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Left ventricular pressure-volume (PV) loops

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1. OBJECTIVE

When intrinsic left ventricular cardiac contractility is studied, it is necessary to differentiate the effects of an intervention/genotype/drug…on intrinsic contractility itself and the effects on preload, afterload and heart rate that indirectly influence left ventricular contractility. This can be performed using the study of left ventricular pressure-volume loops under varying loading conditions.

A catheter is inserted in the left ventricle under anesthesia that continuously measures left ventricular pressure and conductance. Conductance is measured with 4 electrodes on the catheter and is inversely related to volume. Varying load can be obtained by gently occluding the inferior caval vein. The most robust and load dependent parameter, thus the one preferably used, is the Preload Recruitable Stroke Work (PRSW). Additionally it is often useful to study the effect of increasing doses of dobutamine or isuprel on contractility.

2. SCOPE AND APPLICABILITY

This standard operating procedure will describe the technical aspects of PV loops measurements in anesthetized mice, but not the theoretical background nor the analysis of the acquired pressure and conductance data. This SOP is intended for researchers or technical personnel that are performing the in vivo measurements.

3. CAUTIONS

Caution should be taken for the miniature catheters. They are easily damaged by exerting direct force to the pressure sensor, or by bending the catheter. Coagulation with an electrical bistouri can destroy the catheter and conditioning modules, so switch off these devices (Bovie, Valleylab,..) before inserting the pressure-conductance catheter.

The body temperature should be stable, since this heavily influences contractile parameters. Adjustement of anesthesia during the measurement protocol certainly invalidates the measurements. The anesthesia should be stable and deep enough to allow controlled ventilation and momentarily stopping of the ventilation during PV loop acquisition, without spontaneous breathing of the animal. Arrhythmias make the measurements unreliable.

Advantages:

- load independent contractility can be measured
- reliable results in experienced hands
- Able to study contractility in mice with high heart rates

Disadvantages:

- anesthesia required
- usually a terminal procedure, so no longitudinal follow-up in the same animals can be done
- Time-consuming, therefore not appropriate as rapid screening procedure. Only use this methodology when good theoretical or preliminary results indicate a possible effect on cardiac contractility

4. MATERIALS

Equipment:

- Mouse ventilator (e.g. HSE Minivent, Hugo Sachs Electronics, March Hugstetten, Germany)
- Warming mattress to preserve body temperature, preferably coupled with a rectal temperature probe (many suppliers) to maintain body temperature at 37°C [36.5-37.5°C]
- PV signal conditioning unit (Aria1 or MVPS from Millar Instruments, USA, or equivalent from Scisense, Canada)
- Data-acquisition system (many suppliers, e.g. ADInstruments), analysis software (e.g. PVAN, Millar).
- Syringe driver (e.g. Harvard)

Instruments:

- Pressure conductance catheter (Millar Instruments or Scisense or other manufacturer) adapted to the size of the animal (1-1.4 Fr for mice), and adapted to the access route, which should be preferably through the carotid artery.
- Tracheal tube
- Surgical Instruments (forceps, scissors)

Supplies:

- NaCl 0.9% at 37°C
- Anesthetics should be chosen according to their cardiovascular and respiratory effects.
- Cotton swab

- Operation microscope (e.g. Zeiss, WPI,…)
- Precalibrated cuvets to determine specific conductance.
- Tergazyme or other detergent to soak the catheter after each procedure.
- Sutures silk 5/0-8/0
- 24G IV catheter
- 30% NaCl solution (freshly prepared).
- Microsyringe (1 ml and below)
- Dobutamine (dobutrex)

5. METHODS

- Every month perform a complete 0 mmHg- 100 mmHg calibration of the pressure element with a mercury manometer.
- Connect the catheter to the PV conditioning module and submerge the tip of the catheter with the electrodes and pressure tip in NaCl 0.9% at 37°C for at least 15 minutes.
- Before each experiment, adjust the adjustable resistance of the wheatstone bridge amplifier to correct for baseline drift (0 mmHg adjustment) while keeping the pressure tip immediately below the fluid surface.
- Anesthetize the mouse. Anesthesia should be adapted to the specific aims of the study: examples are given: 1) anesthesia is induced with a single intraperitoneal injection of urethane (960 mg/kg) and of alfa-chloralose (40 mg/kg). The mouse is kept in a quiet environment until fully unconscious. This usually takes about 15 minutes; 2) a mix of ketamine-xylazine-midazolam and/or fentanyl is also possible; 3) other modes of anesthesia are however also possible.
- The mouse is positioned supine on a warming mattress, rectal temperature probe inserted. A midline incision is made in the neck. The trachea is incised, a tracheal tube inserted.
- Positive pressure ventilation is started with room air (100 strokes per minute, volume dependent on weight, see manual ventilator).
- An incision in the right neck is made and the right carotid artery and right jugular vein were exposed. A small size high fidelity pressure-conductance catheter is introduced through the right carotid artery into the left ventricle.
- After stabilisation of the hemodynamic situation, baseline PV-loops are recorded.

- A small laparotomy is performed, and with a cotton swab, the inferior caval vein is compressed between the liver and the diaphragm, while PV-loops are recorded (occlusion loops). Alternatively, the inferior caval vein can be compressed by external abdominal compression, if one takes care to avoid organ displacement that could interfere.
- A 24G IV catheter is introduced in the right jugular vein, and parallel volume is determined by a bolus injection of 1.5 µl of 30% NaCl solution. This is performed three times.
- Afterwards the jugular catheter is replaced with a fresh one, and dobutamine is infused with a syringe driver at a dose of 1, 3, 10 and 30 ng/g/min each time for 2 min until a stable heart rate plateau is reached. During this progressive increase in dobutamine, PV loops are recorded without occlusion. Afterwards the dobutamine dose is progressively reduced from 30, to 10 to 3 to 1, and each time occlusion loops are obtained.
- Afterwards blood is retrieved from the inferior caval vein to measure specific conductivity in 3 precalibrated cuvets. A conductance-volume calibration line is constructed with these cuvet data.
- Since this is usually a terminal experiment, the heart is often excised and processed as required for histology or other in vitro tests.
- Analysis is performed from each experiment, with correction for parallel volume and data are expressed in absolute volumes. Only technically acceptable loops (no arrhythmia, stable baseline,...) are included in the analysis for each experiment. This analysis is performed blinded for the group to which the mouse belonged. This yields data sheets for all experiments, which are used as the data for statistical analysis. Only after all data sheets are established, the group code of the mice is unblinded for statistical analysis.

6. EVALUATION AND INTERPRETATION OF RESULTS

- A conductance-volume calibration line is constructed with the cuvet data.
- Analysis is performed from each experiment, with correction for parallel volume and data are expressed in absolute volumes. Only technically acceptable loops (no arrhythmia, stable baseline,...) are included in the analysis for each experiment. This analysis is performed blinded for the group to which the mouse belonged. This yields data sheets for all experiments, which are used as the data for statistical analysis. Only after all data sheets are established, the group code of the mice is unblinded for statistical analysis.
- First parallel volume is determined to correct RVU, and with the calibration line obtained by the cuvet data, absolute volume excluding parallel volume is calculated.

- Afterwards, technically acceptable loops are selected. Usually the mean of 5-15 loops are used for further calculations.

7. REFERENCES

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Vangheluwe P, Tjwa M, Van Den Bergh A, Louch WE, Beullens M, Dode L, Carmeliet P, Kranias E Herijgers P,, Sipido KR, Raeymaekers L, Wuytack F. (2006). A SERCA2 pump with an increased Ca^{2+} affinity can lead to severe cardiac hypertrophy, stress intolerance and reduced life span. Journal of Molecular and Cellular Cardiology 41: 308-317.

8. APPENDIX

A manuscript with data from the *mdx* mice is published in the European Heart Journal (Buyse G. Van der Mieren G, Erb M, D'Hooge J, Herijgers P, Verbeken E, Jara A, Van den Bergh A, Mertens L, Courdier-Fruh I, Barzaghi P, Meier T. (2009) Long-term blinded controlled efficacy study of SNT-MC17/idebenone in the dystrophin-deficient *mdx* mouse. European Heart Journal 2009; 30: 116-124.). An extract from the relevant table under baseline conditions.

	WT-veh N=8	***mdx*-veh** N=12
HW/TL (mg/cm)	72.2 ± 8.6	82.5 ± 8.8 *
Parameters in steady state		
HR (bpm)	501 ± 37	483 ± 36
Pmax (mmHg)	82.6 ± 7.5	80.6 ± 8.7
Pes (mmHg)	73.7 ± 10.8	72.2 ± 9.5
Ped (mmHg)	3.6 ± 1.4	5.4 ± 2.2 *
Ved (µl)	30.1 ± 11.4	32.0 ± 7.5
SV (µl)	16.1 ± 5.8	15.8 ± 3.6
EF (%)	53.2 ± 11.7	47.6 ± 9.8
CO (µl/min)	8149 ± 3189	7599 ± 1703
SW (mmHg*µl)	1096 ± 445	1021 ± 367
Ea (mmHg/µl)	5.6 ± 3.8	4.8 ± 1.0
dP/dtmax (mmHg/s)	6349 ± 1005	5907 ± 1623
dP/dtmin (mmHg/s)	-6257 ± 777	-5564 ± 1321
Tau (ms)	6.3 ± 0.9	7.0 ± 1.0
Parameters obtained after temporary preload reduction		
PAMP (mWatts/µl²)	88 ± 50	62 ± 27
Ees (mmHg/µl)	8.4 ± 0.9	8.0 ± 1.1
PRSW (mmHg)	71.4 ± 8.3	70.1 ± 13.8
EDPVR (mmHg/µl)	0.30 ± 0.17	0.42 ± 0.20
dP/dt_EDV (mmHg*µl/s)	303 ± 91	359 ± 203
PVA (mmHg*µl)	1328 ± 472	1310 ± 584
Efficiency (%)	58.1 ± 10.3	55.2 ± 9.8