Video Article

Cardiac Stress Test Induced by Dobutamine and Monitored by Cardiac Catheterization in Mice

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Abstract

Dobutamine is a β-adrenergic agonist with an affinity higher for receptor expressed in the heart (β_1) than for receptors expressed in the arteries (β_2). When systemically administered, it increases cardiac demand. Thus, dobutamine unmasks abnormal rhythm or ischemic areas potentially at risk of infarction.

Monitoring of heart function during a cardiac stress test can be performed by either ecocardiography or cardiac catheterization. The latter is an invasive but more accurate and informative technique that the former.

Cardiac stress test induced by dobutamine and monitored by cardiac catheterization accomplished as described here allows, in a single experiment, the measurement of the following hemodynamic parameters: heart rate (HR), systolic pressure, diastolic pressure, end-diastolic pressure, maximal positive pressure development (dP/dtmax) and maximal negative pressure development (dP/dt_{min}), at baseline conditions and under increasing doses of dobutamine.

As expected, in normal mice we observed a dobutamine dose-related increase in HR, dP/dt_{max} and dP/dt_{min}. Moreover, at the highest dose tested (12 ng/g/min) the cardiac decompensation of high fat diet-induced obese mice was unmasked.

Video Link

The video component of this article can be found at https://www.jove.com/video/50050/

Protocol

Protocol was approved by the Ethic Committee of Facultad de Medicina Clinica Alemana-Universidad del Desarrollo.

I. Preparing Dobutamine Infusion

1. Dissolve 10 mg of dobutamine in 20 ml of sterile distillated water, in order to obtain a stock solution of 500 µg/ml dobutamine. Aliquot and store at -20 °C. This solution can be used at least for 3 months.
2. Thaw an aliquot of dobutamine stock solution at room temperature.
3. Dilute dobutamine stock solution in sterile 0.9% NaCl, in order to obtain dobutamine working solution, which concentration is calculated using the formula: dobutamine (µg/ml) = body weight x 0.2.
4. Fill a 1 ml syringe 29Gx1/2" with dobutamine working solution.
5. Insert the needle of the syringe into a 20 cm PE-10 tube.
6. Adjust the syringe in the infusion pump following manufacturer instructions.
7. Set up the ramp infusion in a step-to-step format with an increase of 10 µl/min for each step, for 6 steps.

II. Preparing Pressure Sensor

1. To minimize signal drift, submerge pressure sensor in sterile water at 37 °C for at least 15 min. Do not soak the catheter more than 0.5 cm deep, in order to prevent that hydrostatic pressure affects the pressure sensor.
2. Electronically calibrate the pressure sensor at 25 and 100 mmHg. Electric input (Volt) is converted to pressure signal (mmHg).
3. Set the sampling rate of 2 k/s and use the filter low pass with a cut off at 100 Hz. Set pressure signal to zero mmHg.

4. Mark the catheter 15 mm from the tip. Distant to reach the heart from the introduction point was estimated by echocardiography, detecting the presence of the catheter into the left ventricule.

III. Preparing Mouse for Catheterization

1. Weight C57BL/6 male mice, 30-32 week-old.
2. Inject intraperitoneally 60 µg/kg ketamine and 4 µg/kg xylazine[1]. Note: Other anesthetics might be used, for example: 350 - 450 µg/kg avertin, 50 µg/kg pentobarbital or 1.5 - 2% isoflurane[2-3].
3. Shave the neck with an electric razor.
4. Place the anesthetized mouse in supine position on a warmed isothermal heating plate. Secure its limbs with paper tape.
5. Perform a toe pinch to confirm complete sedation.
6. Gently insert a rectal probe to monitor body temperature. Using vaselinized probe is recommended.
7. If body temperature differs from 37 °C ± 0.5 °C, adjust it via the heating plate.
8. Put mouse snout near the oxygen supply.
9. Place the mouse neck region under the stereomicroscope.

IV. Data Acquisition

1. In the LabChartPro 7 software, select one channel for pressure registration and one channel for heart rate (HR) registration. For the latter, select the option "Cyclic Measurements" and setup measurement as rate.
2. For the pressure channel set the scale range: 0 to 150 mmHg.
3. For HR channel set scale range: 200 to 600 bpm.
4. Press the start key to begin the registration.
5. Insert comments indicating procedures performed, for example: anesthesia administration, start of dobutamine infusion, dobutamine concentration, breathing changes.

V. Cardiac Catheterization[4,5]

1. Perform a small incision on the right side near the jaw. With scissors separate the skin-muscular connective tissue.
2. Perform a longitudinal dissection (1.5 - 2 cm) on the right side of the trachea. Separate the connective tissue, fat and muscle with curved forceps, in order to expose the right carotid artery near the trachea.
3. Place an expander in the animal right side to expose the carotid artery. Pulsatile pressure generated by the heart facilitates the identification of the artery. The jugular vein, which is dark red, is on the right.
4. Separate the artery from adjacent tissues with curved forceps. The vagus nerve, which resembles a white thread, lies along the artery.
5. Cut a 20 cm piece of 6/0 silk thread and "double" it.
6. Pass the "double" thread under the artery from left to right. Cut the thread, in order to obtain separate ends.
7. Pass a third thread (10 cm) below the artery.
8. Tie a tight knot in the thread positioned near to the head, and a loose one in the more distal thread.
9. Tie a loose knot in the middle thread, and fix the right end of the middle thread to the heating pad with a paper tape.
10. Keep carotid artery moist by dropping sterile 0.9% NaCl. Dry off excess of liquid with cotton buds.
11. Stretch the lower thread with a hemostat clamp.
12. Fix the position of the hemostat scissor by pinching the skin of the abdomen, stretching the upper thread, in order to occlude blood flow. Verify that connective tissue around the artery has been removed. The artery should be full of blood and deprived of pulse. Prevent threads from producing a torque force on the artery.
13. Make a cross section nick near the bottom of the artery with a Vannas micro-scissor. Blood drops will be spilled.
14. Insert the catheter into the carotid artery. Be sure to introduce the entire pressure sensor. Verify that there is no blood loss.
15. Gently adjust the middle thread knot, in order to hold the catheter in place. Do not compress too much, the pressure sensor is very fragile.
16. Release the hemostat scissors from the animal abdomen.
17. Hold the catheter with the hand and push the middle thread, in order to avoid blood loss. Note: artery should be full of blood.
18. Start recording pressure signals.
19. When the catheter is inside, the arterial pressure signal fluctuates from 60-70 to 100-120 mmHg. The shape of the pressure signal is shown in **Figure 1.A.** Note: if you are interested on, at this time point you can record arterial pressure if signal is stable for at least 5 min. HR values were obtained from the pressure waveforms considering an interval of 30 sec of record signal. It is possible to use also an ECG method for direct measurement of HR, according with the investigation goals.
20. Gently push the catheter up to observe a change in the shape of the pressure signal (**Figure 1.B**). Once the catheter is inside the left ventricle, the pressure signal fluctuates from 0 to 100-120 mmHg. If it is difficult to slide the catheter, pinch animal chest with two fingers.
21. Continuously control breath rate, body temperature, anesthesia level and pressure signal. All of them should remain stable.

VI. Infusion of Dobutamine

1. For jugular vein cannulation be sure to peel back the adipose tissue around the vein, in order to prevent its perforation. The surgical procedure for vein occlusion is similar to the procedure shown for carotid artery.
2. Introduce a PE-10 tube into the vein. Confirm that blood flow is not blocked moving backward the syringe plunger.
3. Dobutamine infusion starts with 10 µl/min and finish with 60 µl/min. In every step, the infusion rate is maintained for 2 min[6].
4. After the last dobutamine dose, euthanize the animal with an overdose of anesthesia.

VII. Data Analysis

1. For data analysis, choose the section of the recorded data of your interest. Be sure to consider a time interval where pressure signal is stable.
2. Select the Setup icon in the Blood Pressure module. Indicate the selected type of pressure signal.
3. Automatically the LabChartPro 7 software displays mean, maximum and minimum values for HR, systolic pressure (P_{max}), diastolic pressure (P_{min}), end-diastolic pressure (EDP), maximal positive pressure development (dP/dtmax) and maximal negative pressure development (dP/dt_{min}). In addition, cardiac parameters can be depicted on the pressure trace.

Representative Results

The arterial pressure signal is defined by systolic and diastolic pressure. When the pressure sensor is inside the left ventricle, its pressure (LVP) waveform is characterized by a drop to zero of the diastolic pressure and the appearance of the left atrial contraction before ventricle contraction (**Figure 1**). At baseline condition, ketamine-xylazine anesthetized normal mice had HR of 280 ± 24, P_{max} of 107 ± 8, P_{min} of 5 ± 1, EDP of 14 ± 2, dP/dt_{max} of 6081 ± 365 and dP/dt_{min} of 5230 ± 526.

As seen in **Figure 2**, in normal mice LVP and HR progressively increased along dobutamine infusion, and normalized after stopping the infusion. As expected, all the hemodynamic parameters assessed also increased in a dobutamine dose-dependent way (**Figure 3**). Thus, dobutamine chronotropic (HR increase) and positive inotropic (LVP and dP/dt_{max} increase) effects are evidenced.

Compared to normal mice, in high fat diet-induced obese mice we observed a lower increase of HR and dP/dt_{max} when cardiac stress is induced, been statistically significant at the highest dobutamine doses tested (**Figure 4**). These differences were not observed at baseline conditions (0 ng/g/min).

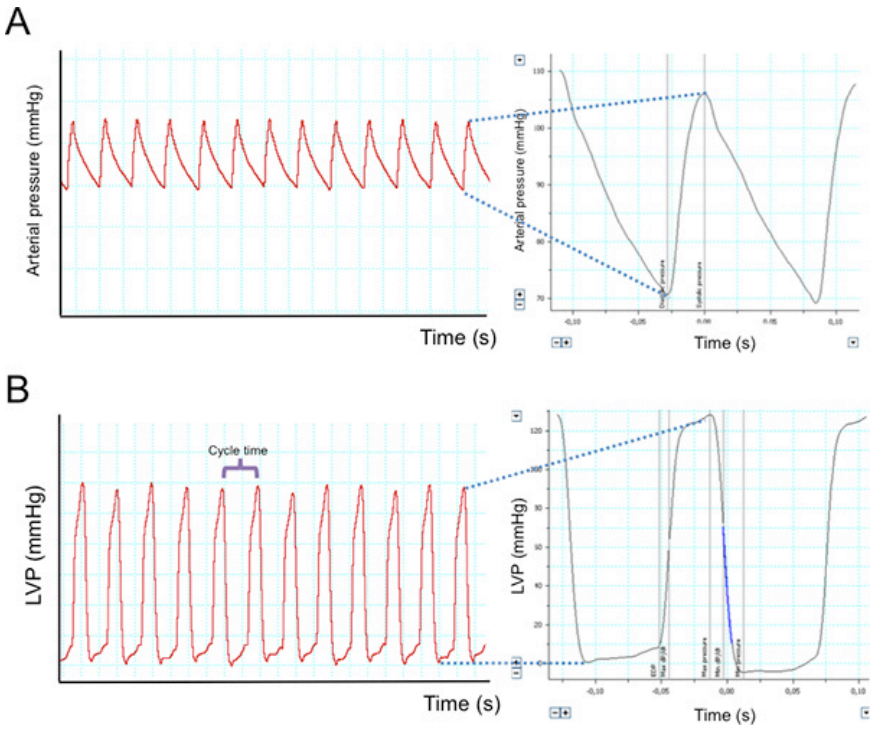

Figure 1. Arterial (A) and ventricular (B) pressure registers obtained after catheterization of normal mice. Hemodynamic parameters were determined from representative LVP vs. time cardiac cycles. Representative data of 5 animals. Click here to view larger figure.

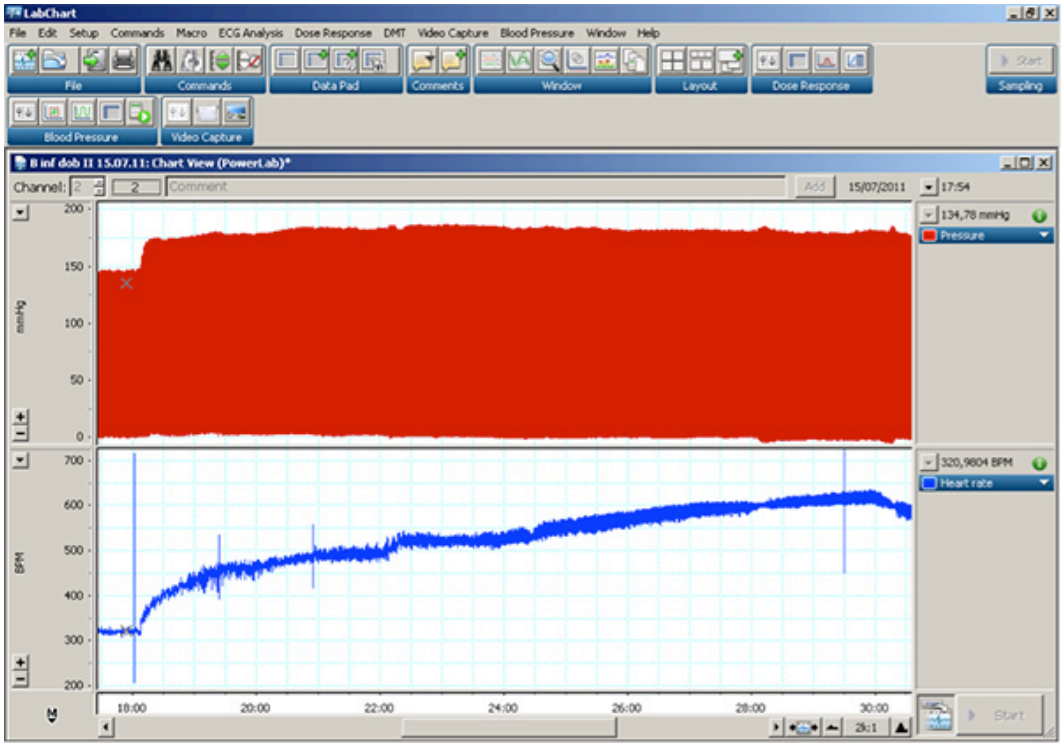

Figure 2. LVP (A) and HR (B) registers during dobutamine infusion in normal mice. Representative data of 5 animals.

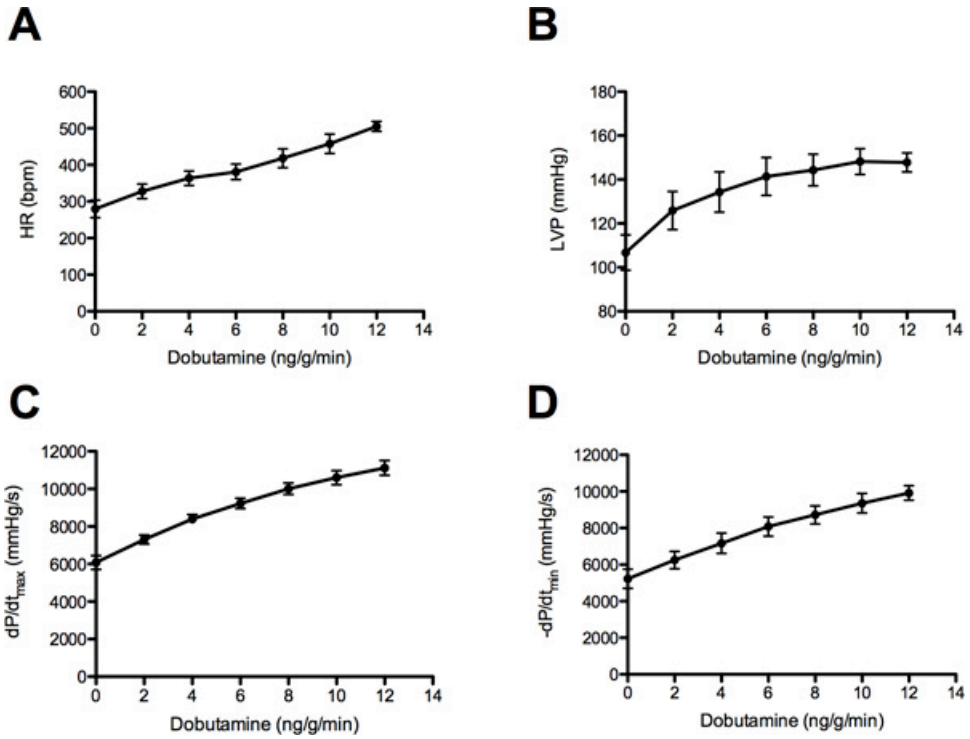

Figure 3. HR (A), LVP (B), dP/dt_{max} (C) and dP/dt_{min} (D) changes during dobutamine infusion in normal mice. Data are expressed as mean ± SEM. n = 5

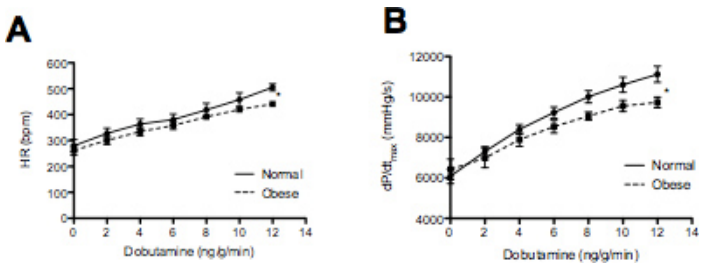

Figure 4. HR (A) and dP/dt_{max} (B) changes during dobutamine infusion in normal and high fat diet-induced obese mice. Data are expressed as mean ± SEM. *= $p<0.05$, n = 5

Discussion

Cardiac stress test induced by dobutamine and monitored by cardiac catheterization is laborious. Nonetheless, following the protocol here describe and with a short time of training, it is possible to assess six hemodynamic parameters in a single experiment that last approximately one hour.

The critical steps of the protocol here presented are the cannulations of blood vessels. Regarding the cannulation of carotid artery, the incision performed should be deep enough to break the three tissue layers of the artery, and large enough to allow the passage of the catheter. Regarding the cannulation of jugular vein, while the risk of bleeding is low, the possibility of vein occlusion is high. Thus, protocol repeatability amply hangs on the standardization of the nicking strategy used.

To minimize the impact of the depressive effect of anesthesia, hypothermia and hypoxia should be prevented and, if necessary, corrected. Respiratory rhythm should be keep regular because deep or irregular breathing affects LVP recording.

Dobutamine doses used should be adjusted according to the: i) route of administration (intravenously: 0.5 to 40 ng/g/min[7,8]; intraperitoneally: 1 to 1.5 µg/g/min[9,10]), ii) magnitude of cardiac dysfunction and iii) ethiology of cardiac alteration.

And last but not least, three practical advices: i) in animals with abundant adipose tissue surrounding the jugular vein, allow a small amount of blood to come out, in order to detect the incision borders; ii) keep the working area moisten because the cannulation procedure is simpler when the catheter is well lubricated; iii) tagging the catheter facilitates its visualization under the microscope.

Disclosures

No conflicts of interest declared.

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