special communication

End-systolic pressure-volume and $M\dot{V}O_2$-pressure-volume area relations of isolated rat hearts

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Wannenburg, Thomas, Steven P. Schulman, and Daniel Burkhoff. End-systolic pressure-volume and $M\dot{V}O_2$-pressure-volume area relations of isolated rat hearts. *Am. J. Physiol.* 262 (*Heart Circ. Physiol.* 31): H1287–H1293, 1992.—We tested the utility of a standard isolated, crystalloid-perfused, isovolumic rat heart preparation for studying ventricular metabolism in terms of the myocardial oxygen consumption-pressure-volume area ($M\dot{V}O_2$-PVA) relations. The end-systolic pressure-volume relations (ESPVRs) determined between volumes of 0.15 and 0.65 ml were fit equally well by linear and nonlinear regression analysis within the data range but predicted widely differing volume-intercept (V_o) values. Linear regression analysis of the ESPVRs provided a mean slope (E_{es}) of 419 ± 186 $mmHg \cdot g \cdot ml^{-1}$ and V_o of 0 ± 0.12 ml, respectively ($n = 6$). The $M\dot{V}O_2$-PVA relations were linear with a slope and $M\dot{V}O_2$ intercept of 1.30 ± 0.31 × 10^{-5} ml $O_2 \cdot mmHg^{-1} \cdot ml^{-1}$ and 0.38 ± 0.09 × 10^{-3} ml $O2 \cdot beat^{-1} \cdot g^{-1}$, respectively. These $M\dot{V}O_2$-PVA parameters were not significantly different from those obtained when nonlinear regression analysis was applied to the ESPVR. Decreasing perfusate calcium concentration ($[Ca^{2+}]$) ($n = 7$) resulted in a downward shift in the ESPVR, a decrease in the $M\dot{V}O_2$-PVA intercept (0.52 ± 0.26 vs. 0.34 ± 0.20 × 10^{-3} ml $O_2 \cdot beat^{-1} \cdot g^{-1}$, $P < 0.01$), and no significant change in the $M\dot{V}O_2$-PVA slope (1.33 ± 0.47 vs. 1.57 ± 0.69 × 10^{-5} ml $O_2 \cdot mmHg^{-1} \cdot ml^{-1}$, NS). We conclude that this preparation may be a useful alternative to more expensive preparations for selected experiments in cardiac energetics.

cardiac energetics; calcium; contractility

SIGNIFICANT INSIGHTS have been gained into the interrelations between ventricular mechanical performance and myocardial metabolism from studies of isolated, cross-perfused canine hearts (5). This preparation, in which an isolated dog heart is metabolically supported by continuous blood flow from a "support" dog, offers the important advantages over standard in situ heart preparations of being able to measure intraventricular volume (10) and to control afterload and preload conditions (11). Two pivotal concepts studied with this model include *1*) quantitative assessment of ventricular contractile state (contractility) through analysis of the end-systolic pressure-volume relation (ESPVR; 5, 9); and *2*) quantitative assessment of total mechanical energy generation by the ventricle in terms of the pressure-volume area (PVA; 6). Studies in which PVA has been correlated with measured myocardial oxygen consumption ($M\dot{V}O_2$) under a variety of hemodynamic loads and contractile states have provided new understanding about the determinants of myocardial energy demands. These same concepts have also been shown to apply to the isolated cross-perfused rabbit heart (4).

Isolated cross-perfused canine and rabbit heart preparations, however, are technically complex and expensive and have limited application for studies that require easy control of perfusate composition (e.g., electrolytes, metabolites, pH, and drugs). Manipulation of these components is usually technically difficult and is frequently associated with detrimental effects on the support animal.

The purpose of this study was to test the validity of using a standard Langendorff crystalloid-perfused rat heart preparation for studies of cardiac energetics in the same format developed originally for dog (and later rabbit) hearts. This preparation offers the advantages of economy, simplicity, and the ability to control perfusate constituents. The data show that the general features of rat heart ESPVRs and $M\dot{V}O_2$-PVA relations are similar to those measured in dogs and rabbits. Furthermore, these relations respond to changes in contractile state induced by changing perfusate calcium concentration ($[Ca^{2+}]$) as expected from results obtained from dog hearts (8). It is concluded that the Langendorff rat heart model is useful for selected studies of cardiac energetics through evaluation of ESPVRs and MVO_2-PVA relations. Limitations of this model are reviewed.

METHODS

Surgical Preparation

In each experiment, a retired male breeder Sprague-Dawley rat weighing ~500 g was heparinized (1,000 U ip) and then heavily anesthetized with pentobarbital sodium (140 mg ip). Bilateral sternotomy was performed, and the inferior, left superior, and right superior venae cavae were ligated near their insertions into the right atrium. The heart was excised and immediately submerged into oxygenated, warmed perfusate (37°C, composition provided below). The severed end of the aorta was fed over a 16-gauge needle that was connected to a Langendorff perfusion system. Perfusate flow was adjusted to provide a perfusion pressure of ~70–80 mmHg, and flow was kept constant throughout the experiment.

A thin piece of Tygon tubing with a fenestrated tip was advanced into the right ventricle via the main pulmonary artery and held in place by a suture placed at the base of the pulmonary artery. This tube served to collect all of the coronary sinus and right ventricular Thebesian perfusate flow.

The left atrium was opened with care to ensure that the interatrial septum was not damaged. A balloon, made of thin latex, was attached to the end of a 10- to 15-cm length of stiff

polyethylene tubing with fenestrations on the distal 3 mm of its tip. The fenestrated portion of the tube was advanced into the balloon to facilitate removal of all volume from within the balloon without having the balloon wall occlude the tip of the tube. The balloon was inserted into the left ventricle (LV) and held in place by a purse string suture around the mitral annulus. The balloon and tubing were filled with water and connected to a Statham pressure transducer for measurement of ventricular pressure. Balloon volume was controlled using a calibrated 1-ml syringe. Before each experiment the pressure-volume relationship of the balloon was measured, and the balloon was used only if the pressure was zero to an intraballoon volume ≥0.4 ml. The volume of the balloon wall plus the tip of the tubing within the balloon was measured by water displacement after withdrawing all fluid from within the balloon (range, 0.15–0.2 ml); this value was added to the volume infused inside the balloon to obtain total intraventricular volume.

Pacing electrodes were placed at the right ventricular outflow tract and the remnants of the left atrium. Pacing stimuli were provided by a Grass SD-9 stimulator set at a rate above the native sinus node rate (150–215 beats/min) and was kept constant throughout each experiment.

The perfusion system consisted of a warmed storage vat for perfusate solutions, a Masterflex adjustable speed rotary pump (model 7523–10), and a condenser. The vat and condenser were warmed by a VWR Constant Temperature Circulator (model 1135) set to heat the solutions to 37°C. Perfusate was composed of (in mM) 15 glucose, 140 Na, 5 K, 0.9 Mg, 1.5 Ca, 152 Cl, and 6 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). Lidocaine (0.5 μg/ml) was added to suppress ventricular ectopy. All chemicals were obtained from Sigma Chemical (St. Louis, MO). The solution was warmed to 37°C. The pH was adjusted to 7.40, and the solution was equilibrated with 100% O_2. Perfusate was not recirculated. After attachment of the heart to the Langendorff perfusion system, the hearts were allowed to stabilize for at least 30 min, during which time left ventricular pressure (LVP) and venous oxygen tension (P_{O_2}) were monitored (as described below).

$M\dot{V}O_2$ Measurement

$M\dot{V}O_2$ was calculated by multiplying the coronary arterial flow by the difference in O_2 content between arterial and venous perfusate. To estimate O_2 contents, we measured P_{O_2} using a commercially available platinum O_2 electrode system (Instech, Plymouth Meeting, PA) that allowed for continuous measurements. Sodium dithionate (a compound which extracts O_2 from solution) was used to zero the electrode at the start of each experiment. The gain of the electrode system was calibrated using the perfusate solution, which had been equilibrated with 100% O_2. As determined by multiple measurements using a Lex-O_2-CON (model K) machine, the O_2 content of this solution was 2.3 vol/100 ml, which is in good agreement with standard values. Linearity of the O_2 electrode was confirmed by comparison of electrode readings at various P_{O_2} with measurements using the Lex-O_2-CON machine. A tubing system was devised to divert either all of the pulmonary effluent or all of the oxygenated perfusate flow through the electrode system for on-line sampling.

Protocols

Mechanical and metabolic stability. In the first series of hearts (n = 6), the stability of ventricular mechanical performance and $M\dot{V}O_2$ at various workloads was tested. This was done by measuring venous P_{O_2}, end-diastolic pressure, and end-systolic pressure (P_{es}) at several ventricular volumes between 0.2 and 0.55 ml (in increments of 0.05 ml). Measurements were obtained over a ~60-min time period (range, 50–70 min). After each change in volume was made, enough time was allowed for all recorded signals to attain a steady state (usually 5 min). The order in which volume was changed was varied randomly. Arterial P_{O_2} was measured at the beginning and at the end of a run, and this served to test the stability of the electrode system during the data acquisition period, as well as to provide the upper limit calibration signal for the electrode. Stability of the preparation was checked by making measurements at different times but at the same volume settings over the experimental period.

Contractile and metabolic effects of perfusate [Ca^{2+}]. In the second series of hearts (n = 7), the influences of alterations in perfusate [Ca^{2+}] on contractile strength (as assessed by the ESPVR) and the relationship between $M\dot{V}O_2$ and workload were tested. For this series, two perfusate solutions were prepared: one identical to that listed above (1.5 mM Ca^{2+}) and the second differing only with respect to the [Ca^{2+}], which was reduced to 0.75 mM. The hearts were randomly perfused initially with either the high (n = 3) or low (n = 4) calcium perfusate. The protocol outlined above for determining the ESPVR and the relationship between $M\dot{V}O_2$ and workload was executed during sequential high and low calcium perfusion. For this protocol, however, data were acquired at only four or five different ventricular volumes within a 20-min time period.

Data Analysis

To assess ventricular contractile state, ESPVRs were constructed by plotting P_{es} as a function of left ventricular volume (LVV). These data were subjected to linear and nonlinear regression analysis

$$P_{es} = E_{es} (LVV - V_{o,lin}) \quad (1)$$

$$P_{es} = E'_{es} (LVV - V_{o,nonlin}) + \alpha (LVV - V_{o,nonlin})^2 \quad (2)$$

where E_{es} and $V_{o,lin}$ are the slope and volume axis-intercept of the linear fit to the ESPVR, respectively. $V_{o,nonlin}$ is the volume axis-intercept of the nonlinear fit to the ESPVR, E'_{es} is the slope of the extrapolated ESPVR at a volume of $V_{o,nonlin}$, and α is related to the degree of ESPVR curvilinearity. The statistical significance of α, and thus the statistical significance of ESPVR curvilinearity, was determined by t test. E_{es} and E'_{es} provide quantitative indexes of contractile state (9, 2). Because, however, these measures are dependent on both the shape of the measured ESPVR and the volume range over which data are collected, $P_{0.4}$ (an alternative index of contractile strength that is independent of these factors) was also quantified. $P_{0.4}$ was defined as the interpolated P_{es} at a fixed volume of 0.4 ml (a volume always within the range of data collected). This parameter was determined by calculating P_{es} from the linear fit to the ESPVR (*Eq. 1*); E_{es} was normalized to 1 g for this calculation.

Total energy liberated by the ventricle under the isovolumic conditions studied was quantified by the PVA. PVA is defined as the area on the pressure-volume plane circumscribed by the ESPVR, the end-diastolic pressure-volume relation (EDPVR), and the systolic portion of the ventricular pressure-volume trajectory (6). The calculation of PVA is therefore dependent on how the ESPVR is extrapolated back to the volume axis. This is important because, as detailed below, it was not possible to measure the ESPVR in a low pressure range due to the substantial size of the intraventricular balloon. Therefore, the sensitivity of PVA determination to the mathematical fit to the ESPVR (linear vs. nonlinear) was also assessed, as will be detailed below. For PVA calculations, the EDPVR was described by a second-order polynomial above the minimum volume at which data were collected and was assumed to be equal to zero below that volume. Finally, PVA was normalized

by LV mass to 1 g.

$M\dot{V}O_2$ of the whole heart was estimated by multiplying perfusion flow by the difference in arterial and venous perfusate O_2 contents. This value represents the $M\dot{V}O_2$ of the LV (which will vary with LV workload) plus a small amount of O_2 consumed by the non-work-performing right ventricle, which theoretically would not vary with changes in LV workload. Right ventricular $M\dot{V}O_2$ was thus approximated by multiplying total $M\dot{V}O_2$ estimated for unloaded contractions by the ratio of right ventricular mass to total heart mass (4, 6, 8). Values of $M\dot{V}O_2$ were reported as milliliters oxygen per beat per gram LV after subtracting estimated right ventricular $M\dot{V}O_2$. Linear regression analysis was then performed to quantify the slope (A) and intercept (B) parameters of the following relationship

$$M\dot{V}O_2 = A\text{PVA} + B \quad (3)$$

Statistical analysis was also performed to test whether a second-order polynomial provided a better fit to these data. The statistical significance of alterations of this relationship noted when contractile state was changed were tested using multiple linear regression analysis (3). All statistical tests were performed using commercially available software (SYSTAT).

RESULTS

ESPVR and $M\dot{V}O_2$-PVA Relations

Results from one experiment in which the stability of the preparation was tested are shown in Fig. 1. Ventricular volume was varied between 0.25 and 0.65 ml in a random manner; a total of 13 separate measurements was made over a ~60-min time period. The ESPVR (Fig. 1, *top*) was reasonably linear with relatively little scatter about the regression (solid line), indicating that the mechanical function of the heart was stable over the experimental period. It is evident that a nonlinear regression (dotted line) also provided a good fit to the data; statistical analysis indicated that this fit was no better than the linear fit ($P > 0.05$). The major difference between the linear and nonlinear ESPVR was the extrapolated V_o value, which in turn impacts on the estimation of the PVA. It will be shown in detail below how this difference impacts on the parameters of the $M\dot{V}O_2$-PVA relationship.

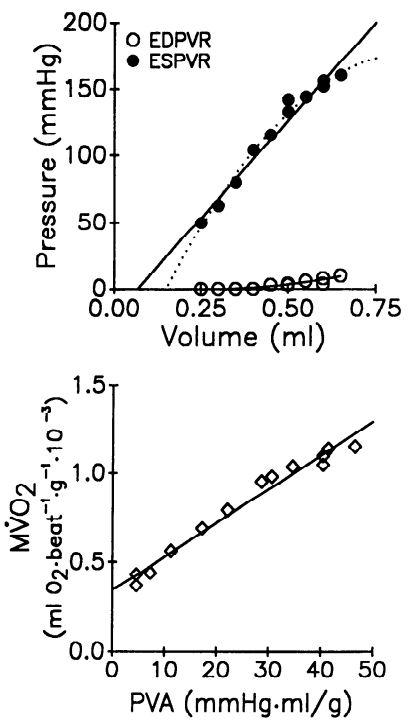

Fig. 1. Results from representative experiment (Table 1, *expt 3*). *Top*: end-systolic pressures (filled circles) and end-diastolic pressures (open circles) plotted as function of left ventricular volume (LVV). Solid line, linear regression through data points; dotted line, nonlinear regression. ESPVR and EDPVR, end-systolic and end-diastolic pressure-volume relations. *Bottom*: myocardial oxygen consumption ($M\dot{V}O_2$) per beat plotted as function of pressure volume area (PVA).

There was a reasonably linear relationship between PVA and $M\dot{V}O_2$ (Fig. 1, *bottom*). (Note that these PVA values were calculated assuming a linear ESPVR.) The data exhibited relatively little scatter about the regression line, once again indicating metabolic stability of the heart. Statistical analysis indicated that a second-order polynomial did not provide a better fit to the data than did the linear fit.

Data from the six hearts studied in this protocol are summarized in Table 1, which provides both the linear and nonlinear fit to the ESPVRs, and the parameters of the $M\dot{V}O_2$-PVA relation determined assuming both the linear and nonlinear ESPVR. In all cases the ESPVR was found (statistically) to be linear. In most of the six hearts, mechanical and metabolic data obtained at different times but at the same volumes were nearly identical. In *Expt 4* there was some scatter in the ESPVR, and in *Expts 1* and *2* there was some scatter in the $M\dot{V}O_2$-PVA relationship, as indicated by the relatively lower r^2 values. However, even in these worst cases, the degree of scatter was not physiologically large. Mean E_{es} was 418.5 ± 186 (SD) mmHg·g LV·ml^{-1}. Mean V_o values extrapolated from linear and nonlinear regression analyses were 0.00 ± 0.12 and 0.09 ± 0.09 (SE) ml, respectively, which were statistically different ($P < 0.01$). The mean slope and intercept of the $M\dot{V}O_2$-PVA relationships (with PVA derived from linear ESPVR) were 1.30 ± 0.31 × 10^{-5} ml O_2·ml^{-1}·mmHg^{-1} and 0.38 ± 0.09 × 10^{-3} ml O_2·beat^{-1}·g^{-1}, respectively. These numbers changed only slightly if the ESPVR was fit with nonlinear regression (see below for further analysis). $M\dot{V}O_2$-PVA relations fit to both linear and nonlinear quadratic analyses. In only one case (*expt 2*) was the $M\dot{V}O_2$-PVA relation found statistically to be fit better by a second-order polynomial than a linear function.

Influence of Contractile State on ESPVR and $M\dot{V}O_2$-PVA Relationship

The results from one experiment in which contractility was varied by changing the perfusate [Ca^{2+}] are shown in Fig. 2. The end-systolic pressure-volume data points were fit equally well by linear and nonlinear regression analyses for both the high- and low-calcium conditions (Fig. 2, *top*). The linear fit to the data obtained with the 1.5 mM calcium had a slightly steeper slope and a significantly smaller extrapolated V_o than the corresponding values determined at 0.75 mM calcium. In contrast, when these ESPVRs were fit by nonlinear regression, the extrapolated V_o values obtained with high and low [Ca^{2+}] were closer together.

The $M\dot{V}O_2$-PVA relationships (Fig. 2, *bottom*) were fit

Table 1. *Summary of contractile and metabolic data from six experiments with ESPVRs analyzed by linear and nonlinear regression*

Expt. No.	n	Linear ESPVR			Nonlinear ESPVR			$P_{0.4}$	$M\dot{V}O_2$-PVA					
		E_{es}	V_o	r^2	E'_{es}	α	V_o		A_{lin}	B_{lin}	r^2	A_{nonlin}	B_{nonlin}	r^2
1	12	718	0.14	0.978	990	−1,039	0.17	187	1.30	0.26	0.821	1.25	0.26	0.814
2	15	239	−0.20	0.962	421	−242	−0.07	144	1.36	0.49	0.862	1.31	0.56	0.813
3	13	381	0.07	0.974	655	−608	0.15	126	1.87	0.34	0.975	1.74	0.39	0.945
4	11	236	−0.09	0.868	506	−419	0.13	116	1.16	0.31	0.924	1.07	0.40	0.825
5	10	392	0.00	0.986	468	−162	0.03	158	1.03	0.47	0.901	1.02	0.47	0.897
6	9	545	0.05	0.998	835	−909	0.11	190	1.07	0.40	0.900	1.04	0.41	0.886
Mean		419	−0.01		645	−563	0.09	153	1.30	0.38		1.24	0.41	
±SD		±186	±0.12		±226	±355	±0.09	±31	±0.31	±0.09		±0.27	±0.1	

ESPVR, end-systolic pressure-volume relation; $M\dot{V}O_2$-PVA, myocardial oxygen consumption-pressure-volume area; E_{es} (mmHg·g·ml^{-1}) and V_o (ml), slope and volume axis-intercept of ESPVR derived from linear regression analysis. E'_{es} (mmHg·g·ml^{-1}), α (mmHg·g^2·ml^{-2}), and V_o (ml): parameters of ESPVR derived from nonlinear regression analysis. In each expt, ESPVR is statistically fit as well by linear as by nonlinear regression. $P_{0.4}$ (mmHg), interpolated end-systolic pressure at left ventricular volume of 0.4 ml; A_{lin} (ml O_2·mmHg^{-1}·ml^{-1} × 10^{-5}) and B_{lin} (ml O_2·beat^{-1}·g^{-1} × 10^{-3}), slope and intercept of $M\dot{V}O_2$-PVA relation with linear extrapolation of ESPVR; A_{nonlin} (ml O_2·mmHg^{-1}·ml^{-1} × 10^{-5}) and B_{nonlin} (ml O_2·beat^{-1}·g^{-1} × 10^{-3}), slope and intercept of $M\dot{V}O_2$-PVA relation with nonlinear extrapolation of ESPVR. n = no. of measurements.

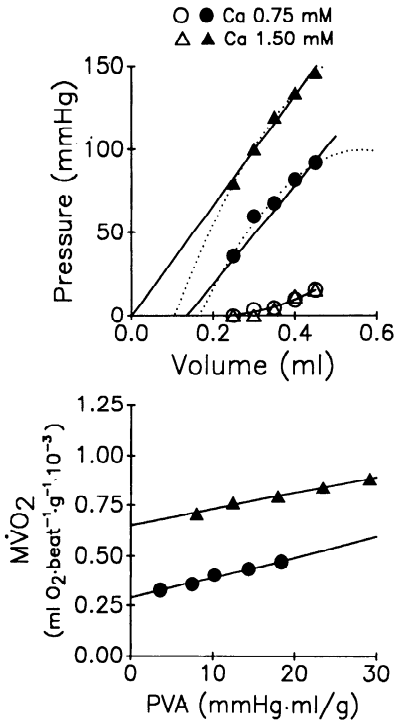

Fig. 2. Results from representative experiment. *Top*: impact of perfusate [Ca^{2+}] on ESPVR. Solid lines, linear regression to data points; dotted lines, quadratic fit. *Bottom*: relation between $M\dot{V}O_2$ and PVA. See text for details.

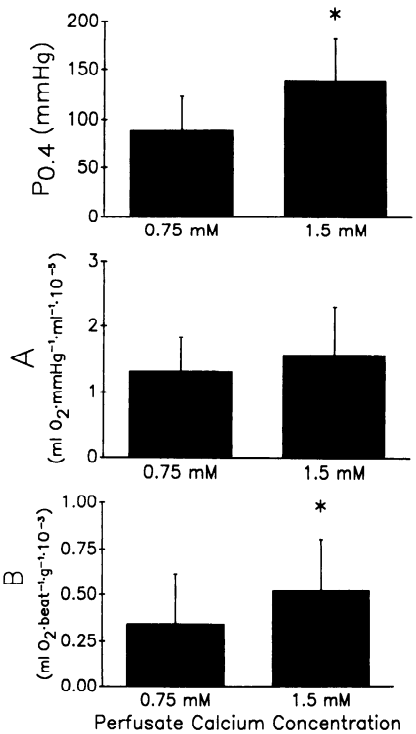

Fig. 3. *Top*: mean normalized end-systolic pressure with high and low calcium at ventricular volume of 0.4 ml ($P_{0.4}$). *Middle*: average slope (A) of $M\dot{V}O_2$-PVA for high and low calcium. *Bottom*: mean $M\dot{V}O_2$ intercept (B) for high and low calcium. * $P < 0.05$.

well by linear regression for both the high and low [Ca^{2+}]. The effect of an increased [Ca^{2+}] was an increase in the $M\dot{V}O_2$ intercept B without a significant change in the slope A.

The results from the seven hearts studied in this series are summarized in Fig. 3. $P_{0.4}$ increased from 94.1 ± 31.9 to 144.0 ± 41.3 mmHg ($P < 0.001$) when perfusate [Ca^{2+}] was increased from 0.75 to 1.50 mM. The slope of the $M\dot{V}O_2$-PVA relationship, A, averaged 1.33 ± 0.47 × 10^{-5} ml O_2·mmHg^{-1}·ml^{-1} in low calcium and 1.57 ± 0.69 × 10^{-5} ml O_2·mmHg^{-1}·ml^{-1} in high calcium (NS). The intercept of the $M\dot{V}O_2$-PVA relationship, B, increased from 0.34 ± 0.20 × 10^{-3} to 0.52 ± 0.26 × 10^{-3} ml O_2·beat^{-1}·g^{-1}. Multiple linear regression analysis revealed that, whereas the change in mean slope was not statistically significant, the change in intercept value was statistically significant ($P < 0.01$).

Impact of Mathematical Fit to ESPVR on $M\dot{V}O_2$-PVA Relationship

Accurate determination of PVA depends on reliable measurement of V_o, which could not be done because of the relatively large volume of the balloon wall and cannula placed within the LV. Therefore, V_o had to be estimated by extrapolation of the ESPVR to the volume

axis. It is therefore important to assess how possible errors in V_o estimation would impact quantitatively on the parameters of the $M\dot{V}O_2$-PVA relationship. This was done for each data set by applying both linear and nonlinear (*Eq. 2*) regression analysis to the data for each ESPVR. Figure 4 shows a worst case example. At the lowest volume (0.2 ml), isovolumic pressure was ~90 mmHg, necessitating extrapolation of the ESPVR over a wide pressure range to reach the volume axis. Between volumes of 0.20 and 0.45 ml, both linear (dotted line) and nonlinear (dashed line) regression provided excellent fits to the data and were not statistically different from each other. Below 0.20 ml, the linear fit fell above the nonlinear fit, resulting in a smaller estimate of V_o (which in this case was a negative value). Thus PVA for each beat calculated from the linearly extrapolated ESPVR (PVA_{lin}) would be greater than the corresponding PVA calculated from the nonlinearly extrapolated ESPVR (PVA_{nonlin}) by an approximately fixed amount, referred to as PVA_{dif}, which is equal to the area contained between the linear and nonlinear ESPVR regression lines below the lowest volume at which data were obtained. Thus for any beat in the run

$$PVA_{lin} = PVA_{nonlin} + PVA_{dif} \quad (4)$$

For the present analysis, assume that the $M\dot{V}O_2$-PVA_{lin} relationship is linear

$$M\dot{V}O_2 = A PVA_{lin} + B \quad (5)$$

By substituting *Eq. 4* into *Eq. 5* and rearranging, one obtains the following equation

$$M\dot{V}O_2 = A PVA_{nonlin} + (B + A PVA_{dif}) \quad (6)$$

Thus, in comparison of *Eqs. 5* and *6*, it is predicted that errors in the calculation of PVA resulting from inaccuracies in V_o estimation would introduce a change in the intercept value but no change in the slope of the $M\dot{V}O_2$-PVA relationship. This prediction is supported for the example of Fig. 4, *bottom*; filled triangles show PVA_{lin} values whereas filled squares show PVA_{nonlin} values. As predicted, the relation derived from the PVA_{lin} values has nearly the same slope but a lower intercept value than that derived from the PVA_{nonlin} values. Note that the magnitude of the shift was relatively small despite a rather large difference in extrapolated V_o values.

Analysis of all the experiments in this study produced similar findings, which are summarized in Fig. 5. Note that four ESPVRs obtained during low calcium perfusion were excluded from this analysis because the nonlinear fit did not intersect the volume axis. The values for V_o, A, and B determined by linear extrapolation of the ESPVR ($V_{o,lin}$, A_{lin}, B_{lin}) for each data set are plotted versus the corresponding values determined by nonlinear

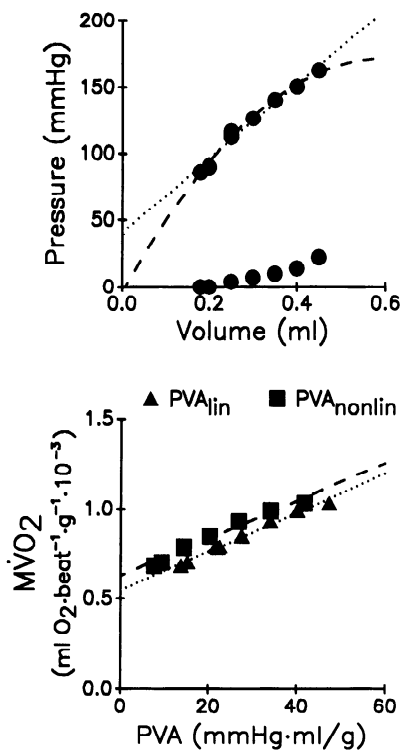

Fig. 4. Impact of linear vs. nonlinear ESPVR analysis on $M\dot{V}O_2$-PVA relationship in extreme example. *Top*: dotted line, linear regression; dashed line, nonlinear regression. *Bottom*: PVA_{lin}, PVA values determined from linear ESPVR; PVA_{nonlin}, PVA determined from nonlinear ESPVR.

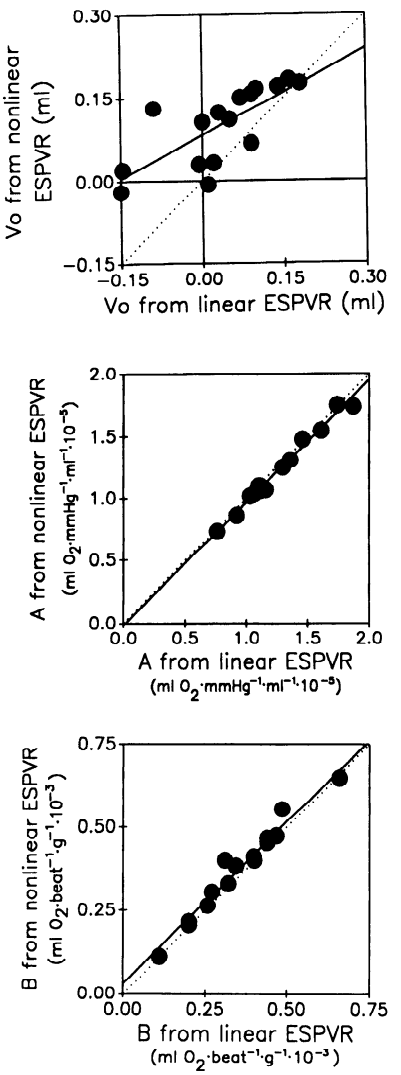

Fig. 5. Impact of linear vs. nonlinear ESPVR analysis on volume axis-intercept of ESPVR (V_o, *Top*), slope (A) of $M\dot{V}O_2$-PVA (*Middle*); and intercept (B) of $M\dot{V}O_2$-PVA (*Bottom*). For each parameter, values derived when nonlinear ESPVR regression is used are plotted against values derived when linear regression is used. Solid lines, linear regression through points; dotted lines, lines of identity.

extrapolation ($V_{o,nonlin}$, A_{nonlin}, B_{nonlin}). The relationship between $V_{o,lin}$ and $V_{o,nonlin}$ (Fig. 5, *top*, solid line) fell far from the line of identity (Fig. 5, *top*, dotted line): $V_{o,nonlin} = 0.52\ V_{o,lin} + 0.08$ ($r^2 = 0.55$). The relationship between A_{lin} and A_{nonlin} is shown in Fig. 5, *middle*: $A_{nonlin} = 0.98A_{lin} - 5 \times 10^{-8}$ ($r^2 = 0.992$); this relation was statistically, though not physiologically, different from the line of identity ($P = 0.002$). Despite the large differences between $V_{o,lin}$ and $V_{o,nonlin}$, the relation between B_{lin} and B_{nonlin} (Fig. 5; *bottom*) revealed that B was not strongly influenced by the method of extrapolation of the ESPVR: $B_{nonlin} = 0.97\ B_{lin} + 0.000033$ ($r^2 = 0.986$), which was significantly different than the line of identity ($P = 0.007$). In summary, in a comparison of the parameters of the $M\dot{V}O_2$-PVA relations determined with linear and nonlinear ESPVRs, there was an average 3% difference between the slopes and 5% difference between the intercept values.

DISCUSSION

It is demonstrated that ESPVRs and $M\dot{V}O_2$-PVA relations can be determined in a standard isolated, crystalloid-perfused rat heart preparation. These relations are typically stable over a period of 60 min after an initial 30-min equilibration period. The general characteristics of the ESPVR are similar to those reported previously for isolated, blood-perfused dog and rabbit hearts. With linear regression applied to the ESPVRs, the average E_{es} was 418.5 $mmHg \cdot g \cdot ml^{-1}$ in a standard [Ca^{2+}] (1.5 mM), which is slightly less than values obtained in blood-perfused dog hearts (630 ± 140 $mmHg \cdot g \cdot ml^{-1}$; Ref. 8) but quite similar to values obtained in blood-perfused rabbit hearts (410 ± 110 $mmHg \cdot g \cdot ml^{-1}$; Ref. 4). On a statistical basis, the ESPVR was usually fit as well by linear regression as by nonlinear regression. This, however, should be considered to apply only for the volume range over which data were acquired, which did not include low volumes near V_o. Linear regression frequently provided negative V_o values suggesting, for those cases, that the linear fit likely did not predict the actual ESPVR in the lower volume range. Despite this limitation, a detailed analysis indicated that the parameters of the $M\dot{V}O_2$-PVA relation are not influenced significantly by how the ESPVR is extrapolated back to the volume axis.

In agreement with previous studies in other species (6, 4), the $M\dot{V}O_2$-PVA relationship of the rat heart was described well by linear regression analysis. The mean slope of this relation (from all 20 relations obtained in this study) was $1.40 \pm 0.50 \times 10^{-5}$ ml $O_2 \cdot mmHg^{-1} \cdot ml^{-1}$, which is slightly lower than the values reported for dog ($1.3\text{–}2.1 \times 10^{-5}$ ml $O_2 \cdot mmHg^{-1}ml^{-1}$; Ref. 8) and for rabbit ($1.1\text{–}2.5 \times 10^{-5}$ ml $O_2 \cdot mmHg^{-1}ml^{-1}$; Ref. 4). The inverse of the slope of this relation has been proposed to reflect overall efficiency of the contractile apparatus. Whether the slightly lower slope obtained in the rat than dog and rabbit preparations represents a species difference or is peculiar to the crystalloid perfusion cannot be determined from the results of this study. With a decrease in perfusate [Ca^{2+}], there was no statistically significant change in this slope of the $M\dot{V}O_2$-PVA relation; as in the dog heart subjected to negative inotropic agents, the relation shifts downward. It is important to note that the magnitude of the change in the offset measured on going from high to low perfusate calcium (mean decrease, 37 ± 6%) was much larger than the maximal 5% uncertainty introduced because of the uncertainty in defining V_o. Another similarity to the isolated blood-perfused dog heart is the seemingly large interanimal variability in the intercept of the $M\dot{V}O_2$-PVA relation, which could not be accounted for simply by differences in baseline contractile states (2).

Limitations

The limitation of not being able to reduce ventricular volume below 0.15 ml because of the combined volume of the balloon wall and cannula has been dealt with extensively above. In our hands, the use of other material, such as a thin-walled condom, did not reduce this volume. Because the nonlinearity of the ESPVR is relatively small within the range of volumes tested, there is relatively little uncertainty introduced into the $M\dot{V}O_2$-PVA slope (as shown in Fig. 4). In the event of a wider volume range of data collection over which the ESPVR would actually demonstrate significant nonlinearity, the simple assumptions made in deriving *Eq. 6* would no longer hold and the conclusions made regarding a comparison of results between linear and nonlinear ESPVR fitting would be invalid. However, in such a case, there would be no indication for using the linear fit to the ESPVR. The uncertainty in defining V_o introduces an uncertainty in the $M\dot{V}O_2$-PVA intercept as shown by *Eq. 6*. According to this equation the magnitude of the effect is dependent not only on the uncertainty in V_o but also on the $M\dot{V}O_2$-PVA slope. Thus this analysis indicates that the magnitude of the uncertainty would be larger under circumstances in which the slope is greater (e.g., blood-perfused dog hearts).

Another limitation of the preparation relates to the crystalloid perfusate, which has one-tenth the O_2-carrying capacity of blood. In response to reduced O_2 content, there is significant vasodilation so that coronary flow is roughly 10 times greater than normal at a physiological perfusion pressure. Whereas the rate of O_2 delivery is roughly normal, tissue oxygenation should not be regarded as normal. O_2-carrying capacity of the perfusate can be enhanced by the addition of washed red blood cells. With the added expense of obtaining and preparing these cells, there would be little advantage of this preparation over larger heart preparations (e.g., rabbit), for which more sophisticated left ventricular loading systems are easy to construct (12).

With the use of the current techniques, the ventricle is constrained to contract isovolumically. This contrasts with sophisticated afterload control systems available for studying isolated canine (11) and rabbit hearts (12). It may be technically possible to construct a volume servo system for rat hearts comparable to those used for these larger hearts. However, the ability to impose physiological ejection patterns is not a necessity for many of the types of studies to which this preparation is suited.

Economy

The cost of one rat and of two dogs (required for the isolated heart preparation) differs by more than a factor of 50 (based on 1991 prices in Maryland). The added costs of nonreusable supplies (sutures, anesthetics, solutions, blood filters, etc.) increase the disparity in per experiment costs between the two preparations. Whereas the cost differential between rabbits and rats is on the order of a factor of two to three, the cost of purchasing and preparing whole animal blood for use as a perfusate (e.g., separating the red blood cells, washing them multiple times, incorporating albumin) for the rabbit studies are significant and must be included. Thus, provided the use of crystalloid perfusion and size of the rat heart preparation do not limit its utility for a particular application, this preparation offers an economic alternative to more expensive methods.

Summary

End-systolic pressure-volume and $M\dot{V}O_2$-PVA relations can be measured in the isolated crystalloid-perfused rat heart. Both relationships respond to changes in contractile state caused by changing the $[Ca^{2+}]$ in a similar manner as in the isolated canine heart. Unlike the canine model, the ESPVR cannot be experimentally defined in the lower-volume range because of the relatively large size of the balloon placed inside the ventricular chamber. Because indexes of contractility based on a mathematical fit to the ESPVR (such as E_{es} and E'_{es}) are dependent on the range over which data are collected, contractile strength may better be quantified in this model using an index such as pressure at a common volume (e.g., $P_{0.4}$). This limitation does not significantly impact on the parameters of the $M\dot{V}O_2$-PVA relation. The advantages of this preparation over cross-perfused preparations include greater ability to control perfusate composition, simplicity, and economy.

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