2.9. Closed-chest left ventricular (LV) hemodynamic assay with the Millar catheter (Duan lab)

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A. OBJECTIVE

To evaluate pressure-volume change of the left ventricle during heart contraction in an intact mouse. The left ventricular catheterization of mice has been described using both a closed-chest internal carotid approach and an open-chest apical stab approach. We describe the closed-chest approach. This approach yields more physiologic hemodynamics than the open-chest approach. Further, the closed-chest approach can be performed without ventilation. However, for mouse models of muscular dystrophy, we recommend utilization of a ventilator during left ventricular hemodynamic study because respiratory function is often impaired in these mice.

B. CAUTIONS

- For all animal experiments, make sure to get approval from the Institute's Animal Care and Use Committee and follow NIH guidelines.
- An appropriate anesthetic regimen is pivotal. Numerous anesthetics have been tested in mice with significant variations in heart rate and blood pressure (Bostick et al, 2011). Isoflurane is increasingly becoming the anesthetic of choice because it is readily available and easy to titrate. Further, isoflurane provides rapid induction and recovery.
- Mouse heart rate is strongly dependent on temperature and the mouse should be kept warm during all phases of the procedure. Induction chamber and operating tables should be monitored with a thermometer to ensure the temperature is kept between 36-38°C.
- Mice with severe muscle disease (such as utrophin/dystrophin double knockout mice and DBA background mdx mice) require extra care during anesthesia because of poor respiratory function. Isoflurane should be carefully titrated to prevent respiratory depression and maintain a respiratory rate above 80 per minute.
- Negotiation of the PV catheter into the left ventricle requires gentle in an out movement of the catheter especially when negotiating the aortic valve. Ideal placement of the PV catheter in the left ventricle may require adjusting the angle and rotation of the catheter into the carotid artery.

C. MATERIALS

- Millar MPVS-400 (Millar Instruments, Houston, TX, USA).
- Millar ultra-miniature P-V catheter model SPR-839 (Millar Instruments).
- Millar cuvette block (Millar Instruments).
- Chart software v5.5.6 (AD Instruments).
- Isoflurane portable anesthesia aystem (Summit Medical Equipment, Bend, OR, USA).
- Isoflurane (VetOne, Median, ID, USA).
- Oxygen tank containing 100% oxygen (Airgas National, Charlotte, NC, USA).
- Thermophore heating pad (Medwing).
- Heating lamp (Tensor Lighting Company).
- Vicks digital thermometer (Kaz).
- Thermolyne 589 rectal digital pyrometer (Barnstead International).
- Hair clippers (Wahl, Sterling, IL, USA)
- Stereo microscope (Nikon, Melville, NY, USA).
- Mini-vent mouse ventilation system type 845 (Hugo Sachs Elektronik, Hugstetten, Germany).
- Tracheotomy cannula, 1.3 mm outer diameter (Harvard Apparatus, Holliston, MA, USA).
- Surgical instruments: microsurgical spring scissors, straight serrated fine tip forceps, Dumont type or other fine tip straight and angled forceps, Kilner curved fine sharp point scissors, hemostats (World Precision Instruments), and Guthrie double hook retractor (Fine Science Tools, Foster City, CA, USA).
- Bread silk suture # 4-0 (SofSilk USSC Sutures, Norwalk, CT, USA).
- 25µL 33G gas-tight Hamilton syringe and needle (Hamilton Company, Reno, NV, USA).
- PE 10 polyethylene tubing (Clay Adams Division of Becton Dickinson and Company, Parsippany NJ, USA).
- 30% hypertonic saline (Abbott Laboratories, North Chicago, IL, USA).
- 0.9% isotonic saline (Abbott Laboratories).
- 27G ½ inch and 30G ½ inch needles (Becton-Dickinson Medical Supply, Franklin Lakes, NJ, USA).
- Cotton tipped wooden applicators (Fisher Scientific, Pittsburgh, PA, USA).
- Heparin multidose vial (Baxter Healthcare Corporation, Deerfield, IL, USA).
- Dobutamine (Sigma, St. Louis, MO, USA).
- 30G ½ cc insulin syringes (Becton-Dickinson Medical Supply).
- PVAN data analysis software (Millar Instruments).

D. METHODS

1. Mouse anesthesia. Place the mouse into a clean, empty cage by itself for 5 minutes prior to anesthetizing. Gently transfer the mouse into a pre-warmed

induction chamber and anesthetize with 3% isoflurane at an oxygen flow rate of 2 l/min for about 2.5 minutes.

2. Promptly remove the mouse from the induction chamber. Place the mouse onto a pre-warmed recording table with snout inserted into nose cone. Provide maintenance anesthesia of 1-1.5% isoflurane at an oxygen flow rate of 0.5 – 0.6 l/min.
3. Secure mouse limbs to the recording table using tape placed over the paws. Leave one lower limb unsecured for monitoring the depth of anesthesia with toe pinch.
4. Lubricate the rectal temperature probe with surgical lubricant and gently insert 2-3 mm into the rectum. Maintain mouse body temperature between 37-38°C during entire procedure.
5. Make a skin incision at the anterior neck and separate the parotid glands and subcutaneous tissue overlying the trachea using blunt dissection. Under a stereo microscope, expose the trachea by cutting away the cricothyroid muscles. Make an incision in the trachea just above the cricoid cartilage (swollen region just under the cricothyroid muscle) using a 20 G needle bent at a 90° angle. Enlarge the incision using microsurgical spring scissors. Remove the mouse snout from the nose cone. Place a looped suture around the teeth and secure above the head to stretch the mouse neck and create traction on the trachea. Cannulate the trachea using a 1.3 mm OD tracheotomy tube. Tie a suture around the trachea and the tracheotomy tube to hold in place. Connect the anesthesia supply tube from the nose cone to the intake tubing on the mouse ventilator and set the oxygen flow rate to 0.2-0.3 l/min. Ventilate the mouse with a tidal volume of 8-10 µL per gram bodyweight at a respiratory rate of ~ 200 respirations per minute.
6. Locate the right carotid sheath running along the posteriolateral region of the right side of the trachea. Dissect away the right carotid sheath with blunt dissection. Separate the larger pulsating internal carotid artery from the internal jugular vein and vagus nerve. The vagus nerve should be carefully separated and left intact to prevent altering the autonomic innervation of the heart. Place looped sutures at the distal and proximal ends of the internal carotid artery and secure them with hemostats. Stretch and occlude the artery by pulling on the hemostats. Place a third looped suture between the outer sutures and leave it loosely tied.
7. Pre-soak the 1.4 F Millar pressure-volume (PV) catheter in 37°C normal saline for a few minutes. Open the Chart software to enable visualization of the pressure tracing while inserting the PV catheter. Stretch the carotid artery using the proximal and distal sutures to occlude blood flow. Grasp the end of the PV catheter with serrated straight fine-toothed forceps which have the tips covered with PE 10 tubing. Incise the carotid artery with a 27 G needle which has the tip bent at a 90 angle. While holding the 27 G needle in the carotid artery provide gentle upward traction and

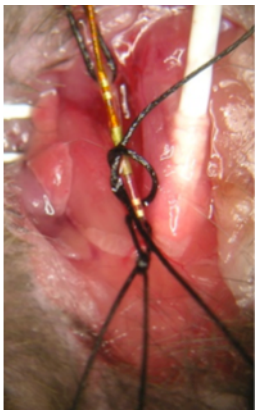

Figure 1. Right carotid artery catheterization.

simultaneously advance the PV catheter into the carotid artery underneath the 27 G needle. Gently tie the middle suture around the carotid artery and the PV catheter. Release traction on the suture occluding the proximal end of the carotid artery. Advance the catheter further into the carotid artery until the proximal suture is above the volume electrodes on the PV catheter. Tie the proximal and middle sutures around the carotid artery and the catheter. The knots should be tight enough to prevent blood from leaking around the catheter but loose enough to allow the catheter to slide within the artery (**Figure 1**).

8. Carefully advance the PV catheter from the carotid artery into the aorta. Use the pressure tracing from the Chart software window to guide advancement. Confirm aortic location of the PV catheter by visualizing the pressure tracing oscillating with the dichrotic notch evident. Negotiate the PV catheter through the aortic valve with gentle in and out advancement. A drop in the diastolic pressure to near zero with the systolic pressure unchanged confirms the correct placement of the PV catheter in the left ventricle. Once inside the left ventricle, adjust the depth and angle until the best change in relative volume units (RVU) is obtained
9. After signal stabilization, record baseline PV loops at the steady state.
10. Make a small sub-xiphoid incision and open up the peritoneum. Mobilize the liver away from the diaphragm by cutting the falciform ligament between the diaphragm and superior edge of the liver. Carefully spread the liver and diaphragm with a cotton tipped applicator to visualize the inferior vena cava (IVC) along the posterior abdominal wall to the right of the spinal column.
11. While visualizing the pressure-volume tracings in the Chart window, occlude the IVC with a cotton tipped application for ~ 2 seconds. The pressure and volume tracings should exhibit a uniform 20-30 % decrease during the occlusion.
12. Prepare 1 µg/µL solution of dobutamine in normal saline. Inject 5 µg/gram body weight of dobutamine via intraperitoneal injection.
13. At 5 minutes post dobutamine injection, record PV loops to determine the dobutamine response. .
14. Carefully expose the right external jugular vein by bluntly dissecting the surface of the right neck along the mid-clavicular line. Cannulate the vein with a 30 G needle attached to a 25 µL Hamilton syringe with PE 10 tubing. Quickly and evenly inject 5 µL of 30% hypertonic saline into the right external jugular vein while visualizing the PV tracings in the Chart window. During injection, the pressure tracing should remain unchanged while the volume tracing exhibits a uniform rise.
15. At the end of PV recording, remove ~ 200 µL of blood from the mouse by either direct cardiac puncture or large vein sampling using a heparinized syringe. Fill each well in the Millar supplied cuvette with heparinized blood and partially submerge the cuvette in a 37°C water bath. Place the PV catheter into each well and record the resulting RVU. Using a Microsoft Excel graph, plot the measured RVU for each well on the *x*-axis against the known well volumes on the *y*-axis. Determine the slope and y-intercept for the line.

16. After finishing these calibrations and other experimental manipulations, euthanize the mouse and save your Chart file for future analysis.

E. EVALUATION AND INTERPRETATION OF RESULTS

1. PV loop analysis is best performed off-line once the experiment has been completed. **Figure 2** shows a representative left ventricle PV loop tracing.

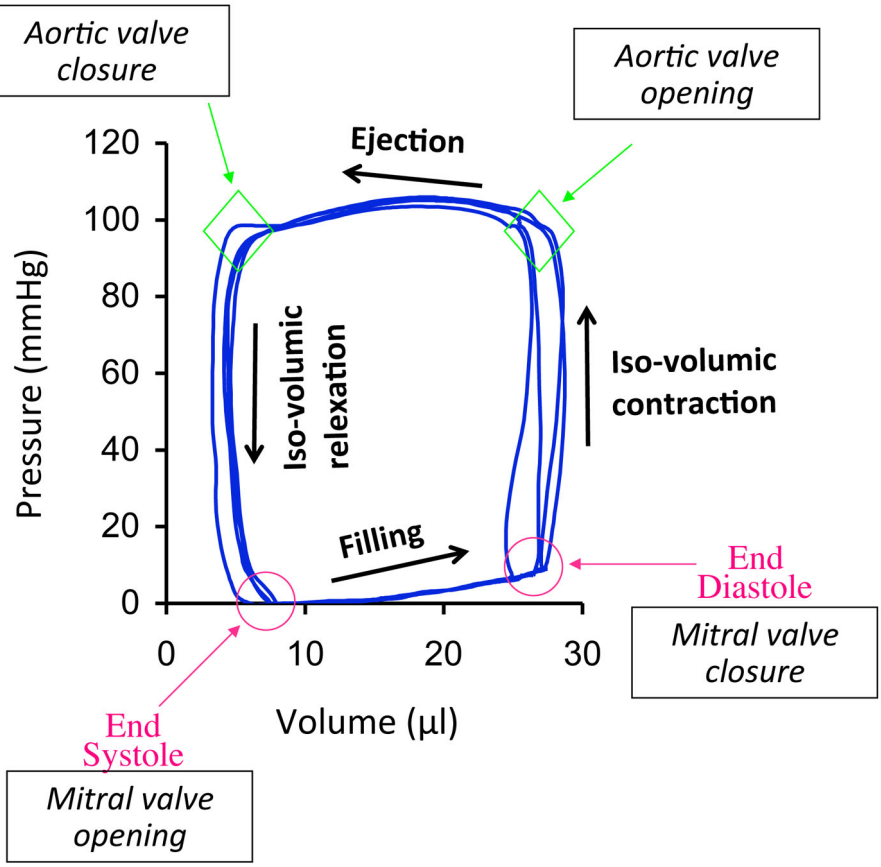

Figure 2.

2. Determination of offset voltage (Vp). Select the region of the hypertonic saline injection beginning with the first volume loop before injection to the maximum volume achieved during saline injection. Import this data into PVAN by clicking on the heart icon in the Chart window. Click on the steady state/saline icon and enter the slope and y-intercept values determine from the blood cuvette calibration. Perform the saline calibration analysis to construct a linear regression graph. Save the Vp, offset voltage, value for use in determining the actual volume of the left ventricle.
3. Baseline and dobutamine stress analysis. Select the corresponding loops to be analyzed and click on the heart icon in the Chart window. Select steady state/saline icon and enter the slope, y-intercept and Vp from cuvette calibration and offset voltage determination. Select hemodynamics to complete the data analysis and view the left ventricular functional results. These data may be exported to Microsoft excel by saving the data file.

4. Pre-load reduction IVC occlusion analysis. Select the IVC occlusion loops to be analyzed beginning with the first loop before occlusion started and ending at the point where the pressure tracing is 20-30% decreased from baseline. Click on the heart icon in the Chart window and select occlusion. Enter the slope, y-intercept and offset voltage for the mouse as determined above. Click on the contractility icon to determine the end-systolic pressure volume reserve (ESPVR) and end-diastolic pressure volume reserve (EDPVR). Determine the pre-load recruitable stroke work by clicking on the icon to compare the stroke work to the maximum volume.

F. REFERENCES

Bostick B, Yue Y, Duan D (2011) Phenotyping cardiac gene therapy in mice. Methods Mol Biol **709:** 91-104