2.10. Closed-chest right ventricular (RV) hemodynamic assay with the Millar catheter (Duan lab)

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A. OBJECTIVE

To evaluate pressure-volume change of the right ventricle during heart contraction in an intact mouse (Li & Wehrens, 2010; Wasala et al, 2013).

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.
- RV catheterization is performed via external (right) jugular vein and it is extremely fragile. Take extra precautions during dissection tot to damage or stretch the vessel too much.
- Max Pressure of RV is lower (30-50 mmHg) than that of LV.

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. Make a small incision in the midline at the level of clavicle. Carefully separate the subcutaneous tissues and underneath glands to visualize the right jugular vein. Place looped sutures at the distal and proximal ends of the right jugular vein and secure them with hemostats. Gently straighten and secure the right jugular vein using a 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. Make a small incision in the longitudinal direction of the right jugular vein using micro-scissors and a pre-bent 27G puncture needle. Insert the PV catheter through the incision and carefully advance through the right jugular vein to the right ventricle.
8. Proper catheter positioning is verified by real-time monitoring the pressure changes during catheter advance using Lab Chart software.
9. Once a stable tracing is reached (body temp is between 37°C ±1°C and heart rate is approximately between 550-650bpm), record the tracings for at least 10 minutes.
10. After finishing recording, 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.
2. Select the corresponding loops to be analyzed and click on the heart icon in the Chart window. Select hemodynamics to complete the data analysis and view the right ventricular functional results. These data may be exported to Microsoft excel by saving the data file.

F. REFERENCES

Li N, Wehrens XH (2010) Programmed electrical stimulation in mice. *J Vis Exp***:** pii 1730

Wasala NB, Bostick B, Yue Y, Duan D (2013) Exclusive skeletal muscle correction does not modulate dystrophic heart disease in the aged mdx model of Duchenne cardiomyopathy. *Hum Mol Genet* **22:** 2634-2641