in anesthetized, open-chest dogs under autonomic blockade. This phenomenon might be related to length-dependent calcium activation.

Key Words: Cylinder model Myocardial contractility Loading intervention Anrep effect Length-dependent calcium activation

In 1912, Anrep1) as well as Knowlton and Starling2) reported that an abrupt increase in left ventricular (LV) pressure had a delayed positive inotropic effect on the LV myocardium of the isolated heart. Since that time, many studies have been performed and various hypotheses have been proposed regarding the mechanism of this phenomenon. The positive inotropic effect
has been variously suggested to result from an increase in coronary blood flow,¹⁻³ the initiation of homeometric autoregulation,⁴ the recovery from pressure-induced transient subendocardial ischemia,⁵ or the release of a peripheral circulating factor(s).⁶ Since the phenomenon was not observed in myocardial strip preparations in earlier studies,⁷,⁸ it was considered to be related to changes in the coronary blood flow.⁹ However, more recent studies have also detected this phenomenon in isolated myocardial preparations.¹⁰,¹¹ Concerning the effect of changes in coronary blood flow on LV myocardial contractility, many investigators in this field have not reached common conclusions. A number of studies have been carried out using various indices to assess myocardial contractility, but the underlying mechanism of the enhancement of contractility during pressure loading still remains unclear.

Recently, we developed two new ventricular models (an active cross-bridge model¹²,¹³ and a cylinder model¹⁴) to mathematically express the dynamics and energetics of cardiac contraction. On the basis of these models, we proposed a new index for the evaluation of LV myocardial contractility,¹⁴ which is the slope of the LV end-systolic force-length relation (Ec). Experiments in dogs have suggested that Ec is a reasonable index for evaluating the myocardial performance of the intact left ventricle.¹⁵,¹⁶ In the present study, this index was used to examine the effect of pressure loading by gradual aortic occlusion on LV myocardial contractility in anesthetized, open-chest, and autonomically blocked dogs. In addition, we discuss the mechanism(s) underlying the effect of pressure loading by a comparison of the results of the present and earlier studies.

**Methods**

**Animal Preparation:**

Eleven healthy adult mongrel dogs (11.1 ± 1.6 kg) were anesthetized with intravenous pentobarbital sodium (26 mg/kg) and were fitted with instruments as previously described in detail.¹⁵ In brief, the dogs were artificially ventilated via an endotracheal tube using positive pressure respiration. Thoracotomy was performed in the left fourth intercostal space, the pericardium was opened, and the heart was suspended in a pericardial cradle. A micro-manometer-tipped catheter (MPC500, Millar Instruments) was then inserted through the LV apex and held in place by a purse-string suture. A catheter inserted into the right femoral vein served for the infusion of drugs and fluid, while another catheter was inserted into the right femoral artery for blood gas analysis. Ties were loosely placed around the inferior vena cava (IVC) and the descending aorta for the purpose of manipulating LV pressure. A
pair of ultrasonic crystals (UDM5C, MECC, Japan) were implanted in the LV endocardium of each dog to provide continuous measurements of the antero-posterior LV diameter. Arterial blood gases were monitored (ABL4 Analyzer, Radiometer, Copenhagen) and after a 20-sec period of apnea the arterial PO2 and PCO2 were respectively maintained at greater than 100 mmHg and less than 40 mmHg (irrespective of alterations in the inspired oxygen fraction and/or ventilation rate). The base excess was maintained within a −5 to +3 mEq/l range by the intravenous infusion of 7% bicarbonate solution whenever the excess fell to below −5 mEq/l. The stability of the conformation of the LV pressure-diameter loops was tested with an oscilloscope both during steady state and during the manipulation of LV pressure.

**Data Collection:**

A micromanometer was balanced on the surface of a constant temperature water bath (37°C). Lead II of the surface electrocardiogram was recorded. The LV diameter was obtained from the implanted ultrasonic crystals. The following variables were recorded: LV pressure, dp/dt, electrocardiographic data, and the LV antero-posterior diameter. Data for these variables were simultaneously stored in a hard disk memory at 1-msec intervals (1,000 Hz) by a computer system (PC9801VX, NEC, Japan). Digital data stored on the hard disk were then evaluated without the use of digital filtering.

**Experimental Protocol:**

The open-chest dogs were mechanically ventilated. Autonomic blockade was produced with atropine (0.2 mg/kg) and propranolol (2 mg/kg) given intravenously. Adequate autonomic blockade was defined as an increase or decrease of less than 10 beats/min in the heart rate over the course of any vena caval or aortic occlusion procedure. To eliminate changes in intrathoracic pressure due to respiration, data were recorded during a steady-state period at least 10 min after the initiation of autonomic blockade. First, the IVC was gradually occluded by tightening the ties around it for the collection of control data. IVC occlusion resulted in a 27±2 mmHg drop in the LV end-systolic pressure. The IVC was then released and the pressure was allowed to return to the baseline level. After these measurements were completed, the descending aorta was then gradually occluded by tightening the ties around it, producing a 44±9 mmHg elevation in LV end-systolic pressure. After the study was completed, arterial blood gas analysis was repeated after a 20-sec period of apnea, and the positions of the ultrasonic crystals were examined at necropsy.

**Data Analysis and Theoretical Background:**

In all the dogs, no arrhythmias occurred during the manipulation of LV
pressure, the final arterial blood gas analysis gave a PO2 greater than 70 mmHg and a PCO2 less than 50 mmHg (after a 20-sec period of apnea), the micromanometer drift was less than 1.0 mmHg throughout the course of the study, and the positions of the ultrasonic crystals were appropriate. The digital data recorded on the hard disk were analyzed using software developed at our laboratory.

Instantaneous LV pressure (P, mmHg) and diameter (D, cm) data recorded from several cardiac cycles during the manipulation of preload and afterload were used for the construction of LV force-diameter loops. LV circumferential force (F, g) was calculated from the equation F = P\cdot D/2a,\textsuperscript{15} where a denotes the conversion factor (0.735 mmHg\cdot g\textsuperscript{-1}\cdot cm\textsuperscript{2}). The loops constructed for one dog are shown in Fig. 1 as a representative example. End-systole was defined as the upper left corner of the LV pressure-diameter loop.\textsuperscript{17},\textsuperscript{19},\textsuperscript{20} In the present model, the LV end-systolic circumferential force (F\textsubscript{es}) was assumed to be linearly proportional to the myocardial length. Thus, F\textsubscript{es} was expressed as follows:\textsuperscript{14}

\[
F_{es} = \pi E_e \cdot (D_{es} - D_o)
\]  

where \( E_e \) denotes the slope of the LV end-systolic force-length relation [g/cm], \( D_{es} \) is the end-systolic LV diameter [cm], and \( D_o \) is the basal LV diameter [cm]. Instantaneous LV end-systolic force (F\textsubscript{es}) and diameter (D\textsubscript{es}) data were used to obtain values for \( E_e \) and \( D_o \). The linear regression equation for the LV F\textsubscript{es}-D\textsubscript{es} relation was then obtained as follows:

\[
F_{es} = A \cdot (D_{es} - B)
\]  

where \( A \) is the regression coefficient and \( B \) is the regression constant. Finally, \( E_e \) and \( D_o \) were derived in the following manner from Eqs. 1 and 2:

\[
E_e = A/\pi
\]

\[
D_o = B
\]

Data was processed using a computer system (PC9801VX21, NEC, Japan). Software was developed in our laboratory to obtain force-diameter loops, to determine the end-systolic point in each cardiac cycle, and to estimate the regression equation for the F\textsubscript{es}-D\textsubscript{es} relation and the values of \( E_e \) and \( D_o \).

**Statistical Analysis:**

Data were expressed as the mean\( \pm \)SD. The statistical significance of the differences between means was assessed using the paired t-test. Linear regression by the least squares method was used to fit data to the LV F\textsubscript{es}-D\textsubscript{es} relation.
RESULTS

Values for $E_c$, $D_0$, and each parameter during the vena caval occlusion and aortic occlusion are shown in Table I. These values were used for comparisons. The LV end-systolic pressure was decreased from $117 \pm 16$ to $90 \pm 14$ mmHg ($p < 0.001$) by IVC occlusion and increased from $122 \pm 17$ to $167 \pm 16$ mmHg ($p < 0.001$) by aortic occlusion. Heart rate showed little change.

Table I. Effect of Blood Pressure Elevation on $E_c$ and $D_0$

<table>
<thead>
<tr>
<th>Condition</th>
<th>$E_c$ [g/cm]</th>
<th>$D_0$ [cm]</th>
<th>$P_{es}$ [mmHg]</th>
<th>HR [bpm]</th>
<th>$D_{es}$ [cm]</th>
<th>$F_{es}$ [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vena caval occlusion (VCO)</td>
<td>62.8 $\pm$ 15.6</td>
<td>1.20 $\pm$ 0.36</td>
<td>117 $\pm$ 18/90 $\pm$ 14*</td>
<td>134 $\pm$ 18/134 $\pm$ 16</td>
<td>2.1 $\pm$ 0.4/1.8 $\pm$ 0.4*</td>
<td>164 $\pm$ 28/110 $\pm$ 31*</td>
</tr>
<tr>
<td>Aortic occlusion (AOO)</td>
<td>122.6 $\pm$ 18.6</td>
<td>1.66 $\pm$ 0.38</td>
<td>122 $\pm$ 17/167 $\pm$ 16*</td>
<td>135 $\pm$ 17/132 $\pm$ 17*</td>
<td>2.1 $\pm$ 0.4/2.4 $\pm$ 0.4*</td>
<td>168 $\pm$ 30/262 $\pm$ 48*</td>
</tr>
</tbody>
</table>

*p < 0.001 (a vs. b).

Abbreviations: $E_c$ = slope of the LV end-systolic force-length relation; $D_0$ = extrapolated diameter intercept of the LV end-systolic force-diameter relation; $P_{es}$ = LV end-systolic pressure; HR = heart rate; $D_{es}$ = LV end-systolic diameter; $F_{es}$ = LV end-systolic force.

$a$ and $b$ represent data at two different pressure conditions: $a =$ baseline and $b =$ the lowest or highest pressure condition.

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Fig. 1. Representative left ventricular circumferential force-diameter loops recorded in one dog during vena caval occlusion (VCO) and aortic occlusion (AOO). The slope of the end-systolic force-length relation ($E_c$) was significantly increased by aortic occlusion compared with vena caval occlusion ($E_c$ during VCO, 70.9 g/cm. $E_c$ during AOO, 135.0 g/cm).
with either IVC occlusion (134±18 vs. 134±16 bpm, NS) or aortic occlusion (135±17 vs. 132±17 bpm, p<0.001). The LV end-systolic diameter decreased from 2.1±0.4 to 1.8±0.4 cm (p<0.001) with IVC occlusion and increased from 2.1±0.4 to 2.4±0.4 cm (p<0.001) with aortic occlusion. Similarly, the LV end-systolic force decreased from 164±28 to 110±31 g (p<0.001) with IVC occlusion and increased from 168±30 to 262±48 g (p<0.001) with aortic occlusion. The LV F_{es-D_{es}} relation remained nearly linear during these interventions.

Ec and D_{o} values for the LV F_{es-D_{es}} relation were obtained by the linear regression analysis of a set of end-systolic data (Fig. 1), and comparisons were made between preload reduction and afterloading. It was found that the values of Ec (62.8±15.6 vs. 122.6±18.6 g/cm, p<0.001) and D_{o} (1.20±0.36 vs. 1.66±0.38, p<0.001) were both significantly increased by pressure loading in comparison with IVC occlusion.

**DISCUSSION**

We recently developed a new contractility index for evaluating myocardial performance, which is the slope of the LV end-systolic force-length relation (Ec). The present study assessed the acute effect of an increase in systolic pressure on myocardial contractility by using this index. It was shown that under beta-blockade Ec and D_{o} were both greater during AOO than during VCO. It has previously been reported that when the contractility of papillary muscle preparations or the isolated heart is increased, the length intercept of the myocardial force-length relation or the volume-axis intercept of the LV end-systolic pressure-volume relation remain almost constant, whereas the slopes of these relations become steeper. On the basis of these earlier findings, it would appear that Ec progressively increases during pressure loading while D_{o} remains almost constant. In other words, myocardial contractility may progressively increase with pressure loading, as shown in Fig. 2.

Many investigators have reported that a sudden increase of ventricular pressure causes an increase in LV myocardial contractility. This effect has been variously suggested to be the result of an increase in coronary blood flow, the initiation of homeometric autoregulation, the recovery from pressure-induced transient subendocardial ischemia, or the release of a peripheral circulating factor(s). However, the underlying mechanism(s) still remains unclear.

In 1912, Anrep and Knowlton and Starling reported that an abrupt increase in ventricular pressure had a delayed positive inotropic effect on the
myocardium of the isolated heart. Knowlton and Starling suggested that this effect was due to improved perfusion of the myocardium, since it is accompanied by an increase in coronary blood flow. However, Sarnoff et al. found that the effect (they designated it as the Anrep effect) also persisted in isolated hearts even when coronary blood flow remained constant. They considered that this positive inotropic effect was independent of coronary blood flow, and suggested that it reflected some form of intrinsic homeometric autoregulation. Since the Anrep effect was not observed in muscle strip preparations at that time, Monroe et al. suggested that a transient negative inotropic effect occurred after a sudden increase in ventricular pressure as a result of pressure-induced transient subendocardial ischemia, and that this phenomenon was immediately modified by autoregulation of the coronary
vascular bed with a redistribution of coronary blood flow. Thus, the Anrep effect was considered to be caused by changes in the coronary blood flow and to represent the recovery from the negative inotropic effects of an abrupt increase in pressure.

In more recent times several investigators have detected the Anrep effect in isolated cardiac muscle preparations. When cardiac muscle preparations are subjected to an afterload, either negative or positive inotropic responses, or even a biphasic change in myocardial contractility have been observed. The first phase of the biphasic change in contractility may be related to a transient change in the Ca$^{2+}$ level and the second phase (the delayed increase in contractility) may correspond to the Anrep effect for the whole heart. These findings are inconsistent with the hypothesis of Monroe et al.

It is possible that changes in coronary blood flow and/or coronary arterial pressure could affect the myocardial contractility of the left ventricle. Knowlton and Starling concluded that enhanced myocardial contractility was attributable to improvement of the coronary circulation by an increase in pressure. After their experiments, a large number of studies were carried out to assess the effects of changes in coronary blood flow and/or perfusion pressure on LV contractility. It has been reported that increasing the coronary blood flow does not enhance the contractility of the myocardium. In contrast, Abel et al. examined the influence of changes in coronary blood flow on $V_{\text{max}}$ and reported that coronary blood flow was a major determinant of myocardial contractility. They suggested that the phenomenon was caused by catecholamine release, changes in intracellular metabolism, or the release of other substances from somewhere in the body following the increase in coronary blood flow. However, although contractile force was found to be highly dependent on the coronary blood flow at low flow rates where coronary flow may have already been influenced by autoregulatory mechanisms, it was relatively unaffected at higher flow rates.

A study which examined the $E_{\text{max}}$ of isolated hearts under conditions of constant coronary blood flow showed that $E_{\text{max}}$ was little affected by coronary blood flow. On the other hand, it was reported that the $E_{\text{max}}$ of the in situ heart increased after coronary blood flow was increased at a constant coronary perfusion pressure. The study which examined the influence of coronary perfusion pressure on the $E_{\text{max}}$ of isolated hearts showed that $E_{\text{max}}$ was unchanged when the perfusion pressure remained above a critical level ($67\pm22.1\text{ mmHg}$), but became increasingly more sensitive to changes in coronary perfusion pressure below this critical level. The result was a downward bending of the LV end-systolic pressure-volume relation and a decrease
in $E_{\text{max}}$ at low perfusion pressure. When in situ hearts were studied, $E_{\text{max}}$ was found to increase after an increase in coronary perfusion pressure at a constant coronary blood flow rate. Conflicting results have been obtained, i.e., an increase in coronary perfusion pressure induces a positive LV inotropic effect, but does not influence LV contractility. Thus, the effects of changes in coronary blood flow and/or perfusion pressure on LV contractility remain ambiguous. It may be reasonable to assume that LV contractility is affected by coronary blood flow and/or perfusion pressure only when these variables fall below a critical level, whereas when they are maintained sufficiently, the effects of changes in either parameter on the LV contractility are not obvious.

Using the conductance catheter technique, Baan et al. investigated the influence of pressure or volume interventions on LV contractility. They found that $E_{\text{max}}$ under a pressure load was always larger than under a volume load. They suggested that the difference may be due to a factor(s) released into the peripheral circulation, which is especially produced distal to the site of aortic occlusion. They concluded that the positive inotropic effect noted after aortic occlusion was not caused by the Anrep effect, because of the use of gradual aortic occlusion in their experiments (over 5 to 10 sec) and because of the lack of transient changes in end-diastolic pressure or volume as observed with the typical Anrep effect. They also hypothesized that the positive inotropic effect of an increase in pressure was to allow the heart to eject a sufficient cardiac output to the peripheral organs under various conditions, as described by Ehrlich et al.

Since the pressure was also increased gradually in the present study, our experimental results may also not be identical to the Anrep effect, as Baan et al. hypothesized. LV wall contractility appeared to increase progressively during pressure loading and there was no biphasic change of the contractile state. Accordingly, it is obvious that the increase in contractility was not caused by the recovery from transient subendocardial ischemia as suggested by Monroe et al. LV pressure was increased by a relatively small amount (from approximately 120 to 160 mmHg) in our experiments. Although coronary blood flow and perfusion pressure were not controlled and thus were not measured, these factors probably do not contribute to the increase in $E_{c}$, since cardiac function is little affected by changes in the distribution of blood in the coronary arteries induced by small increases in pressure. In addition, since the time until the onset of $E_{c}$ elevation was very short, this positive inotropic effect may not be caused by a peripheral factor as proposed by Baan et al. The positive inotropic effect that we observed was also not caused by changes of the cardiac sympathetic tone, because the adrenergic
and vagus nerves were almost completely blocked by the infusion of propranolol and atropine. Accordingly, the effect observed in our experiments is suggested to be induced by an intrinsic homeometric autoregulation mechanism in the myocardium, as previously proposed by Sarnoff et al., which is initiated immediately after an increase in LV pressure.

We cannot rule out one possibility that our result reflects the essential non-linearity of the force-length relation, because the force-length relation during volume-loading was not examined in the present experiments. Experimental studies for the force-length relations during volume-loading and volume-unloading are needed to validate this possibility.

A more likely underlying mechanism of the homeometric autoregulation is “length-dependent calcium activation”, i.e., augmented contractile performance of the myocardium by the change in sensitivity of calcium and/or calcium concentration released from the sarcoplasmic reticulum with increasing muscle length. In our experiments the initial muscle length was prolonged by afterloading (Fig. 1). It is theoretically predicted from our model that $E_c$ may correspond to calcium concentration released from the sarcoplasmic reticulum. Thus, the rise in calcium concentration with initial muscle lengthening would lead to the phenomenon of “homeometric autoregulation” of the myocardium. Accordingly, our model’s prediction reconciles the result of our experiments (increased $E_c$ by afterloading) with “length-dependent calcium activation”.

In conclusion, the LV end-systolic force-length relation was nearly linear both during the decrease of pressure produced by inferior vena caval occlusion and during pressure loading produced by descending aortic occlusion. The slope of the relation was significantly steeper during pressure loading than during pressure reduction. The change in the slope under pressure loading was observed immediately after the initiation of loading, and progressively increased as the pressure rose. These findings suggest that afterloading due to aortic occlusion immediately increases the myocardial contractility of the in situ autonomically blocked canine left ventricle. This increase in LV myocardial contractility with pressure loading is thought to be generated by “length-dependent calcium activation” with initial muscle lengthening.

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