Systolic pressure-volume relationship (ESPVR) of the in situ left ventricle shows contractility-dependent curvilinearity. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1429–H1434, 1998.—We developed a miniaturized conductance catheter for in situ left ventricular (LV) volumetry. After the validation study of the conductance volumetry in 11 rats, we characterized the end-systolic pressure-volume relationship (ESPVR) in 24 sinoaortic-denervated, vagotomized and urethan-anesthetized rats. Stroke volume (SV) measured with the conductance catheter correlated closely with that measured by electromagnetic flowmetry (r = 0.95). No significant difference was found between the in situ LV end-diastolic volumes measured by conductance volumetry and postmortem morphometry; a linear regression analysis indicated that the correlation coefficient was 0.934, that the slope was not significantly different from 1, and that the intercept was not significantly different from 0. During cardiac sympathotonic depression, the ESPVR was curvilinear. The estimated slope of ESPVR (end-systolic elastance, Ees) by quadratic curve fitting at end-systolic pressure of 100 mmHg was 2,647 ± 846 mmHg/ml. Bilateral cervical and stellate ganglionectomy depressed contractility and made the ESPVR linear; a quadratic equation did not improve the fit. Ees was 946 ± 55 mmHg/ml with the volume axis (V0) intercept of 0.076 ± 0.007 ml. Administration of propranolol (1 mg/kg) further reduced Ees (573 ± 61 mmHg/ml, P < 0.001) and increased V0 slightly (0.091 ± 0.011 ml). We conclude that the conductance catheter method is useful for the assessment of the ESPVR of the in situ left ventricle and that the ESPVR displays contractility-dependent curvilinearity.

Comparison With Flowmetric Stroke Volume

We compared the stroke volume measured by conductance volumetry with that calculated from aortic flow measured by electromagnetic flowmetry in 11 open-chest urethan-anesthetized rats weighing 280–500 g. The conductance catheter was inserted into the LV cavity through the apex and was moved 1.5–2.0 cm toward the aortic valve along the longitudinal axis of the LV cavity. We also inserted a 2-Fr catheter-tip microcatheter (SPC-320, Millar Instruments, Houston, TX) into the LV cavity from the apex. After confirming that the tip of the conductance catheter was placed into the ascending aorta, we withdrew the conductance catheter so that the loop of the pressure and the segmental volume measured between the distal pair of sensing electrodes appeared to be normal. Then we fixed the catheter to the apex with suture. An electromagnetic flow probe (MFV-2100, Nihon Kohden, Tokyo, Japan) was placed around the ascending aorta. To change the preload, we placed a cuff occluder around the inferior vena cava. First, a parallel conductance volume was measured by the hypertonic saline-dilution method (3, 7, 9) in which 0.02 ml of saturated NaCl solution was injected into the pulmonary artery. When the conductance catheter was placed into the in situ LV cavity from the apex, the actual measured conductance...
volume was the sum of the LV blood conductance volume and the parallel conductance volume resulting from other structures extrinsic to the LV blood volume. The true LV conductance volume, therefore, was estimated by the subtraction of the parallel conductance volume from the actual measured conductance volume. The procedure for calculating parallel conductance volume is explained in Fig. 2. With the assumption that actual LV volume does not change but conductance volume changes as a result of an increase in blood conductivity after the injection of the small amount of hypertonic saline (Fig. 2A), parallel conductance volume can be determined as the volume when intraventricular blood volume is supposed to be zero [i.e., LV end-diastolic volume ($V_{ed}$) = LV end-systolic volume ($V_{es}$)]. For practical reasons, we plotted the relationship between $V_{ed}$ and $V_{es}$ and then calculated the intersecting point of the regression line between the two volumes with the line of identity as shown in Fig. 2B. After this procedure, we recorded the electrical signals of the aortic flow, conductance volume, and left ventricular pressure (LVP) during a gradual caval occlusion, and then we measured blood conductivity. Finally, we arrested the heart with potassium chloride solution and excised it for a morphometric study.

Comparison With Morphometric LV Volume

To evaluate whether conductance LV volume after the correction of parallel conductance volume corresponded with the absolute values of in situ LV volume, we compared the $V_{ed}$ measured by conductance volumetry with the morphometric $V_{ed}$ in 11 rats. The excised heart was fixed with 10% Formalin, while LVP was maintained at the in situ LV end-diastolic pressure for 40 min. Sections of 10-µm thick were cut and stained with a hematoxylin-eosin mixture. Histological images were digitized through a frame grabber and analyzed.

ESPVR Study

The rats weighing 290–320 g were anesthetized with urethan (1–1.5 g/kg ip) and ventilated artificially. Anesthesia was maintained with urethan (0.1 g·kg$^{-1}$·h$^{-1}$ iv). Bilateral vagi, aortic depressor nerves, and carotid sinus nerves were cut. For the drug administration and sampling of blood, a polyethylene tubing was cannulated into the femoral vein. A pair of pacing leads was placed on the right ventricle, and the cuff occluder was placed around the aortic arch. The conductance catheter and the 2-Fr catheter-tip micromanometer were inserted into the LV cavity through the apex. To examine the effects of stimulation of cardiac sympathetic nerves on the ESPVR, we attempted to identify superior cervical and stellate ganglions and superior and inferior cardiac nerves (6, 11) under a dissecting microscope in 24 rats. We could identify the superior or inferior cardiac nerve in 7 of 24 rats.

Protocol 1. In 17 rats in which we could not identify the superior or inferior cardiac nerve, we recorded conductance volume and LVP during gradual aortic occlusions, before and after surgical cardiac sympathectomy (removal of cervical and stellate ganglions) and β-blockade (propranolol 1 mg/kg iv), while right ventricular pacing maintained heart rate at 320–360 beats/min. When the heart was not paced, heart rates before and after sympathectomy were 320–360 and 260–280 beats/min, respectively. We repeatedly measured blood conductivity and performed the procedure for calculating parallel conductance volume immediately before recording each set of pressure-volume data to estimate the ESPVR.

Protocol 2. In seven rats in which we could identify the superior or inferior cardiac nerve, we recorded conductance volume and LVP during gradual aortic occlusions after surgical cardiac sympathectomy (cut of cardiac nerves), during the electrical stimulation of the cardiac nerve, and after β-blockade (propranolol 1 mg/kg iv), while right ventricular pacing maintained heart rate at 320–360 beats/min. Intensity of the electrical stimulation was adjusted to increase the heart rate by 60–80 beats/min above the control level. We repeatedly measured blood conductivity and performed the procedure for calculating parallel conductance volume immediately before recording each set of pressure-volume data to estimate the ESPVR.

Data Acquisition

Electrical signals were digitized through an analog-to-digital converter mounted on a personal computer at a sampling frequency of 1 kHz with a 12-bit resolution.
Estimation of ESPVR

The points of the ESPVR were determined by an iterative technique reported previously (3, 7, 8). In five consecutive pressure-volume loops, the points of each cardiac cycle with the maximal pressure-to-volume ratio were first determined. Linear regression of these points with the expression

\[ P_{es} = E_{es} (V_{es} - V_0) \]

yielded estimates for the slope, or end-systolic elastance \( (E_{es}) \), and the volume-axis intercept \( (V_0) \), where \( P_{es} \) and \( V_{es} \) are end-systolic pressure and volume, respectively. This initial \( V_0 \) estimate was used for the determination of the points of maximal \( P/(V^2 - V_0) \) for each cardiac cycle, and these new points were again fitted by linear regression, leading to new \( E_{es} \) and \( V_0 \) estimates. This procedure was iterated until convergence was achieved.

In addition to the linear model expression (Eq. 1), ESPVR values were also represented by a nonlinear model. End-systolic pressure-volume points obtained from five consecutive cardiac cycles were fitted to the parabolic curvilinear model proposed by Kass et al. (8)

\[ P_{es} = a \cdot (V_{es} - V_0)^2 + b \cdot (V_{es} - V_0) \]

where \( a \) is the coefficient of curvilinearity, \( b \) is the slope of the tangential line at 0 mmHg of \( P_{es} \), and \( V_0 \) is the volume-axis intercept of the curvilinear ESPVR. If \( a \) is negative, the ESPVR is considered convex toward the pressure axis. We also defined \( E_{es} \) as the slope of the tangential line of the ESPVR at 100 mmHg of \( P_{es} \). We used the Levenberg-Marquardt least-square algorithm to search for the coefficients that minimized the discrepancy between the fitted \( (P_{es, fitted}) \) curve and raw \( (P_{es, raw}) \) data. For each ESPVR, the standard error of the estimate (SEE) by linear and nonlinear analyses was calculated as a measure of the goodness of fit. When the number of model parameters was increased, reduction in SEE was a natural result. Therefore, we also calculated an Akaike's information criterion (AIC) value, based on the principle of parsimony (1)

\[ AIC = 5 \ln \left( \sum_{n=1}^{5} [P_{es, fitted}(n) - P_{es, raw}(n)]^2 \right) + 2(m + 1) \]

where \( m \) is the number of model orders. The order that minimizes the AIC value would be considered better.

Statistical Analysis

Data are expressed as means ± SD. Multiple comparison tests were performed by a Scheffe’s procedure after analysis of variance. Differences were considered significant at \( P < 0.05 \).

RESULTS

Validation Study

The relationships between conductance stroke volume and flowmetric stroke volume during caval occlusions are shown in Fig. 3A from one rat and in Fig. 3B from 11 rats. Conductance stroke volume was calculated from \( V_{ed} \) and \( V_{es} \) in each beat; flowmetric stroke volume was computed as the time integral of aortic flow. The stroke volumes measured by the two methods were correlated closely. In 11 rats, correlation coefficients were between 0.956 and 0.986, the slopes of the regression lines were between 0.962 and 1.002, and the intercepts of the axis of conductance stroke volume were between 0.011 and 0.024 ml. The SEE was distributed between 0.0010 and 0.0019 ml.

The relationships between in situ conductance \( V_{ed} \) and the correction of parallel conductance volume and postmortem morphometric \( V_{ed} \) in 11 rats are presented in Fig. 4. The parallel conductance volume was distributed between 0.216 and 0.374 ml. The difference between the conductance and morphometric \( V_{ed} \) was 0.09 ± 0.013 ml, since the latter was reference and was not statistically significant. A linear regression analysis indicated that correlation coefficient was 0.934, slope was not significantly different from 1, and that intercept was not significantly different from 0.
not statistically significant. A linear regression analysis indicated that the correlation coefficient was 0.934, the slope was 1.01, and the intercept was statistically negligible. These results revealed the validity of the estimates of parallel conductance volumes and thus absolute volume by conductance volumetry.

ESPVR Study

Representative examples of pressure-volume loops and ESPVR values during various contractile conditions from one rat are shown in Fig. 5. Under baseline conditions (before surgical sympathectomy), which would be equivalent to sympathotonic conditions inducing the heart rate increase of 60–80 beats/min (see METHODS), the quadratic curve was well fitted to end-systolic pressure-volume points (Fig. 5A). The quadratic model markedly reduced both SEE and AIC (SEE = 7.6, AIC = 19.6, by linear regression analysis; SEE = 1.7, AIC = 14.2, by nonlinear regression analysis). The quadratic coefficient a was −8,712, indicating that the ESPVR was convex toward the pressure axis. The slope $E_{es}$ of the linear regression line was 1,470 mmHg/ml; on the other hand, the slope $E_{es}$ of the tangential line of the quadratic curve at 100 mmHg was 2,367 mmHg/ml. The volume-axis intercepts by linear ($V_0$) and nonlinear ($V_0'$) analyses were 0.065 and 0.10 ml, respectively.

After sympathectomy, the linear model described the ESPVR well (Fig. 5B). Nonlinear regression analysis decreased the SEE but increased the AIC (SEE = 1.1, AIC = 10.8, by linear regression analysis; SEE = 0.74, AIC = 11.2, by nonlinear regression analysis). Sympathectomy reduced the $E_{es}$ by 40% and the $E_{es}'$ by 64%.

After β-blockade, the nonlinear model concave toward the pressure axis was better fitted to the ESPVR points than the linear model (Fig. 5C). Nonlinear regression analysis decreased both SEE and AIC (SEE = 0.71, AIC = 9.0, by linear regression analysis; SEE = 0.32, AIC = 7.5, by nonlinear regression analysis). The slopes of ESPVR became lower after β-blockade ($E_{es}$ = 666, $E_{es}'$ = 685).

The results from 17 rats studied in protocol 1 are presented in Table 1. According to the principle of parsimony, the ESPVR values under baseline and β-blockade conditions were considered nonlinear; the ESPVR under sympathectomized conditions was consid-

Fig. 5. Representative examples of end-systolic pressure-volume relationships (ESPVR) under baseline (A), sympathectomized (B), and β-blockade (C) conditions in a rat. ESPVR was estimated from 5 consecutive end-systolic pressure-volume points (open circles) by linear (solid line) and quadratic curvilinear (dotted line) regression analyses (see Eqs. 1 and 2). Middle and bottom panels show residuals of end-systolic pressure-volume points from linear and quadratic curvilinear fits, respectively. ESPVR values appear convex toward pressure axis under baseline conditions, linear under sympathectomized conditions, and concave toward the pressure axis under β-blockade conditions. See text for details.
Table 1. Summary of results from protocol 1 of ESPVR study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Sympathectomy</th>
<th>β-Blockade</th>
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</thead>
<tbody>
<tr>
<td>Linear ESPVR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEE, mmHg</td>
<td>6.9 ± 1.6</td>
<td>1.3 ± 0.7</td>
<td>3.2 ± 12</td>
</tr>
<tr>
<td>AIC</td>
<td>18.9 ± 0.9</td>
<td>11.7 ± 0.6</td>
<td>15.6 ± 0.8</td>
</tr>
<tr>
<td>$E_{es}$, mmHg/ml</td>
<td>1,587 ± 94</td>
<td>946 ± 55b</td>
<td>573 ± 61de</td>
</tr>
<tr>
<td>$V_0$, ml</td>
<td>0.067 ± 0.008</td>
<td>0.076 ± 0.007</td>
<td>0.091 ± 0.011d</td>
</tr>
<tr>
<td>Curvilinear ESPVR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEE, mmHg</td>
<td>1.8 ± 1.2c</td>
<td>1.1 ± 0.6</td>
<td>0.9 ± 0.6b</td>
</tr>
<tr>
<td>AIC</td>
<td>15.1 ± 0.9</td>
<td>12.0 ± 0.7</td>
<td>12.0 ± 0.6b</td>
</tr>
<tr>
<td>a, mmHg/ml²</td>
<td>-9,546 ± 4,822</td>
<td>-883 ± 1.419de</td>
<td>499 ± 922e</td>
</tr>
<tr>
<td>b, mmHg/ml</td>
<td>3,239 ± 776</td>
<td>1,191 ± 308f</td>
<td>452 ± 395e</td>
</tr>
<tr>
<td>$E_{es}$, mmHg/ml</td>
<td>2,647 ± 846</td>
<td>1,071 ± 85i</td>
<td>638 ± 59i</td>
</tr>
<tr>
<td>$V_0$, ml</td>
<td>0.096 ± 0.014f</td>
<td>0.081 ± 0.014</td>
<td>0.042 ± 0.010cde</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 17 rats. ESPVR, end-systolic pressure-volume relationship; SEE, standard error of estimate; AIC, Akaike’s information criterion; $E_{es}$, end-systolic elastance; $V_0$, volume-axis intercept; $V_0$, volume-axis intercept of curvilinear ESPVR; a, coefficient of curvilinearity; b, slope of curvilinear ESPVR at $V_0$; $E_{es}$, slope of linear ESPVR at 100 mmHg of end-systolic pressure. *P < 0.05 from linear ESPVR; **P < 0.05 compared with $E_{es}$; ***P < 0.05 compared with $V_0$; ****P < 0.05 from baseline; *****P < 0.05 from sympathectomy.

As shown in Table 2, similar results were obtained from another seven rats studied in protocol 2. Before the electrical stimulation of the cardiac sympathetic nerves, the ESPVR was considered linear. The electrical stimulation of the cardiac cut end of the superior or inferior cardiac nerve made the ESPVR convex toward the pressure axis and increased the slope slightly. Intravenous β-blockade made the ESPVR concave toward the pressure axis and decreased the slope significantly. Taken together, these results obtained from protocols 1 and 2 indicated that the ESPVR was well fitted to the quadratic curvilinear model and that a measure of curvilinearity was changed by sympathetic stimulation, sympathectomy, and β-blockade. The slopes of the ESPVR estimated by both linear and nonlinear regression analyses were dependent on the cardiac sympathetic activity.

Table 2. Summary of results from protocol 2 of ESPVR study

<table>
<thead>
<tr>
<th></th>
<th>Sympathectomy</th>
<th>CSN Stimulation</th>
<th>β-Blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear ESPVR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEE, mmHg</td>
<td>1.9 ± 0.8</td>
<td>7.2 ± 1.9</td>
<td>4.0 ± 1.8</td>
</tr>
<tr>
<td>AIC</td>
<td>13.3 ± 0.8</td>
<td>19.1 ± 0.9</td>
<td>16.5 ± 0.9</td>
</tr>
<tr>
<td>$E_{es}$, mmHg/ml</td>
<td>1,010 ± 62</td>
<td>1,864 ± 154d</td>
<td>573 ± 61de</td>
</tr>
<tr>
<td>$V_0$, ml</td>
<td>0.074 ± 0.006</td>
<td>0.070 ± 0.010</td>
<td>0.081 ± 0.011d</td>
</tr>
<tr>
<td>Curvilinear ESPVR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEE, mmHg</td>
<td>1.2 ± 0.6</td>
<td>1.5 ± 0.4b</td>
<td>0.8 ± 0.2e</td>
</tr>
<tr>
<td>AIC</td>
<td>13.3 ± 0.5</td>
<td>14.3 ± 0.7c</td>
<td>11.5 ± 0.6e</td>
</tr>
<tr>
<td>a, mmHg/ml²</td>
<td>-1,026 ± 1,537</td>
<td>-10,512 ± 5,102d</td>
<td>521 ± 1,066de</td>
</tr>
<tr>
<td>b, mmHg/ml</td>
<td>1,258 ± 515</td>
<td>3,854 ± 802d</td>
<td>473 ± 476e</td>
</tr>
<tr>
<td>$E_{es}$, mmHg/ml</td>
<td>1,024 ± 93</td>
<td>2,859 ± 915d</td>
<td>587 ± 66de</td>
</tr>
<tr>
<td>$V_0$, ml</td>
<td>0.080 ± 0.015</td>
<td>0.089 ± 0.018f</td>
<td>0.042 ± 0.010cde</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7 rats. CSN, cardiac sympathetic nerve. *P < 0.05 from linear ESPVR; **P < 0.05 compared with $E_{es}$; ***P < 0.05 compared with $V_0$; ****P < 0.05 from sympathectomy; *****P < 0.05 from CSN stimulation.

DISCUSSION

The main results of the present study were as follows. First, stroke volume measured by conductance volumetry was in good agreement with that estimated from the aortic flow measured by electromagnetic flowmetry. Second, the conductance $V_0$, was in good agreement with the morphometric $V_{ed}$. Third, the ESPVR was convex toward the pressure axis under sympathetically conditioned, linear under sympathectomized conditions, and concave toward the pressure axis under β-blockade. Finally, the slope of the ESPVR estimated by both linear and nonlinear regression analyses changed in response to cardiac sympathetic activity.

Accuracy of Conductance Volumetry

Although Ito et al. (7) also have developed a conductance-catheter method for the in situ rat left ventricle and examined its accuracy, they only compared 1) the conductance stroke volume with the stroke volume calculated from aortic flow measured by electromagnetic flowmetry in six rats, and 2) the postmortem LV volume measured by conductance volumetry with the true LV volume in three rats (the detailed method is not described in the paper). In their study, no information was provided on the validity of the estimation method for parallel conductance. This is a critical point for the accurate estimation of the volume intercept of the ESPVR (3, 4, 15). Therefore, we must examine the accuracy of the conductance volumetric method for measuring absolute LV volume more extensively in order to characterize the ESPVR of the in situ rat left ventricle. The present results showing that the in situ conductance $V_0$ after the subtraction of parallel conductance volume was in good agreement with the postmortem morphometric $V_{ed}$ in 11 rats indicate that the hypertonic saline-dilution method is valid for the estimation of parallel conductance volume in rats. We, therefore, confirmed the accuracy of our conductance volumetry for the in situ rat left ventricle.
ESPV R of In Situ Rat Left Ventricle

Many important findings about normal and failing hearts have been increasingly derived from the molecular biology of rat cardiac myocytes. Although the heart is a pump of the circulatory system, one of its most unique functions, i.e., chamber contractility, has never been evaluated on an in situ organ basis in terms of the ESPVR. This fundamental description would help comprehensive understanding of the heart from molecular to organ function (12).

A contractility-dependent curvilinearity has been shown in the isolated isovolumically contracting (5, 14) or in situ ejecting canine left ventricle (8, 10). Kass et al. (8) examined the effect of contractile state on the curvilinearity of the in situ canine ESPVR by conductance volumetry and employed a quadratic parabolic curvilinear model for describing the ESPVR. We also used the same model for nonlinear regression analysis of the rat ESPVR, because our experimental setting was similar to that of Kass et al. The in situ ESPVR of the rat left ventricle was convex under a high contractile state and concave under a low contractile state toward the pressure axis as well as that of the canine left ventricle. In the isolated cross-circulated canine heart preparation (5, 14), the isolated heart was denervated and was affected by blood-borne catecholamines from an anesthetized support dog. During baseline conditions where no inotropic drugs were administered, the ESPVR has been reported to be reasonably linear (5). The present study also shows that the in situ ESPVR after surgical sympathectomy was considered linear in rats. Interestingly, a contractility-dependent curvilinearity could be observed beyond differences in species and heart size. Despite a contractility-dependent curvilinearity, the present study indicates that the slope of the ESPVR is a useful description of the in situ rat LV function, provided that the slope is estimated in the physiological range of pressure-volume points such as the tangential slope at 100 mmHg of P_e.

Methodological Limitations

Although the accurate volume of the in situ left ventricle is hardly measurable by any method, we needed it as reference. The morphometric method used in the present study could yield measurement errors, because the end-diastolic pressure-volume relationship of the postmortem left ventricle would be different from that of the in situ contracting left ventricle, and our fixation procedures also would affect the dimension of V_d. The problem that there is no absolute standard as reference appears to be inherent in a validation study about in situ conductance volumetry.

The anesthetic agent and the placement of the two catheters through the apex of the heart with the chest open have deleterious effects on the function of the heart. The values of indexes for cardiac function shown in the present study, thus, should be interpreted carefully.

In conclusion, we developed a conductance volumetry for the in situ rat left ventricle and examined its accuracy. After confirming that its errors were acceptable, we characterized the ESPVR in 24 anesthetized open-chest rats. The ESPVR was convex toward the pressure axis under sympathotonic conditions, linear under surgically sympathectomized conditions, and concave toward the pressure axis under β-blockade. We concluded that the conductance-catheter method was useful for the assessment of the ESPVR in the in situ rat left ventricle and that the ESPVR displayed contractility-dependent curvilinearity.

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