Contractility-dependent curvilinearity of end-systolic pressure-volume relations

DANIEL BURKHOFF, SEIRYO SUGIURA, DAVID T. YUE, AND KIICHI SAGAWA
Department of Biomedical Engineering, The Johns Hopkins School of Medicine, Baltimore, Maryland 21205

BURKHOFF, DANIEL, SEIRYO SUGIURA, DAVID T. YUE, AND KIICHI SAGAWA. *Contractility-dependent curvilinearity of end-systolic pressure-volume relations.* Am. J. Physiol. 252 (Heart Circ. Physiol. 21): H1218–H1227, 1987.—The shape of the end-systolic tension-length relationship (ESTLR) changes when contractile state is changed, whereas the end-systolic pressure-volume relationship (ESPVR) remains linear despite changes in contractility. To investigate this disparity, the ESPVR was determined with contractility altered extensively by dobutamine, BAY K 8644, nifedipine, lowering coronary blood flow, and the introduction of extrasystolic and postextrasystolic stimulations. The ESPVRs were fitted by nonlinear regression analysis to the parabolic equation $P_{es} = aV_{es}^2 + bV_{es} + c$, where P_{es} is end-systolic pressure, V_{es} is end-systolic volume, and a, b, and c are parameters. There was a negative, statistically significant correlation between a, which serves as a shape index of the ESPVR, and E'_{max}, the slope of the ESPVR in a low volume range. When E'_{max} was large a was negative, indicating increasing concavity of the ESPVR to the volume axis at high contractility. When E'_{max} was small a was positive, indicating convexity of the ESPVRs to the volume axis at low contractility. Within the average range of E'_{max} between 3.4 and 7.8 mmHg/ml, however, the parabolic fit to the data was not statistically better than a linear fit over the range of volumes testable in the isolated heart. We conclude that the shape of the ESPVR measured in the isolated canine heart changes with contractile state. In accordance with previous interpretations of shape changes in the muscle ESTLR, these results are consistent with the existence of length-dependent activation of cardiac muscle in the intact heart.

dobutamine; BAY K 8644; coronary perfusion pressure; postextrasystolic potentiation; nifedipine; length-dependent activation

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RECENT STUDIES PERFORMED on isolated mammalian cardiac muscle have led to the concept that the extent of myofilament activation achieved on a beat is dependent not only on the amount of calcium supplied to the myofilaments but also on the length of the muscle. This phenomenon, known as length-dependent activation, was first described for skeletal muscle (3) and then for cardiac muscle by Allen et al. (1) and Jewell (11), who noted that the shape of the end-systolic tension-length relation (ESTLR) of isolated cat papillary muscle was different when contractile state was altered by varying the calcium concentrations of the bathing solution. Evidence for length-dependent activation has since been obtained in numerous experimental preparations with contractile state varied by different methods (2, 4, 7, 9, 10, 15, 16, 21, 31). Although the mechanisms responsible for length-dependent activation are not completely understood, the available experimental data suggest two possibilities. The first is that the affinity of the force-generating machinery for Ca^{2+} increases when cardiac muscle is lengthened (9). The second possibility is that the amount of calcium released to the myofilaments on a beat depends (in the steady state) on muscle length (2, 5). Both of these mechanisms are believed to underlie, in part, not only the length dependence of activation but also the Frank-Starling relation as well.

Despite numerous studies of length-dependent activation in isolated muscle preparations, very little is known about the significance of this phenomenon for a more intact situation. In fact, previous studies of isolated canine hearts have shown that, in the physiological range, the shape of the ventricular end-systolic pressure-volume relationship (ESPVR) (the ventricular level counterpart of the ESTLR) is linear, independent of contractile state (26, 28). These findings raise the question of whether evidence for the existence of length-dependent activation is observable under conditions closer to those encountered physiologically than in excised pieces of ventricular muscle.

The purpose of the present study, therefore, was to investigate whether the shape of the ventricular ESPVR changes with large alterations in contractile state as has been observed for the ESTLR in isolated heart muscle. Specifically, we tested the hypothesis that with marked enhancements of the contractile state the ESPVR would become concave to the volume axis and that with marked reductions in contractile state it would become convex to the volume axis. We employed an isolated canine heart preparation, which allows for precise measurement of ventricular volume. In contrast to previous studies in isolated hearts (26, 28), we varied the ventricular contractile state over a very wide range by different inotropic agents, alterations in coronary perfusion pressure and the introduction of extrasystolic and postextrasystolic stimulations. The results indicated that at high and low contractile states the ESPVR deviates from linearity in a manner predicted from isolated muscle studies.

METHODS

Surgical preparation. The procedures used to isolate and support a canine heart were similar to those described by Suga and Sagawa (26). A pair of mongrel dogs was anesthetized with pentobarbital sodium (30 mg/kg

iv). The femoral arteries and veins of one dog (support dog) were cannulated and connected to a perfusion system that was used to supply oxygenated blood to the isolated heart. The chest of the second dog (donor dog) was opened under artificial respiration, and the heart was removed while metabolically supported by arterial flow from the support dog. The left atrium was opened, and all chordae tendineae were freed from the mitral valve leaflets. A metal adapter that held the isolated heart to the ventricular volume servo-pump system (described below) was sutured to the mitral ring. When the surgical preparation was complete, the isolated heart was positioned such that a water-filled latex balloon was inside the left ventricular cavity. The metal adapter sutured to the mitral annulus has a flange that extended under the aortic valve and prevented herniation of the intraventricular balloon into the aorta. Accumulation of blood in the space between the balloon and ventricular wall was avoided by venting.

Coronary perfusion pressure was controlled at a constant level by a perfusion pump that regulated the flow rate of arterial blood from the support dog (more detail presented below). The blood temperature was maintained between 37 and 38°C.

Ventricular volume servo-system. A servo-pump system was used to control ventricular volume. Details of its design and performance have been reported by Suga and Sagawa (27) and Sunagawa et al. (30). Briefly, a linear motor controls the position of the piston within a rolling diaphragm cylinder. A latex balloon is secured to a tube connected to the outflow tract of the cylinder forming a closed system that is filled with water. A linear displacement transducer senses the position of the piston, thus producing a signal proportional to the balloon (and therefore ventricular) volume. This signal is used in a negative-feedback loop for comparison with a volume command signal that represents the desired ventricular volume. The error signal resulting from this comparison is supplied to a power amplifier, which in turn drives the linear motor.

Protocols. All data were collected with the ventricles contracting isovolumically. The hearts were paced at a constant rate between 100 and 130 beats/min. Data for determining ESPVRs were recorded the same way during each experimental intervention. Left ventricular volume was set to the highest value at which steady pacing could be achieved without arrhythmia; this was typically between 40 and 45 ml (with corresponding end-diastolic pressure between 20 and 30 mmHg). After a steady state was established, recordings of ventricular pressure and volume were made. Ventricular volume was then reduced by 2–3 ml, and new recordings were made after a new steady state was reached. Volume was decreased to a minimum between 5 and 10 ml. ESPVR data were obtained under control conditions and then after contractile state was changed in one of the following five ways.

1) Dobutamine, a positive inotropic agent, was infused into the coronary perfusion line. Two or three different infusion rates were used in each heart to enhance the contractile state to two- or threefold the control level (approximate infusion rates between 5 and 20 μg/min). To judge changes in contractile state resulting from drug infusion during the experiment (in this and the protocols described below), we assessed changes in isovolumic developed pressure at a ventricular volume of ~25 ml. Coronary perfusion pressure was set between 80 and 100 mmHg at the start; the speed of the pump controlling arterial blood flow to the heart was held constant so that drug delivery to the left ventricle (LV) was constant during each ESPVR determination.

2) Coronary perfusion pressure (CPP) was lowered to reduce contractile state. The control value was set between 100 and 80 mmHg and was lowered to values between 50 and 30 mmHg on experimental runs.

3) BAY K 8644, a calcium-channel agonist [a new positive inotropic agent (24)], was administered as described above for dobutamine (typical infusion rates providing concentrations between ~0.5×10^{-8} and 2.5×10^{-8} M). In addition, it was necessary to simultaneously infuse adenosine (1 mg/min) to counteract the potent coronary vasoconstricting effects of this agent. The net result of infusing this combination was a slight reduction in coronary resistance. The speed of the pump controlling coronary blood flow was adjusted as described in *1* above, so that drug delivery was constant during each ESPVR determination.

4) Nifedipine, a calcium-channel antagonist (negative inotropic agent), was infused at two or three different rates, the maximal rate titrated to reduce contractile state to less than one-half of control (typical infusion rates providing concentrations between 1×10^{-7} and 6×10^{-7} M). Nifedipine reduces coronary resistance, and the perfusion pump speed was adjusted as in *1* above.

5) Finally, contractile state was varied by introducing extrasystolic (ES) and postextrasystolic (PES) stimuli. Heart rate was set between 100 and 120 beats/min. After a steady mechanical state was established at a given volume, two test stimuli were introduced. The first was at a test pulse interval TPI_1 less than the steady-state beat-to-beat interval, and the second was at an interval TPI_2 chosen to create a "compensatory pause" (i.e., TPI_2 was chosen such that the sum of TPI_1 and TPI_2 was twice the steady-state pacing interval). ES and PES beats were measured over the same wide range of volumes as described above for the other interventions. By shortening TPI_1, the ES beat becomes progressively weaker than the steady-state beat, and the PES beat becomes progressively more potentiated [see Johnson (13), for a review of the interval dependence of contractile strength]. Several combinations of TPI_1 and TPI_2 values were studied in each heart. CPP was set between 80 and 100 mmHg and held constant during each ESPVR determination.

It was not possible to test each of these interventions in every heart due to the limited duration of viability of the experimental preparation (typically between 3 and 5 h). The actual number of hearts in which each intervention was used are presented in Table 1.

Data analysis. Ventricular volume and pressure, CPP, and bipolar epicardial electrogram were recorded on a strip-chart recorder (Gould model 2800), digitized at a sampling rate of 200 Hz, and stored on magnetic tape for off-line analysis. The end-systolic pressure (P_{es}) (which for isovolumic contraction is equal to the peak systolic

TABLE 1. *Summary of number of experiments*

Intervention	No. of Expts
Dobutamine	5
Decreased CPP	6
Nifedipine	2
BAY K 8644	5
ES/PES pacing	8

CPP, coronary perfusion pressure; ES, extrasystolic; PES, postextrasystolic.

pressure) and end-systolic volume (V_{es}) of each beat measured during an experimental run was determined. From a set of preliminary data, we conceived the end-systolic pressure-volume relation to be of the parabolic form

$$P_{es} = aV_{es}^2 + bV_{es} + c \quad (1)$$

In this equation the weighting factor for V_{es}^2, a, served as a quantitative measure of the curvilinearity of the ESPVR. If the ESPVR is linear, then a will have a value of zero. For an ESPVR convex to the volume axis, a will have a positive value. For an ESPVR concave to the volume axis, a will have a negative value. In general, the larger the absolute value of a, the more strongly curvilinear the ESPVR. For each set of end-systolic pressure-volume data, the values of the parameters a, b, and c that provided the best least-squared fit to the data were determined by multiple regression analysis.

With the nonlinear representation of the ESPVR proposed in *Eq. 1*, contractility cannot be quantified simply by the conventional parameter "E_{max}," which is the slope of the (assumed) linear ESPVR (26). However, to test whether the ESPVR changes shape with contractility, we obviously need to be able to quantify contractility reliably. Therefore, contractility was indexed by the slope of the ESPVR at low volumes and was designated E'_{max}. The value of this index was obtained by evaluating the derivative of *Eq. 1* at P_{es} equal to 0 mmHg, with the parameters a, b, and c determined by regression analysis to the data as outlined above

$$E'_{max} \equiv dP_{es}/dV_{es} \mid_{P_{es}=0} \quad (2a)$$

$$= 2aV + b \mid_{P_{es}=0} \quad (2b)$$

Solving *Eqs. 1* and *2* simultaneously yields

$$E'_{max} = (b^2 - 4ac)^{1/2} \quad (3)$$

Thus, with a linear ESPVR (i.e., with $a = 0$), E'_{max} is equal to b, which is precisely the same as the previously defined slope of the linear ESPVR, E_{max}.

Similarly, the volume-axis intercept of the ESPVR, designated V_0, was determined by setting $P = 0$ in *Eq. 1* and solving for V by the quadratic formula

$$V_0 = [-b + (b^2 - 4ac)^{1/2}]/2a \quad (4)$$

For the case of ESPVRs determined when contractile strength was greatly reduced, these definitions of E'_{max} and V_0 (i.e., *Eqs. 3* and *4*) frequently became problematic due to the shallow approach of the end-systolic pressure-volume data points to the volume axis, which resulted in a large statistical uncertainty in the predicted volume-axis intercept of the regression line. Therefore, for ESPVRs measured under reduced contractile states E'_{max} and V_0 were defined alternatively as the slope and volume-axis intercept, respectively, resulting from linear regression analysis applied to the end-systolic pressure-volume data obtained below a low volume of 20 ml. Although the definitions of E'_{max} and V_0 based on linear regression are not ideal, they provide a reliable means of quantifying these parameters when meaningful application of the analytic definition (i.e., *Eqs. 3* and *4*) was obviated by statistical uncertainty in the setting of low contractility. In fact, when applied to data obtained at higher levels of contractility, the alternate sets of definitions provide estimates of E'_{max} and V_0 that were not significantly different from those provided by *Eqs. 3* and *4*. (See APPENDIX for further details.) The quantification of the shape of the ESPVR by the parameter a was the same under all levels of contractile state.

To determine the statistical significance of curvilinearity of each ESPVR, we employed a Student's t test to test the null hypothesis that there was no statistically significant difference in a from zero. If P resulting from this test was <0.05, then the value of a was considered to be different from zero and the ESPVR was deemed nonlinear. Conversely, for $P > 0.05$, the difference, if any, of a from zero was considered insignificant, and the ESPVR was deemed linear.

A total of 151 end-systolic pressure-volume relationships from eight hearts were analyzed in this manner.

To demonstrate the shape dependence of the ESPVR on contractility, we plotted the resulting values of a as a function of E'_{max} for every ESPVR measured in each heart; data from different hearts were plotted and analyzed separately. Linear regression analysis was used to quantify the trend of the relation between a and E'_{max} in each heart; however, it is not our intention to prove or even imply, that the relationship between a and E'_{max} is linear. Data from different hearts were not pooled for these analyses due to the lack of suitable normalization procedures for E'_{max} and a to account for differences in ventricular mass and geometry. A plot was also made of V_0 against E'_{max} to determine whether any significant shifts in the ESPVR could be identified.

RESULTS

Examples of original experimental recordings of isovolumic left ventricular pressure (LVP), left ventricular volume (LVV), and CPP, taken from a typical experiment are shown in Fig. 1. For the experimental run shown, the contractile state was altered by introducing ES and PES stimulations as described in METHODS. These records, obtained at three different volumes, were part of an experimental run in which data were collected at a total of seven different volumes. In this example the steady-state heart rate was set at 110 beats/min (corresponding to a steady-state beat-to-beat interval of 550 ms), TPI_1 was set at 350 ms and TPI_2 was set at 750 ms. Peak isovolumic LVP was less on the prematurely introduced ES than on the steady-state beats and greater on PES. LVV was varied between 5.9 and 37.2 ml while pacing with the same pattern. Peak LVP decreased with decreases in LVV, but the trends in alterations of LVP with altered pacing intervals were the same at each

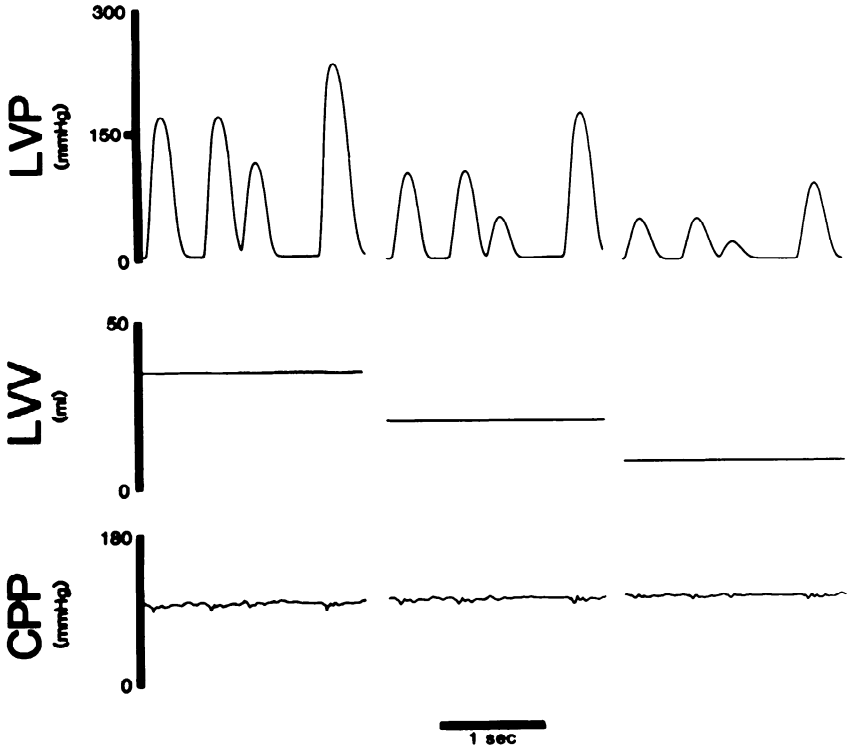

FIG. 1. Original experimental recordings from a typical experiment. LVP, left ventricular pressure; LVV, left ventricular volume; CPP, coronary perfusion pressure. These records were obtained with extra- and postextrasystolic pacing.

volume. Note that the CPP tracing (measured in the aortic root) was not influenced by the high intraventricular pressures, indicating that the metal ring sutured to the mitral annulus did not permit herniation of the balloon into the aorta. From these data we determined the end-systolic (i.e., peak) pressures of the steady-state, ES, and PES beats at each of the volumes studied. These were then plotted as a function of ventricular volume, resulting in ESPVRs. The ESPVRs corresponding to the tracings in Fig. 1, including the rest of the data collected in this run, are presented in Fig. 2, with solid circles corresponding to steady-state data, triangles corresponding to ES data, and squares corresponding to PES data. The solid lines are the parabolic fit to the data (i.e., *Eq. 1*) by nonlinear regression analysis (see METHODS). The

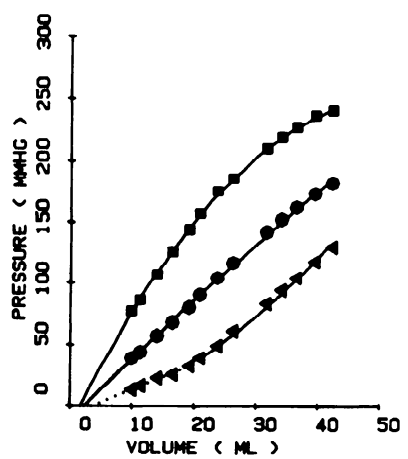

FIG. 2. End-systolic pressure-volume relations for data presented in Fig. 1. *Solid circles*, control data; *triangles*, extrasystolic data; *squares*, postextrasystolic data. *Solid lines* are best-fit quadratic relations ($P_{es} = aV_{es}^2 + bV_{es} + c$) to data as determined by nonlinear regression analysis. *Dotted line* is linear regression to data on extrasystolic beat obtained below 20 ml. See text for further details.

dotted line represents the linear extrapolation of the ESPVR measured on the ES beat and was obtained by linear regression analysis applied to the data below the cutoff point of 20 ml as outlined in METHODS and APPENDIX. The three regression lines intersect the volume axis within 3 ml of each other, indicating very little variation in V_0 (*Eq. 4*) with contractile state. In this example, the steady-state ESPVR had an E'_{max} of 5.3 mmHg/ml and a small a value of -0.017 mmHg/ml^2, resulting in an essentially linear relation. On ES contractions, E'_{max} was 2.02 mmHg/ml with an a value of 0.0506 mmHg/ml^2. The PES E'_{max} was 12.4 mmHg/ml with an a value of -0.152 mmHg/ml^2. The nonlinearities in the regressions to the ES and PES ESPVRs were statistically significant ($P < 0.001$). Thus these data demonstrate ESPVRs that deviated from linearity when contractile state was markedly depressed and enhanced.

Typical ESPVRs obtained while altering the contractile state with the various interventions described in METHODS are presented in Fig. 3; data in a given panel are from a single heart, and the interventions are specified in the graph inserts. Those interventions, which enhanced contractile state (dobutamine, BAY K 8644, and PES pacing), provided ESPVRs that were concave to the volume axis and had parabolic regressions with negative values of a. Note that with the positive inotropic interventions, peak isovolumic pressures at large volumes reached values in excess of 200 mmHg.

In contrast, those interventions that depressed contractile state (nifedipine, decreased CPP, and ES pacing) provided ESPVRs that were convex to the volume axis and had parabolic regressions with positive values of a. With depressed contractility at large volumes, peak isovolumic pressures were typically below 75 mmHg even at large volumes.

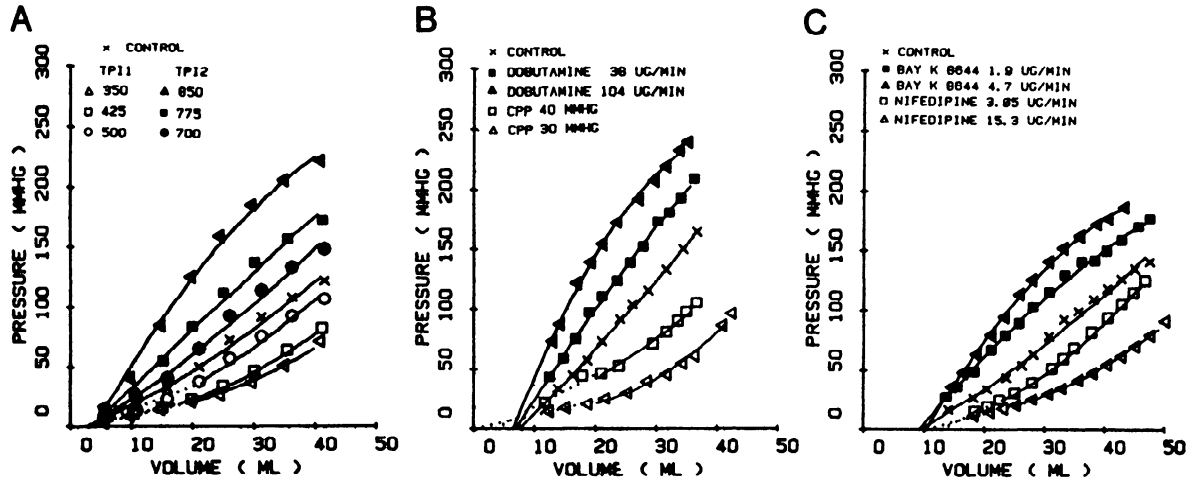

FIG. 3. End-systolic pressure-volume relations obtained with contractile state varied by the different interventions. Data in each panel is from a single heart with different drug dosages, different coronary perfusion flows, or different values of pacing intervals. *Solid lines* are best-fit quadratic equations. TPI_1, TPI_2, test pulse interval 1 and 2, respectively; CPP, coronary perfusion pressure.

It is also seen in each panel that V_0, the volume-axis intercept of the ESPVR, was not significantly influenced by changes in contractile state.

The results of the quantitative analysis of data from a typical heart are summarized in Fig. 4. The relation between contractile state and curvilinearity is illustrated in Fig. 4*A*, where values of a are plotted as a function of E'_{max} for all the ESPVRs measured in this heart. Different symbols indicate the different interventions used to alter the contractile state. As shown, with E'_{max} decreasing below ~6 mmHg/ml there is a trend toward increasing positive values of a, indicating an increasing degree of convexity of the ESPVRs to the volume axis. With E'_{max} increasing above ~6 mmHg/ml there is a trend toward increasing negative values of a, indicating an increasing degree of concavity of the ESPVRs to the volume axis. For this heart, $a = -0.021\ E'_{max} + 0.131$.

The results of the analysis to assess statistical significance of nonlinearity in the ESPVR in this ventricle are presented in Fig. 4*B*, where we plotted P, as a function of E'_{max}. $P > 0.05$ (indicated by the dotted line) means that the value of a is not statistically different from 0; that is, a nonlinear fit to the data is not better than a linear fit. Conversely, $P < 0.05$ indicates that the nonlinear fit to the data is better than the linear fit. Thus, for the data of Fig. 4, those ESPVRs with E'_{max} between ~4 and 10 mmHg/ml can be considered to be linear over the range of volumes tested. The ESPVRs outside this range, however, should be treated as nonlinear.

In Fig. 4*C*, we plot the relation between E'_{max} and V_0 from the same experiment. The solid line was obtained by linear regression. There was no statistical significance to this relation ($P > 0.1$), indicating that V_0 was essentially independent of contractile state.

The results obtained from all eight hearts are summarized in Table 2, where we present the parameters of the lines of regression between a and E'_{max}, and between V_0 and E'_{max}, and the range of E'_{max} over which the ESPVR can be considered linear within the range of volumes tested. In each case there was a negative correlation between a and E'_{max}, indicating a convex ESPVR at low contractile states and a concave ESPVR at high contractile states. The relation between E'_{max} and a was statistically significant in all but one heart (*heart 2*). The relation between E'_{max} and V_0 was only statistically significant in one heart (*heart 8*). The average range over which the ESPVR could be considered linear was 3.4–7.8 mmHg/ml. It should be noted that the results of the statistical analysis performed to assess linearity are dependent not only on the volume range over which the pressure-volume data is collected but also on the actual number of data points measured to define the ESPVR. To minimize this problem we collected data at small intervals of ~3 ml and covered a wide volume range at each contractile state.

In Fig. 5 we have constructed hypothetical nonlinear ESPVRs based on the average results presented in Table 2. For this analysis we assumed that V_0 was 5 ml and constant. E'_{max} was set between 1 and 13 mmHg/ml as indicated in Fig. 5. For a given value of E'_{max}, a was determined by the average relationship: $a = -0.019\ E'_{max} + 0.073$ presented in Table 2. If the values of E'_{max}, V_0, and a are fixed, calculation of the parameters b and c in *Eq. 1* can be made, thus defining the parabolic ESPVR. This plot provides a graphic summary of the results of the present study.

DISCUSSION

We measured ESPVRs in isolated canine hearts in which the contractile state was varied over a very wide range by several different interventions (Table 1). The end-systolic pressure-volume data were fit by nonlinear regression analysis to a parabolic equation (*Eq. 1*), and the quality of this fit was compared with that obtained by linear regression analysis. We quantified contractility by E'_{max}, the slope of the ESPVR at low volumes (*Eq. 3* and APPENDIX). The curvilinearity of the ESPVR was quantified by a, the coefficient of the second-order term in the parabolic equation. There was a statistically significant negative correlation between contractile state and curvilinearity. With high values of E'_{max}, a was negative, indicating concavity of the ESPVRs (over the

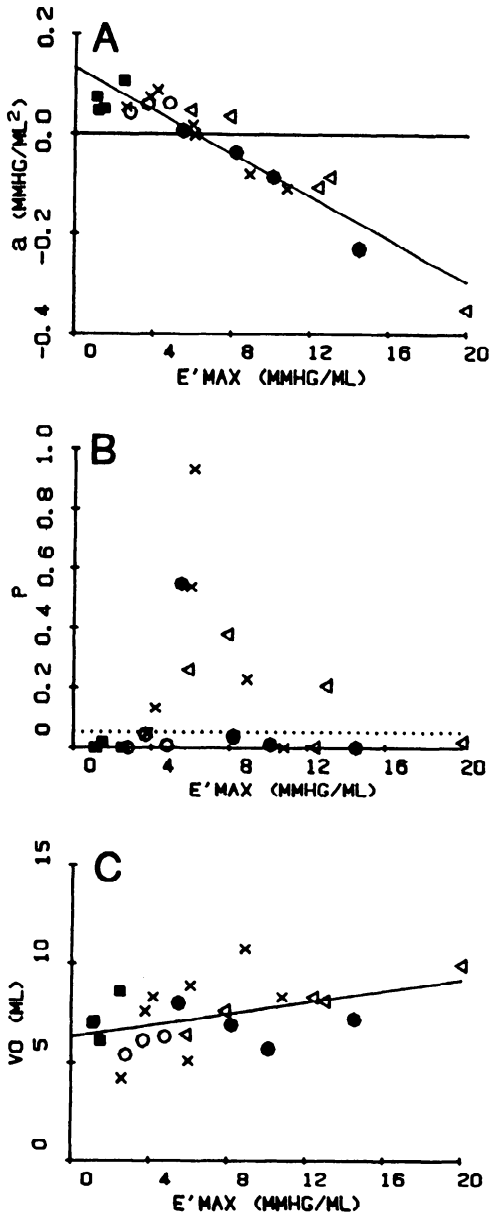

FIG. 4. *A*: relation between E'_{max}, index of contractility, and *a*, index of curvilinearity, for a typical heart (*heart 4* in Table 2). Different *symbols* represent data obtained with different inotropic interventions. *Solid line* was obtained by linear regression to data to assess trend in data. Positive values of *a* indicate convexity of end-systolic pressure-volume relation (ESPVR) to volume axis, whereas negative values of *a* indicate concavity. *B*: results of analysis to determine statistical significance of nonlinearity for data of *A*. If $P < 0.05$ (*dotted horizontal line*), then value of *a* is statistically different than zero, indicating a nonlinear relation. Data indicates that end-systolic pressure-volume relation (ESPVR) can be considered linear when E'_{max} is between 4 and 10 mmHg/ml. *C*: relation between E'_{max} and V_0, volume-axis intercept of ESPVR, for same data presented in *A*. *Solid line* determined by linear regression indicates no statistically significant influence of contractile state on V_0 ($P > 0.1$).

range of volumes tested) to the volume axis at high contractile states. With low values of E'_{max}, *a* was positive, indicating convexity of the ESPVRs to the volume axis at low contractile states. On average, for E'_{max} between 3.4 and 7.75 mmHg/ml, the parabolic fit to the data was not statistically better than that provided by linear regression over the range of volumes tested. Thus the shape of the ESPVR measured in the isolated canine

TABLE 2. *Summary of results from eight hearts*

Expt No.	E'_{max} vs. *a*		E'_{max} vs. V_0		Range of Linear ESPVR
	Slope	Int	Slope	Int	
1	−0.025*	0.145	−0.139	12.0	4–11
2	−0.012	0.035	0.395	0.79	3–6
3	−0.028*	0.182	−0.101	2.57	5–9
4	−0.021*	0.131	0.216	5.53	4–10
5	−0.019*	0.088	0.786	6.93	4–8
6	−0.017*	0.063	0.006	1.51	3–5
7	−0.024*	0.072	−0.351	15.4	1–6
8	−0.013*	0.044	−0.760*	9.9	3–7
Avg	−0.020	0.094	−0.035	6.67	3.4–7.8
SD	0.003	0.019	−0.249	1.40	

Slope and intercept (Int) between specified parameters obtained by linear regression analysis. E'_{max}, slope of end-systolic pressure volume relationship (ESPVR) at low volumes; *a*, index of curvilinearity; V_0, volume-axis intercept. * $P < 0.05$, statistically significant relation.

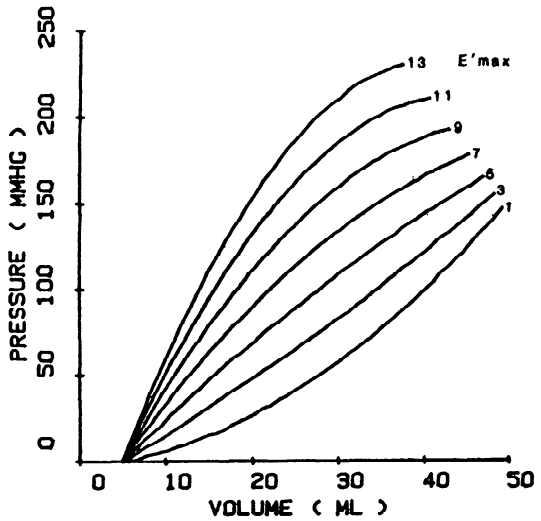

FIG. 5. Hypothetical parabolic end-systolic pressure-volume relations (ESPVR). Volume-axis intercept (V_0) is set at 5 ml and is constant. For a chosen value of slope of end-systolic pressure-volume relationship (ESPVR) at low volumes (E'_{max}) (indicated in graph) value of *a* in *Eq. 1* was determined by average results presented in Table 2. After specifying these 3 parameters, the values of *b* and *c* in *Eq. 1* are determined. These curves graphically illustrate average amount of curvilinearity identified in ESPVRs. See text for further discussion.

heart changes when contractile state is varied over a wide range. This finding was observed with each of the methods used to alter contractile state.

The ESPVR has been studied extensively in the isolated canine heart preparations (22, 26, 28). Results of those previous studies indicated that the ESPVR is linear, independent of the contractile state. There are, however, several differences between the present study and previous works. First, the range of contractile states investigated in the present study is much greater than in previous studies. Second, with enhanced contractile states, ventricular volume was varied over a range wide enough so that peak isovolumic pressure could reach values in excess of 200 mmHg, thus more powerfully revealing the nonlinearity of the relation; i.e., curvilinearities observed in ESPVRs were most apparent when contractile states and end-systolic pressures were outside the range typically investigated in isolated canine hearts. However, because isolated canine hearts have depressed

contractile states compared with in situ hearts, it is possible that the in situ ESPVR may have significant nonlinearity under physiological conditions. In fact, curvilinear ESPVRs have recently been recorded from in situ canine hearts at their base-line state (6). Under pathophysiological conditions, such as those possibly existing in certain human heart diseases, nonlinear ESPVRs might also be apparent in the working range of the ventricle. Finally, in no previous study has the assumption of contractile state-independent linearity of the ESPVR been tested rigorously by statistical analysis as in the present study.

Recently, Suga et al. (29) investigated the ESPVR in a (canine) puppy preparation and found significant nonlinearities under base line, enhanced and depressed contractile states. However, the range of contractile states investigated was again somewhat limited. In contrast to the results of the present study, curvilinearity was found to be in the same direction (always concave to the volume axis) under each of the tested conditions and occurred in a relatively low-pressure range with significant nonlinearity observed when peak isovolumic pressures was in the range of 120 mmHg. Suga et al. (29) cited seven mechanisms that might underlie a difference in the ESPVR between the adult and immature heart: *1*) disorientation of myofibrils, *2*) lower density of myofilaments and contractile proteins, *3*) irregularity of sarcomere length, *4*) lower myofibrillar ATPase activity, *5*) lower density of sarcoplasmic reticulum, *6*) immaturity of excitation contraction coupling, and *7*) lower sensitivity of the myocardium to inotropic agents. Thus the changes in shape observed in the adult canine heart ESPVR with changes in contractility are not likely to result from the same mechanisms as the nonlinearity in the puppy heart ESPVR.

Implications for length dependence of activation. The quantitative relation between the ventricular ESPVR and the ESTLR of cardiac muscle in a given heart is complex. Factors that are important in determining this interrelationship include muscle mass, the arrangement of muscle fibers, activation sequence of the muscles, and the geometry of the ventricular chamber. Since changes in inotropy are not believed to significantly alter any of these factors, the finding of changes in the shape of the ventricular ESPVR with altered contractile state can be considered to result from changes in the shape of the ESTLRs of the muscles comprising the ventricular wall.

Previous studies in isolated cardiac muscle have shown that the shape of the ESTLR changes with changes in inotropic state created by altering bathing Ca^{2+} concentration (16, 31) and with the use of paired pulse stimulation (1, 11). Shape changes in the ESTLR, which are independent of changes in the magnitude of the curves, have been interpreted as evidence that the extent of myofilament "activation" is modulated by muscle length (1, 12, 16, 31). The reason why changes in shape of the ESTLR imply length dependence of activation is most easily understood by consideration of the ESTLRs normalized to peak force at L_{max} (the length at which force development is maximal) presented by Allen et al. (1) and discussed more extensively by Jewell (12). Briefly, these authors have argued that "if muscle length and inotropic state are independent regulators of tension production, then the normalized length-tension curves [obtained at different contractile states] should be superimposable." In reality, however, the normalized length-tension curves obtained at different contractile states were not superimposable. The reader is referred to these original studies for more details (1, 12, 31).

High levels of contractility have been associated with ESTLRs concave to the length axis, and low levels of contractile state have been associated with ESTLRs convex to the length axis. The results of the present study demonstrate exactly the same trends in the ESPVRs.

The length dependence of activation is believed to result in part from length-dependent changes in the affinity of the contractile proteins for Ca^{2+} (9) and, in part, from length-dependent changes in the amount of calcium released to the myofilaments (2, 4). The results of the present study provide no further insight into the mechanisms underlying length dependence of activation but do indicate that this change in the shape of the ESTLR originally identified in isolated muscle preparations is also observable in the more intact situation of the whole ventricle as well. Evidence for length dependence of activation in the intact canine ventricle has also been provided earlier in one study (32).

E_{max} as an index of contractile state. Controversy has existed for many years over the definition and quantification of ventricular "contractility" (23, 25, 33). Recently, E_{max}, defined as the slope of the ESPVR, has gained wide acceptance as an index of contractility (8, 19, 22, 26) based on *1*) linearity of the ESPVR, *2*) sensitivity of the ESPVR to changes in inotropic stimulation, and *3*) relative insensitivity of the ESPVR to changes in pre- and afterload conditions (18). In view of proposed theories of length dependence of activation of heart muscle, one can question whether this parameter, derived from a relation measured by changing ventricular volume, could serve as an ideal index of contractility when volume may modulate contractility. This important question has been raised previously by Jewell (12). Furthermore, the results of the present study suggest that under some circumstances the ESPVR is nonlinear and therefore not totally indexed by a single slope factor. The average extent of nonlinearity of the ESPVR produced by inotropic interventions in the present study is illustrated in Fig. 5 and summarized in Table 2. Statistical analysis revealed that on average, the ESPVR was linear when E'_{max} was between ~3.4 and 7.8 mmHg/ml (within the range of volumes tested). This range appears to correspond to the normal, "unperturbed" contractile state of the isolated heart reported in previous studies (26–28). However, as discussed above, the contractility of the isolated heart is most likely depressed compared with that of the heart in situ, and therefore the normal ESPVR of the intact heart may not necessarily fall within this linear range. Thus the assumption of a linear ESPVR under physiological conditions requires further justification. Furthermore, for ESPVRs measured in hypo- or hyperkinetic states (e.g., dilated cardiomyopathy, myocarditis, during exercise, or hypertrophy), the assumption of linearity is challenged by the results

of the present study.

In the intact animal and humans, the range over which end-systolic pressure and volume can be varied is limited and the number of pressure-volume points that can be measured is also limited (8, 14, 17, 19). E_{max} and V_0 are often determined by linear regression analysis applied to a small number of data points collected over this limited range. With a nonlinear ESPVR, this could result in rather large errors in estimation of the true V_0 and provide misleading estimates of E_{max} depending on the pressure-volume range studied. Other problems could arise when assessing the influence of agents that alter inotropic state as illustrated in Fig. 6. As shown in Fig. 6, when the range over which volume can be varied is relatively limited and fixed (shaded region), it is possible that E_{max}, calculated by linear regression analysis to data obtained within that range (dashed lines), could exhibit little change (as in going from the depressed, convex ESPVR to the midline, linear ESPVR) or even decrease (as in going from the midline, linear ESPVR to the enhanced, concave ESPVR) as contractility is increased. One way to circumvent such misleading results is not to expect E_{max} by itself to quantify contractility but to also take into consideration changes in the extrapolated V_0 as well. Similar problems have been shown to exist when the ESPVR is measured when afterload is increased by methoxamine hydrochloride (6). Clearly, interpretation of V_0 values obtained by linear extrapolation of the ESPVR outside of the measured range of pressures and volumes in terms of changes in the true physiological V_0 should be made with caution.

Limitations and alternative explanations. There are a number of limitations of the present study, and alternative explanations for the results should be considered. First, CPP was held constant during each ESPVR determination and thus uncoupled from LVP generation. Therefore regional myocardial perfusion (i.e., endocardial-to-epicardial flow ratio) may have varied when ventricular volume was changed (especially at high volumes and contractile states), a situation that may have caused volume-dependent changes in contractile state, independent of the mechanisms discussed above. We believe that this played a relatively insignificant role in the present study, since similar contractile state-dependent changes in curvilinearity were observed whether contractile state was altered in a steady fashion by pharmacological agents or in an instantaneous fashion created by pacing interval alterations, during which time such mechanisms most likely could not develop. Second, although it has been shown by Olsen et al. (20) that inotropic state does not influence the dynamic geometry of the heart, the range over which they varied contractility was smaller than in the present study. Therefore, it is possible that, with the greatly enhanced and depressed states we obtained, there may have existed some alterations in ventricular geometry. If that were the case then the dependence of pressure-volume relations on length-tension relations could be different at these extremes and conceivably create changes in the shape of the ESPVR.

Furthermore, a few aspects of the experimental design caution against immediate extrapolation of our results to in vivo conditions. First, studies were performed under the influence of pentobarbital anesthesia, which itself is known to have a negative inotropic effect. Second, all experiments were performed under isovolumic conditions and therefore the influences of afterloading conditions on the shape of the ejecting ESPVRs were not assessed. Therefore further work is required to assess the role of ventricular volume in modulating contractile properties in the conscious animal.

We have demonstrated that the shape of the ventricular ESPVR changes with contractile state in a manner consistent with changes in the ESTLR observed earlier under comparable conditions in isolated ventricular muscle preparations. Along the line of interpretations of these changes offered by investigators of heart muscle, we submit that the data of the present study provide evidence for length dependence of muscle activation in the intact left ventricle.

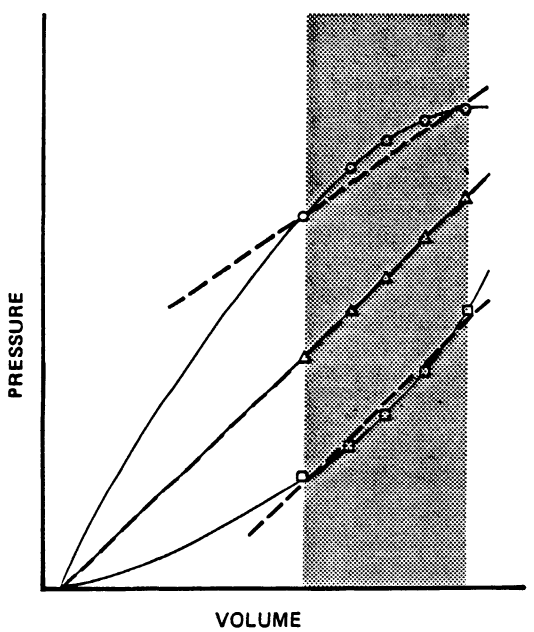

FIG. 6. Schematic illustration of how contractility-dependent curvilinearity of end-systolic pressure-volume relation (ESPVR) could cause misleading changes in slope of linear ESPVR (E_{max}) when linear regression analysis is applied to data within a relatively limited volume range. *Solid lines* represent "true" ESPVRs; *shaded area* indicates volume range over which data can be measured; *open symbols* represent hypothetical data points; *dashed lines* show linear regression through those data points. There is relatively little change in E_{max} when contractility is increased from convex ESPVR to linear ESPVR. E_{max} goes down when contractility is increased from linear ESPVR to enhance ESPVR. See text for further discussion.

APPENDIX

The use of a polynomial to fit the ESPVR became problematic in some of the relations measured under low contractile states. This is illustrated in Fig. 7, in which end-systolic pressure-volume data from a typical experiment are plotted; note the expanded pressure scale in relation to Figs. 2 and 3. The solid circles are the end-systolic pressure-volume points measured with contractile state depressed by lowering CPP. The solid line is of the form of *Eq. 1* with the parameters obtained by nonlinear regression analysis. As shown, this regression line comes close to, but does not intersect the volume axis. Therefore, it is impossible to define E'_{max} or V_0 for this set of data using *Eqs. 3* and *4*, respectively. For these data, we applied linear regression analysis to the data obtained below the arbitrary cutoff volume of 20 ml, and defined E'_{max} as the slope of

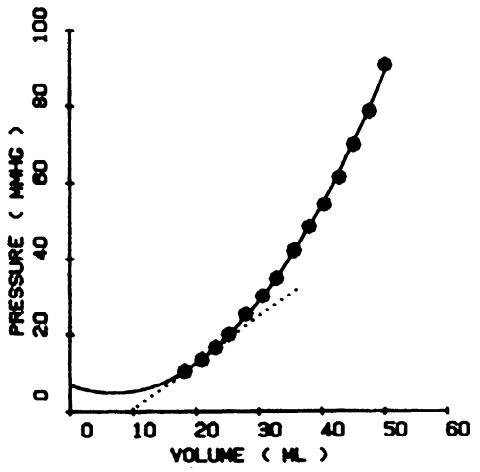

FIG. 7. *Dots* are data from a typical experiment in which contractile state was depressed by reducing coronary perfusion pressure. Note expanded pressure scale compared with those of previous figures. *Solid line* is parabolic regression to data. As shown, this line does not intersect volume axis, making it impossible to define slope of end-systolic pressure-volume relationship at low volumes (E'_{max}) and volume-axis intercept (V_0) by analytical expressions of *Eq. 3* and *4*, respectively. For such curves, E'_{max} and V_0 were determined by applying linear regression to data points collected below arbitrary cutoff volume of 20 ml indicated by *dotted line*.

this regression and V_0 as its volume-axis intercept. The result of this analysis is presented in Fig. 7 by the dotted line. This problem is due to the shallow approach of the data to the volume axis and the need to extrapolate the ESPVR to lower volumes than were actually measured. Although this problem was not apparent for all the ESPVRs measured under depressed contractile state, we did use this procedure to define V_0 and E'_{max} for all these cases so as to maintain consistency and avoid introducing bias into our analysis. For completeness, we also analyzed the data obtained under control and enhanced contractile states in this same manner and compared these determinations of E'_{max} and V_0 with those obtained by the analytic expressions of *Eqs. 3* and *4*. There was no significant difference in either E'_{max} or V_0 determined by these two methods. The value of the cutoff volume we used in the present study obviously cannot be applied to a heart whose size is significantly different than that of a 20-kg dog (e.g., it would not be appropriate for human hearts). Thus the analytic definitions of E'_{max} and V_0 are preferable.

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Address for reprints requests: D. Burkhoff, Dept. of Biomedical Engineering, The Johns Hopkins School of Medicine, Traylor Building, Room 223, 720 Rutland Ave., Baltimore, MD 21205.

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