Basic Res Cardiol 99: 351 – 359 (2004)
DOI 10.1007/s00395-004-0476-5

ORIGINAL CONTRIBUTION

Daniel J. Lips
Theo van der Nagel
Paul Steendijk
Meindert Palmen
Ben J. Janssen
Jan-Melle van Dantzig
Leon J. de Windt
Pieter A. Doevendans

Left ventricular pressure-volume measurements in mice: Comparison of closed-chest versus open-chest approach

Received: 8 January 2004
Returned for 1. revision: 29 January 2004
1. Revision received: 24 February 2004
Returned for 2. revision: 12 March 2004
2. Revision received: 25 March 2004
Accepted: 31 March 2004
Published online: 16 April 2004

Abstract *Objective* We investigated whether *in vivo* closed-chest left ventricular pressure-volume measurements would yield similar values for LV hemodynamics compared with open-chest PV measurements under several anesthetics. *Methods* The right common carotid of C57Bl/6 mice was cannulated with a combined pressure-conductance catheter and inserted retrogradely into the left ventricle in the closed-chest model. The open-chest model consisted of an abdominal approach involving the opening of the thoracic cavity by transverse opening of the diaphragm and ventricular catheterization by apical stab. Measurements were performed under urethane or pentobarbital intraperitoneal injection anesthesia. *Results* Cardiac function in the open-chest model was characterized by larger ejection fraction and stroke volume with a leftward shift in ventricular volume compared to the closed-chest model. Further observed characteristics include low end-systolic pressure and arterial-ventricular coupling mismatch in the open-chest model. Arrhythmias were not detected in either model. *Conclusion* Murine cardiac function determination via open-chest or closed-chest protocols is sensitive, reproducible and comparable. The choice for open- or closed-chest pressure-volume measurements in mice depends on the aims of the study.

Key words Heart – cardiac physiology – mouse – pressure-volume loops – ventricular function

D. J. Lips, M.sc
P. A. Doevendans, M.D., Ph.D. (✉)
Heidelberglaan 100
3584 CX Utrecht, The Netherland
Tel.: +31-30/2509111
Fax: +31-30/2542155
E-Mail: p.doevendans@hli.azu.nl

Th. v. d. Nagel, B.sc · M. Palmen, M.D., Ph.D.
Department of Cardio-Thoracic Surgery
University Hospital Maastricht
PO box 5800
6202 AZ Maastricht, The Netherlands

P. Steendijk, Ph.D.
Department of Cardiology
Leiden University Medical Center
PO box 9600
2300 RC Leiden, The Netherlands

B. J. Janssen, Ph.D.
Department of Pharmacology and Toxicology
Maastricht University
PO box 616
6200 MD Maastricht, The Netherlands

J.-M. v. Dantzig, M.D., Ph.D.
Department of Cardiology
Catherina Ziekenhuis Eindhoven
PO box 1350
5602 ZA Eindhoven, The Netherlands

L. J. de Windt
Hubrecht Laboratorium
Uppsalalaan 8
3584 CT Utrecht, The Netherlands

P. A. Doevendans, M.D., Ph.D.
Interuniversitary Cardiology Institute the Netherlands
Postbus 19258
3501 DG Utrecht, The Netherlands

BRC 476

Introduction

The use of murine transgenic and gene-targeted models in cardiovascular research has shown an exponential growth, since this approach provides essential genetic and molecular insight into human congenital and acquired heart disease. By manipulating and investigating cardiac gene expression with the availability of accurate techniques to analyze the resultant phenotype, the molecular basis for cardiac dysfunction may be uncovered.

To date, multiple techniques allow assessment of murine left ventricular hemodynamic behavior [6, 12], such as MRI [3], transthoracic ultrasonography [22], Langendorff perfusion systems [4], aortic flow probes [18], micromanometers [19] and, more recently, conductance-micromanometers [11]. The use of conductance-micromanometers allows generation of instantaneous pressure and volume signals to create pressure-volume (PV) relations for highly accurate assessment of left ventricular performance. The PV-loop method is regarded as the gold standard for assessment of intrinsic myocardial function in large animals [5] and humans [20]. Recently, it has been shown that contractile parameters derived from PV relationships best detect small changes in contractility in mice [17].

The murine left ventricle can theoretically be entered via several ways for invasive hemodynamic measurements. To date, the most frequently reported method is an open-chest approach, with opening of the thoracic cavity via a transverse substernal incision of the diaphragm, and, subsequently introduction of the catheter into the left ventricle by an apical stab [11]. This method is theoretically disadvantageous, since collapse of the lungs, destruction of myocardial integrity and relatively large trauma can be anticipated. Furthermore, in myocardial infarction or ischemia/reperfusion studies the open-chest approach is hampered by extensive left ventricular remodeling, since the scar tissue is not accessible for the conductance catheter and provides no stable position.

A closed-chest approach would theoretically circumvent several of these disadvantages. First, in a closed-chest, the lungs remain untouched. Second, the cardiac position and myocardial structures remain intact. Third, surgical trauma and hemodynamic stress would be reduced to a minimum. Finally, a closed-chest approach would allow accurate assessment of LV hemodynamic behavior in mice that underwent reversible or permanent occlusion of the left anterior descendent coronary artery.

To investigate whether PV measurements in a closed-chest approach yield similar values for LV hemodynamics compared with open-chest PV measurements, the right common carotid artery was cannulated and a conductance catheter was advanced through the aortic valve into the LV cavity. Values for various hemodynamic parameters were recorded in this set-up and compared with those obtained following PV determinations in an established open-chest model [11].

Methods

Animals

For this study 3 – 4 month old male C57Bl/6 mice (Iffa Credo, Lyon, France) were used. All animals were kept under standard housing conditions with an artificial 12 h light cycle with free access to standard rodent food and tap water. The animal studies were performed conform the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85 – 23, revised 1996) and were approved by the Animal Ethical Committee of the Maastricht University.

Echocardiographic measurements

Adult C57Bl/6 male mice were used for echocardiographic measurements for determination of the longitudinal length of the murine heart and the correction factor α. Left ventricular long-axis and ventricular volume measurements were made with a Hewlett Packard Sonos 5500 ultra-sound machine and a 13 MHz transducer (Hewlett Packard Company, Palo Alto, USA). A standoff of 0.5 – 1.0 cm was used for recordings. Mice were anesthetized with ketamine (100 mg/kg, intramuscular) and xylazine (5 mg/kg, subcutaneous). The anterior thorax was shaved following complete anesthesia. Body temperature was kept constant at 37 °C with a warming lamp.

Electrocardiographic measurements

Continuous ECG-recordings were made with a two-lead ECG Hemodynamic Data Acquisition System apparatus (Instrument Services, Maastricht, the Netherlands) to detect arrhythmias during cardiac and arterial catheterization in both surgical protocols. The first lead was positioned in the left lower leg, the second lead in the right upper leg. The recordings were made in trend save mode (every 5 seconds) with a sample interval of 1 ms and were started at the beginning of the surgical procedure.

In vivo left ventricular pressure-volume measurements

The Sigma SA (CDLeycom, Zoetermeer, the Netherlands) single segment data acquisition module was used for

assessment of left ventricular function through the simultaneous measurement of pressure and volume. The system operated on a constant excitation current of 30 μA to prevent interaction with the murine cardiac conduction system. The time-varying ventricular volume V(t) was estimated from

$$V(t) = \rho L^2 [G(t) - G^P]$$

where ρ (rho) is the mouse specific blood resistivity, L indicates the distance between the sensing electrodes and G(t) the instantaneous conductance. Parallel conductance G^P was determined by hypertonic saline injection and subtracted offline. CONDUCT 2000 software (CDLeycom) was used for data acquisition and Circlab software (LUMC, Leiden, the Netherlands) was used for offline data analysis.

A 1.4 Fr Millar pressure-conductance catheter (SPR-719, Millar Instruments, Houston, TX, USA) was used for the LV pressure-volume measurements. The pressure-signal was calibrated with a mercury manometer at the beginning of each experiment. Baseline zero reference was obtained by placing the sensor in 37 °C normal saline before insertion. Acquired resistance was converted to relative volume units (RVU) by the Sigma SA acquisition module. The correction for electric field inhomogeneity α was calculated by conductance and echocardiographic measurements of end-diastolic volume.

The specific resistance of murine blood (ρ) from 10 mice originating from distinct genetic backgrounds (SWISS, N = 5; C57Bl/6, N = 3; FVB/N, N = 2) was determined using a Rho-cuvette (CDLeycom). The murine specific Rho-cuvette has a length of 0.5 and a diameter of 0.2 cm resulting in a content of 150 μl.

Surgical protocols

Closed-chest method (CCM)

C57Bl/6 mice (N = 13) were anesthetised (1000 mg/kg urethane or 100 mg/kg sodium pentobarbital intraperitoneally). The anterior thorax and the neck of the mouse were shaved upon complete anesthesia. The animals were fixed on a warming plate. Care was taken to maintain body temperature constant at 37 °C. The neck of the mouse was opened with a saggital incision. The trachea was exposed to visually guide the intratracheal cannula (20-Gauge), whereafter the cannula was connected to a mouse ventilator, Minivent type 845 (Hugo Sachs Electronics, Germany), set at 150 strokes per minute and a tidal volume of 200 μl. The external jugular veins were cannulated with flame-stretched PE-50 catheters for saline and drug infusion. The right common carotid was prepared for insertion of the ultraminiature conductance-micromanometer. The catheter was inserted into the left ventricle under guidance of the online pressure signal. The combination of pressure and volume signals allows accurate positioning of the catheter in the left ventricle. A transverse abdominal incision was performed to expose the inferior caval vein, without opening the thorax. During data acquisition ventilation was stopped to avoid influence from ventilation of the lungs on the pressure and volume signals. The acquisition protocol consisted of measurements of baseline cardiac function, hypertonic saline injection (3×), inferior caval vein occlusions with and without β-adrenergic stimulation (isoproterenol, 1000 pg).

Open-chest method (OCM)

C57Bl/6 mice (N = 15) were anesthetised. The anterior neck and abdomen were shaved. The abdomen was opened subcostally. The diaphragm was incised by a transverse substernal approach leaving the pericardium intact. The left ventricle was entered through an apical stab with a 25 1/2 G needle, followed immediately by the Millar conductance-micromanometer. The catheter was positioned correctly in the left ventricle under guidance of the online pressure and volume signals.

Hypertonic saline injection

Parallel conductance correction volume (Vc HSI) was determined by injection of 4 μl pre-warmed (37 °C) 30% saline bolus into the external jugular vein with a 25 μl syringe (Hamilton Bonadus AG, Bonadus, Switzerland). Ventilation was stopped during data acquisition.

Statistics

Results are presented as means ± SEM. Data of closed- and open-chest instrumented groups were statistically analyzed by two-way ANOVA using SPSS 8 software (SPSS Inc., Chicago, Illinois, USA). A P-value < 0.05 was considered to be statistically significant.

Results

Echocardiographic measurements

Echocardiographic measurements of the LV longitudinal axis revealed a length of 4.9 ± 0.1 mm in 22 adult C57Bl/6 mice. The longitudinal length of these hearts is different from the height of the segment in which volume was measured by the Millar catheter (i.e. 4.5 mm). This size difference poses a limitation for the accuracy of *in vivo*

Table 1 Comparison of volume derived by ultrasonography and conductance

	Alpha Mean ± SEM	Range	*N*
EDV	0.30 ± 0.02	0.18 – 0.45	13
ESV	0.51 ± 0.07	0.16 – 0.94	13
SV	0.26 ± 0.04	0.09 – 0.58	13

	Regression analysis R	R^2	Adjusted R^2	SEE	*N*
EDV	0.82	0.68	0.65	1.79	13
ESV	0.83	0.69	0.66	1.72	13
SV	0.41	0.17	0.09	1.17	13

Left ventricular volume was measured by both ultrasonography and conductance in adult C57Bl/6 mice. End-diastolic, end-systolic and stroke volume were used to obtain alpha (α). Definition of alpha is V_{conduc}/V_{echo}, with V_{conduc} is $\rho\, L^2\, [G(t) - G^P]$ (see Methods section). Descriptive analysis shows that α determination at the end-diastolic moment results in the most consistent mean alpha with good correlation, whereas correlation for SV was relatively poor. *SEM* standard error of the mean; *SEE* standard error of the estimate

volume measurements and their transfer into absolute LV volume values. An independent volume or cardiac output measurement could provide a correction factor, commonly designated alpha (α).We measured end-diastolic (EDV), end-systolic (ESV) and stroke volume (SV) by conductance and echocardiography in 13 adult C57Bl/6 mice. Alpha is defined by the ratio conductance-derived volume/gold-standard volume, i.e. V_{conduc}/V_{echo}. The mean alpha EDV_{conduc}/EDV_{echo} of 0.30 was used to correct conductance volume (Table 1). The high Pearson's correlation-coefficient *R* (0.82) indicated strong correlation between conductance and echocardiographic derived EDV. The determination-coefficient R^2 was higher than 64%, indicating strong correlation. The adjusted R^2 is depicted in Table 1, because of the low number of cases ($N = 13$). Even this parameter indicates strong correlation between EDV_{conduc} and EDV_{echo}. SV was excluded for α determination in our setup, because of the low correlation between SV_{conduc} and SV_{echo}.

Invasive measurements

Mice, as instrumented according to the closed- or open-chest protocols, did not differ in age, body or heart weight. The values for LV volumes were corrected for ρ, determined at 124 $\Omega \cdot$cm in the used setup ($N = 10$, 123.52 ± 0.88 $\Omega \cdot$cm), α and Vc. Vc was determined during all PV experiments through the hypertonic saline injection method. No significant differences could be detected between CCM and OCM instrumentation (94.69 ± 4.83 µL versus 92.72 ± 2.05 µL, respectively; N.S.). The 4 µL 30% saline was demonstrated to be sufficient to change blood conductivity. Moreover, as this volume encompasses only 15% of the normal murine end-diastolic volume, it proved to be too small to influence LV EDP or ESP (Fig. 1) in either OCM or CCM mice.

ECG recordings were made during both surgical protocols to detect possible heart rate alterations. Arrhythmias were not observed during either CCM or OCM procedures (Fig. 2). Specifically, bradycardia was absent during the CCM protocol. This finding indicates that cannulation of the right common carotid artery does not alter heart rate by carotid sinus stimulation. The OCM approach did not significantly influence heart rate either.

Several differences were found between the closed- and open-chest approaches (Table 2). Cardiac function in the OCM approach was characterized by higher ejection fractions (EF 69 versus 48%, respectively), larger stroke volume (19.98 versus 14.70 µl, respectively) and a leftward shift in left ventricular volume. In contrast, measuring cardiac function with an intact thorax resulted in higher ESP (OCM 52 versus CCM 65 mmHg, with urethane anesthesia) and a higher rate of relaxation as indicated by the dP/dt_{min} and Tau. Relaxation time Tau was calculated by an exponential fit on the isovolumic pressure decay. For this the interval between the time-point of dP/dt_{min} and the time-point at which dP/dt(t) reaches a value of 10% of dP/dt_{min}. No differences were found in the pressure-volume relationships, i.e. the end-systolic pressure-volume relationship (ESPVR), end-diastolic pressure-volume relationship (EDPVR) or preload-recruitable stroke work (PRSW). Figure 3 shows representative PV-loops while venous return was attenuated following inferior caval vein occlusion. Typical pressure and volume effects are depicted, showing the absence of apparent differences in pressure-volume relationships. The mentioned differences in ESP, SV and EF

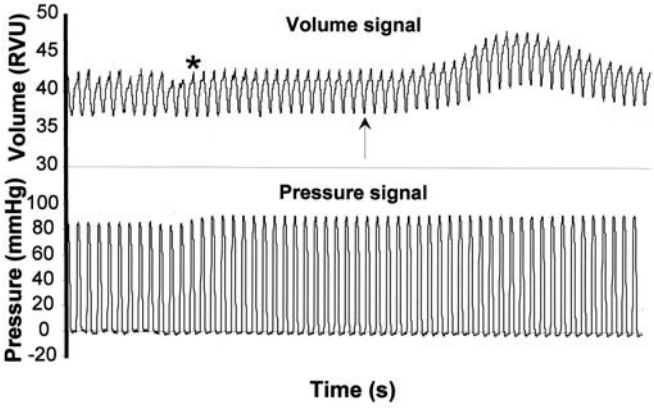

Fig. 1 Conductance and pressure signals during hypertonic saline injection. The total acquired time period was 5.84 s. Ventilation was stopped at the mark (*), after which 4 μL of 30% saline was injected (arrow) into the external jugular vein. The conductivity of blood was increased by the hypertonic saline infusion as demonstrated by the increase in the conductance signal. Parallel conductance is calculated by analyzing the conductance signal obtained during wash-in of the saline. The unchanged pressure indicates that hemodynamics are stable

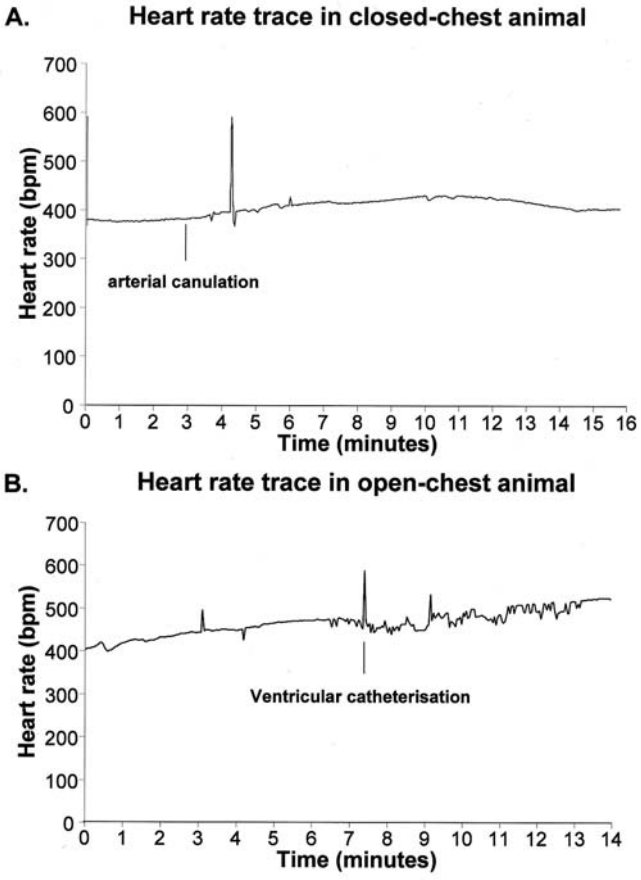

Fig. 2 Heart rate tracings in CCM and OCM instrumented mice. **A** Representative figure of a heart rate tracing in a closed-chest instrumented mouse. The moment of arterial cannulation is indicated. Some arrhythmias are seen in response to introduction of the catheter into the left ventricle. Arrhythmias are only present in a short time-period that is part of the stabilization period following catheter introduction. Heart rate remains unchanged during the measurements. **B** Representative figure of a heart rate tracing in an open-chest instrumented mouse. Somewhat more pronounced chronotropic arrhythmias are seen in response to ventricular introduction of the catheter by apical stab. However, no significant brady- or tachycardias are present during the time of measuring

Table 2 Functional parameters to determine baseline LV functioning during steady-state measurement

	URETHANE		PENTOBARBITAL	
	OCM $N=5$ *Mean ± SEM*	CCM $N=5$ *Mean ± SEM*	OCM $N=10$ *Mean ± SEM*	CCM $N=10$ *Mean ± SEM*
HR	583 ± 20	658 ± 21	493 ± 25*	481 ± 36*
SV	19.6 ± 3.5	15.6 ± 2.5†	20.2 ± 1.8	14.3 ± 1.0†
CO	12 ± 2	10 ± 2	10 ± 1	7 ± 1
V_{max}	17.6 ± 0.5	23.6 ± 2.2†	30.9 ± 2.9*	38.8 ± 2.3*†
V_{min}	2.2 ± 1.3	6.8 ± 1.8†	10.3 ± 1.9*	23.7 ± 2.0*†
EF	79 ± 6	66 ± 8†	66 ± 4*	39 ± 2*†
ESP	52 ± 4	65 ± 5†	42 ± 3*	47 ± 4*†
EDP	1 ± 1	-1 ± 1	-6 ± 2*	1 ± 1*
dP/dt_{max}	7200 ± 1349	8252 ± 1685	4004 ± 629*	3718 ± 684*
dP/dt_{min}	-4233 ± 531	-7577 ± 750†	-3631 ± 424*	-3348 ± 653*†
Tau	10 ± 0	7 ± 0	12 ± 1*	14 ± 2*
SW	1066 ± 245	1147 ± 213	1076 ± 176	666 ± 116
Ea/Ees	0.65 ± 0.20	0.84 ± 0.16†	0.60 ± 0.05	1.03 ± 0.15†
ESPVR	5.7 ± 0.9	5.9 ± 0.7	3.5 ± 0.3*	3.2 ± 0.5*
EDPVR	0.04 ± 0.02	0.01 ± 0.04	0.11 ± 0.04	0.01 ± 0.03
PRSW	79 ± 14	64 ± 6	68 ± 10	53 ± 3

Results from LV pressure-volume measurements in C57Bl/6 mice anesthetized by urethane or pentobarbital. Represented are dependent variables from multivariate analyses of variance with anesthetic (urethane versus pentobarbital; * $P<0.05$) and protocol (OCM versus CCM; † $P<0.05$) as fixed factors. *HR* heart rate in bpm; *SV* left ventricular stroke volume in μl; *CO* cardiac output ml/min; V_{min} minimal left ventricular volume in μl; V_{max} maximal left ventricular volume in μl; *EF* ejection fraction in %; *ESP* left ventricular end-systolic pressure in mmHg; *EDP* left ventricular end-diastolic pressure in mmHg; dP/dt_{max} maximal first derivative of left ventricular pressure in mmHg/s; dP/dt_{min} minimal first derivative of left ventricular pressure in mmHg/s; *Tau* time constant of left ventricular relaxation in ms; *SW* stroke work in mmHg•μl; *Ea* arterial elastance / *Ees* ventricular end-systolic elastance; *ESPVR* left ventricular end-systolic pressure-volume relationship in mmHg/μl; *EDPVR* left ventricular end-diastolic pressure-volume relationship in mmHg/μl; *PRSW* left ventricular preload-recruitable stroke work in mmHg

are evident in the presented loops. The OCM approach did result in a mismatch between arterial afterload and ventricular work. The arterial-ventricular elastance ratio (Ea/Ees) was significantly depressed following opening of the chest, largely due to consistently lower arterial elastance values in OCM instrumented mice independent of anesthetic regime (Ea, 2.45 ± 0.23 versus 3.83 ± 0.40, $P < 0.05$). In contrast, Ea/Ees ratios were normal (i.e. ~ 1.0) in the CCM approach under both regimens.

The steady-state functional parameters from OCM and CCM instrumented mice during either urethane or pentobarbital are depicted in Table 2. The use of pentobarbital anesthesia had a cardiodepressive effect that was represented by significant function decline in most parameters. The cardiodepressive effects of anesthetics are commonly represented in chronotropic and inotropic parameters. Heart rate was significantly lower in pentobarbital anesthetized mice compared to urethane (487 ± 22 bpm versus 621 ± 18 bpm, $P < 0.05$). Also, the inotropic performance was attenuated under the pentobarbital regime, as shown by ESP (44 ± 3 versus 59 ± 4 mmHg, $P < 0.05$) and dP/dt_{max} and $-_{min}$ (max. 3861 ± 453 versus 7726 ± 1032 mmHg/s, $P < 0.05$; min. -3490 ± 380 versus -5905 ± 705 mmHg/s, $P < 0.05$). Cardiac output (CO), however, was not significantly depressed (respectively, 8 ± 1 versus 11 ± 1 ml, N.S.) From these parameters we concluded that pentobarbital has strong negative chronotropic and inotropic effects.

A different situation developed following β-adrenergic stimulation (Table 3). Now, no statistical differences between anesthesia regimes were found in the parameters HR, ESP and dP/dt_{max} and $-_{min}$. This indicates, that

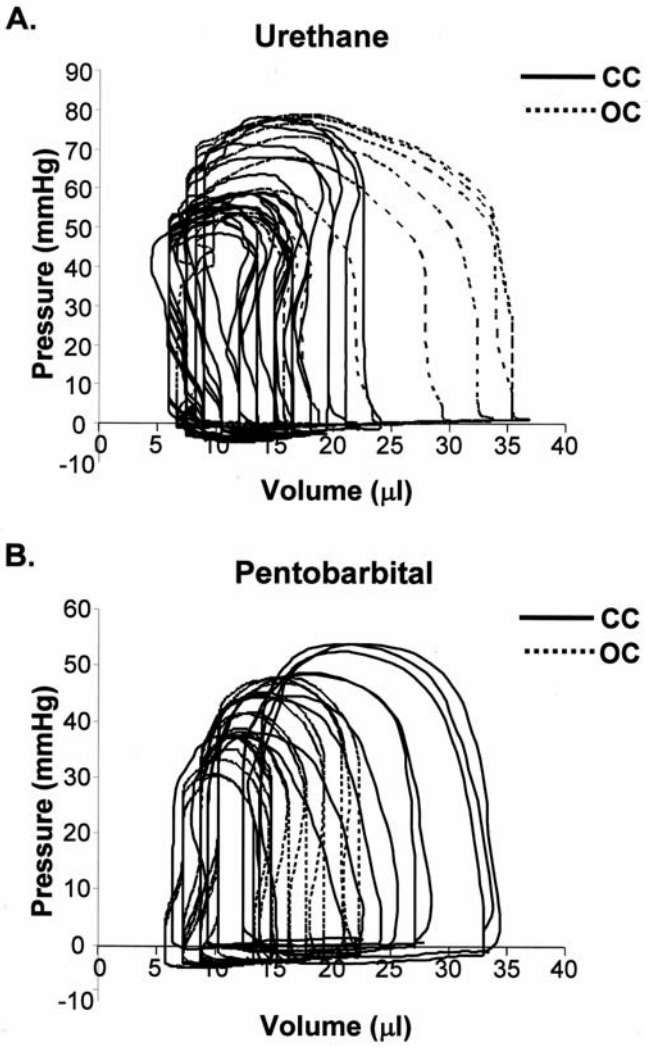

Fig. 3 Left ventricular pressure-volume loops. Pressure presented in mmHg and volume in relative volume units. **A** Representative figure of pressure-volume loops acquired during urethane anesthesia in CCM and OCM instrumented mice. **B** Representative figure of pressure-volume loops acquired during pentobarbital anesthesia in CCM and OCM instrumented mice. Loops are acquired during an occlusion of the inferior caval vein. Venous return decreases, which is represented in the loops by attenuation of both pressure and volume. The differences in ESP, SW and ESPVR between urethane and pentobarbital anesthesia can be observed by analyzing the loops in A and B. The loops show also the larger stroke volume and depressed systolic pressure in OCM instrumented mice

Table 3 Functional parameters to determine steady-state LV functioning during β-adrenergic stimulation

	URETHANE		PENTOBARBITAL	
	OCM	CCM	OCM	CCM
	N = 5	*N* = 4	*N* = 9	*N* = 10
	Mean ± *SEM*	*Mean* ± *SEM*	*Mean* ± *SEM*	*Mean* ± *SEM*
HR	575 ± 16	670 ± 24	684 ± 32	600 ± 39
SV	21.19 ± 2.37	17.62 ± 1.16	27.52 ± 2.47*	27.03 ± 2.15*
CO	12 ± 1	12 ± 1	19 ± 1*	16 ± 2*
ESP	56 ± 11	71 ± 18	66 ± 6	72 ± 16
EDP	2 ± 1	1 ± 1	-8 ± 2*	-1 ± 2*
dP/dt_{max}	6596 ± 942	8656 ± 985	9491 ± 82	8837 ± 1791
dP/dt_{min}	-3884 ± 519	-5732 ± 675	-5724 ± 427	-5556 ± 1226
Tau	8 ± 1	8 ± 1	9 ± 1*	11 ± 1*
SW	1022 ± 217	1132 ± 195	1837 ± 190*	1822 ± 281*

Results from LV pressure-volume measurements in C57Bl/6 mice anesthetized by urethane or pentobarbital during maximal β-adrenergic stimulation. Represented are dependent variables from multivariate analyses of variance with anesthetic (urethane versus pentobarbital; * $P < 0.05$) and protocol (open-versus closed-chest) as fixed factors. *HR* heart rate in bpm; *SV* left ventricular stroke volume in μl; *CO* cardiac output in ml/min; *ESP* left ventricular end-systolic pressure in mmHg; *EDP* left ventricular end-diastolic pressure in mmHg; dP/dt_{max} maximal first derivative of left ventricular pressure in mmHg/s; dP/dt_{min} minimal first derivative of left ventricular pressure in mmHg/s; *Tau* time constant of left ventricular relaxation in ms; *SW* stroke work in mmHg•μl

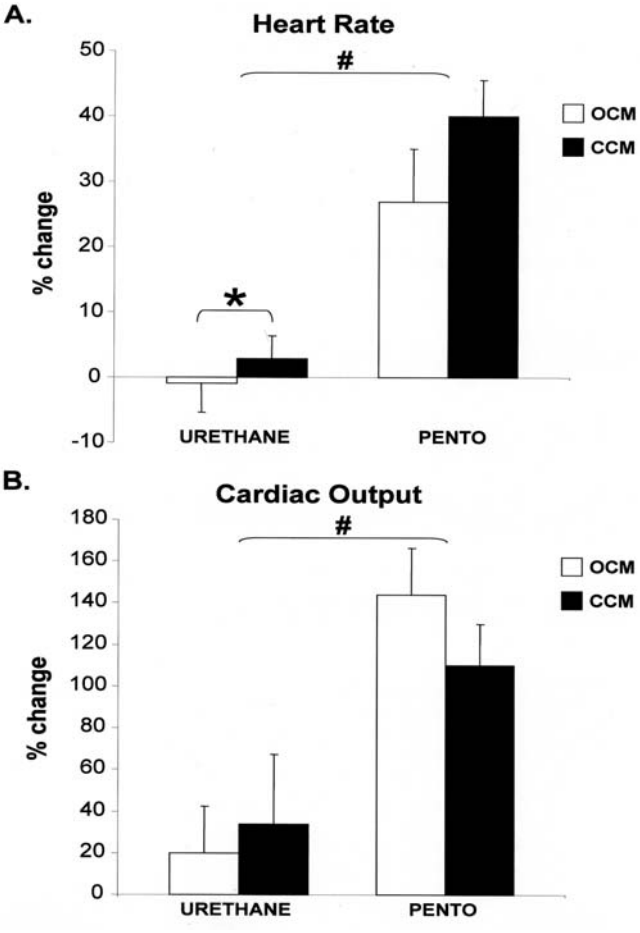

Fig. 4 The effects of isoproterenol infusion on heart rate and cardiac output. **A** Heart rate showed a slight increase of 1% with urethane, while it was augmented by 33% in the OCM group in response to β-adrenergic stimulation. The effect of isoproterenol was significantly more pronounced in the CCM group compared to OCM group under urethane anesthesia (* $P < 0.05$, $N \geq 4$ for all groups). Furthermore, mice under pentobarbital anesthesia showed in general a larger chronotropic response upon isoproterenol stimulation than urethane anesthetized mice (# $P < 0.05$). **B** Cardiac output showed augmentation of more than 120% with pentobarbital in response to β-adrenergic stimulation, compared to a minor increase of 27% during urethane anesthesia (# $P < 0.05$, $N \geq 4$ for all groups) ▶

the effect of β-adrenergic stimulation was larger in mice with pentobarbital anesthesia compared to urethane. Statistical analysis of percent-change revealed significant larger increases in the parameters HR, CO and dP/dt_{max} with pentobarbital anesthesia (Fig. 4).

Discussion

In vivo left ventricular PV measurements provide an accurate view of murine cardiac function. The method is highly sensitive in determining alterations in left ventricular function. Cardiac performance depended on the protocol of left ventricular catheterization. In the open-chest protocol significantly larger ejection fractions and stroke volumes in combination with a leftward LV volume shift were found, indicating a high contractile status. This approach, however, had some significant limitations. Systolic pressure and the ventricular rate of relaxation were low compared to the closed-chest approach. The arterial-ventricular elastance coupling indices indicated an afterload mismatch in the open-chest mice, related to lower arterial elastance: the slope of the ESPVR (Ees) proved to be equal in OCM and CCM instrumented mice. This finding is consistently found in open-chest instrumented mice [11]. The production of stroke work is maximal when the Ees and Ea are approximately equal and stroke work attenuates substantially in relation to its maximal value, whenever Ea and Ees greatly differ [16, 21]. The altered arterial elastance could be explained by the opening of the thorax. It is known, for instance, that overall peripheral vascular resistance significantly decreases from baseline values during thoracoscopy [7].

Several open-chest ventricular catheterization protocols have been described, ranging from an anterior thoracotomy and sternum reflection to a less invasive abdominal approach including subxiphoid incision [9–11, 13, 23]. Current widely used protocols combine minimally invasive techniques with maximal exposure to minimize blood loss and hemodynamic instability. Our open-chest protocol is comparable to those published with respect to the surgical techniques used. However, the presented values from our protocol seem to reflect depressed cardiac performance, especially when compared to values obtained in conscious mice [14, 15]. The observed cardiodepression could partly be due to the used anesthetic regime. The most common anesthetic in open-chest cardiac function measurements is urethane in combination with etomidate, α-chloralose and/or morphine [9–11, 23]. When cardiac performance is defined by heart rate (chronotropy) and systolic pressure (inotropy), large variations in cardiac performance are found between these studies [9–11, 23]. Our data is within the range of these studies. The mixture of ketamine/xylazine led to the most depressed cardiac performance [13]. Besides the specific anesthetics used, other factors like the administered dose, body temperature, agitation by handling and nutritional/hydration status of the mice could importantly influence cardiac performance. Care was taken to mimimize the influence of those factors. The possibility of incorrect calibration was explored and ruled out in the present study. In our study the different surgical and anesthetic approaches were compared in an identical controlled setting, which enables a more direct comparison than comparing results from different individual published studies.

Significant differences between open- and closed-chest cardiac function measurements were found in earlier publications [13]. Although significant cardiodepression was observed in both protocols, probably due to the addition of morphine to the anesthetic regime, the worst cardiac performance was seen in open-chest instrumented mice. The open-chest protocol performed by Hoit et al, consisted of wide opening of the chest via a subxiphoid incision, sternum deflection and bilateral thoracotomies. The traumatic impact of open- and closed-chest protocols in the published study, significantly exceeded those between our protocols [13]. This difference in surgical technique could be the reason for the differences in outcomes between studies.

Murine left ventricular pressure-volume measurements could be distorted by physiological responses to the catheterization. Heart rate alterations are probable, especially due to carotid sinus stimulation via cannulation of the carotid artery. The electrocardiographic analysis did not reveal such an effect for both open- and closed-chest approaches. Also, the hypertonic saline injection could theoretically have unwanted influences on LV hemodynamics. Due to the ionic composition of the 30% saline solution, a substantial flood of volume could arise in the external jugular and superior caval veins, thereby altering the volume in the circulatory system at the time of parallel conductance determination [2, 8]. However, highly consistent Vc measurements were obtained with our protocol, as shown in Fig. 2, without altering hemodynamics. We therefore consider the infusion protocol to be reproducible and accurate for Vc determination.

The conductance-catheter measures besides left ventricular blood volume, also the conductance of other structures (i.e. lungs and mediastinum), due to protrusion of the electric field into these structures (parallel conductance, Vc). The online volume-signal appeared to consist for a large part of parallel conductance (~ 80%) in the current setting. Also, the conductance-catheter tended to underestimate the left ventricular volume with approximately a factor of 3 – 5. An independent left ventricular volume or cardiac output measurement is necessary to correct for the underestimation. Our data showed that echocardiographic volume measurements revealed different correction factors, depending on the moment of

measuring in the cardiac cycle. Those different α-values in single mice are an interesting finding. Correction of relative volume units into absolute volume depends therefore on the measurement timing in the cardiac cycle. Stroke volume proved to be the worst parameter for volume correction by ultrasonography. This conclusion was based on the low correlation between volume measured by ultrasonography and conductance and the wider variance in a in stroke volume measurements. The end-diastolic volume resulted in more accurate and reproducible values and was used for volume correction in our studies.

Barbiturates, including pentobarbital, are notorious cardiodepressive substrates, as shown by decreased systolic and diastolic heart function compared to urethane. In contrast, a remarkable recovery of cardiac function was found following β-adrenergic stimulation. The chronotropic and inotropic reserve of pentobarbital anesthetized mice, therefore, was greater than with urethane. Another explanation could be that enhanced catecholamine stimulation of the heart was already present at baseline measurements in mice anesthetized with urethane. Urethane does increase the central drive to the adrenal medulla leading to the secretion of epinephrine [1]. Both pentobarbital and urethane are anesthetics with important disadvantages. They represent the two extremes of a continuum and should be avoided in cardiovascular studies. The use of inhalation anesthesia using isoflurane or sevoflurane should be subject of new investigations.

In the present paper open-chest and closed-chest protocols for *in vivo* left ventricular pressure-volume acquisition are illustrated. Murine cardiac function determination via these methods is sensitive, reproducible and comparable. The choice for open- or closed-chest pressure-volume measurements in mice depends on the aims of the study. The open-chest approach is technically easy and less time consuming. It is characterized by both low systolic pressures and arterial resistance. The open-chest approach is particularly useful in murine heart failure studies. Cardiac hypertrophy and ischemia/reperfusion studies are probably better done by using the closed-chest approach, because myocardial integrity is guaranteed.

Acknowledgments The authors like to thank Frederick van der Veen, Fritz Prinzen, and Leo Hofstra for their advice and assistance during the development of the experimental set-up and the analyses of the results. This work was supported by grants from the Netherlands Foundation for Scientific Research to L.J.D.W. (NWO 902-16-275), the Netherlands Heart Foundation (NHS 99-114 and NHS 2000-160) and the Interuniversitary Cardiology Institute Netherlands (P.A.D.).

References

1. Armstrong JM, Lefevre-Borg F, Scatton B and Cavero I (1982) Urethane inhibits cardiovascular responses mediated by the stimulation of alpha-2 adrenoceptors in the rat. J Pharmacol Exp Ther 223:524–535
2. Atlan H, Thoma J (1987) Solvent flow in osmosis and hydraulics: network thermodynamics and representation by bond graphs. Am J Physiol 252:R1182–R1194
3. Chacko VP, Aresta F, Chacko SM, Weiss RG (2000) MRI/MRS assessment of in vivo murine cardiac metabolism, morphology, and function at physiological heart rates. Am J Physiol Heart Circ Physiol 279:H2218–H2224
4. De Windt LJ, Willems J, Reneman RS, Van der Vusse GJ, Arts T, Van Bilsen M (1999) An improved isolated, left ventricular ejecting, murine heart model. Functional and metabolic evaluation. Pflugers Arch 437:182–190
5. Dekker AL, Geskes GG, Cramers AA, Dassen WR, Maessen JG, Prenger KB, van der Veen FH (2001) Right ventricular support for off-pump coronary artery bypass grafting studied with bi-ventricular pressure-volume loops in sheep. Eur J Cardiothorac Surg 19:179–184
6. Doevendans PA, Daemen MJ, de Muinck ED, Smits JF (1998) Cardiovascular phenotyping in mice. Cardiovasc Res 39:34–49
7. Faunt KK, Cohn LA, Jones BD, Dodam JR (1998) Cardiopulmonary effects of bilateral hemithorax ventilation and diagnostic thoracoscopy in dogs. Am J Vet Res 59:1494–1498
8. Feher JJ, Ford GD (1995) A simple student laboratory on osmotic flow, osmotic pressure, and the reflection coefficient. Am J Physiol 268:S10–S20
9. Feldman MD, Mao Y, Valvano JW, Pearce JA, Freeman GL (2000) Development of a multifrequency conductance catheter-based system to determine LV function in mice. Am J Physiol Heart Circ Physiol 279:H1411–H1420.
10. Georgakopoulos D, Kass DA (2000) Estimation of parallel conductance by dual-frequency conductance catheter in mice. Am J Physiol Heart Circ Physiol 279:H443–H450
11. Georgakopoulos D, Mitzner WA, Chen CH, Byrne BJ, Millar HD, Hare JM, Kass DA (1998) In vivo murine left ventricular pressure-volume relations by miniaturized conductance micromanometry. Am J Physiol 274:H1416–H1422
12. Hoit BD (2001) New approaches to phenotypic analysis in adult mice. J Mol Cell Cardiol 33:27–35
13. Hoit BD, Ball N, Walsh RA (1997) Invasive hemodynamics and force-frequency relationships in open- versus closed-chest mice. Am J Physiol 273:H2528–H2533
14. Janssen B, Debets J, Leenders P, Smits JF (2002) Chronic measurement of cardiac output in conscious mice. Am J Physiol 282:R928–R935
15. Kass DA, Hare JM, Georgakopoulos D (1998) Murine cardiac function: a cautionary tail. Circ Res 82:519–522
16. Little WC, Cheng CP (1991) Left ventricular-arterial coupling in conscious dogs. Am J Physiol 261:H70–H76
17. Nemoto S, DeFreitas G, Mann DL, Carabello BA (2002) Effects of changes in left ventricular contractility on indexes of contractility in mice. Am J Physiol Heart Circ Physiol 283:H2504–H2510
18. Palmen M, Daemen MJ, Buehler A, Bronsaer RJ, Zimmermann R, Smits JF, Doevendans PA (1999) Impaired cardiac remodeling and function after myocardial infarction in FGF-1 transgenic mice. Circulation 100:250

19. Rockman HA, Hamilton RA, Jones LR, Milano CA, Mao L, Lefkowitz RJ (1996) Enhanced myocardial relaxation in vivo in transgenic mice overexpressing the beta2-adrenergic receptor is associated with reduced phospholamban protein. J Clin Invest 97:1618–1623
20. Schreuder JJ, Steendijk P, van der Veen FH, Alfieri O, van der Nagel T, Lorusso R, van Dantzig JM, Prenger KB, Baan J, Wellens HJ, Batista RJ (2000) Acute and short-term effects of partial left ventriculectomy in dilated cardiomyopathy: assessment by pressure-volume loops. J Am Coll Cardiol 36:2104–2114
21. Sunagawa K, Maughan WL, Sagawa K (1985) Optimal arterial resistance for the maximal stroke work studied in isolated canine left ventricle. Circ Res 56:586–595
22. Tanaka N, Dalton N, Mao L, Rockman HA, Peterson KL, Gottshall KR, Hunter JJ, Chien KR, Ross J (1996) Transthoracic echocardiography in models of cardiac disease in the mouse. Circulation 94:1109–1117
23. Yang B, Larson DF, Watson R (1999) Age-related left ventricular function in the mouse: analysis based on in vivo pressure-volume relationships. Am J Physiol 277:H1906–H1913