Invasive Blood Pressure – Rat Model

Introduction

Blood pressure (BP) measurement is one of the basic procedures in biomedical research. Three methods are most widely used for recording the BP in a rat: tail cuff plethysmography (noninvasive), intra-arterial catheters (invasive), and radio telemetry.

Intra-arterial catheters yield the most precise values, and surgery is required to use them. Most of our physiological and pharmacological knowledge related to BP, and its regulation has been derived from acutely prepared, anesthetized, or immobilized laboratory animals.

Invasive blood pressure (IBP) is the gold standard against which the accuracy of noninvasive blood pressure method (NIBP) is compared. IBP is the arterial pressure directly measured in any artery such as the radial, femoral, or brachial artery using a saline-filled catheter/cannula.

NIBP is more suitable as a basal BP value when a compound is to be screened for anti-hypertensive activity, whereas the invasive technique is usually suitable for measuring the vascular reactivity to various agonists and antagonists.

Invasive measurements yield the correct basal BP, but sometimes there are fluctuations in the basal BP due to the anesthesia which interferes with the normal BP. The best anesthesia for conducting invasive Rat blood pressure measurements is urethane or pentobarbitone.

Materials

IX-RA-834 or any one of the IX-4xx Recorders with the IA-400D Amplifier

BP-100 or BP-102 Intravascular Blood Pressure sensor (replacement elements available)

LabScribe software

Blood Pressure Analysis Module

PE-50 tubing, fits 22ga, .023x.038in

22 gauge Blunt needle with Luer connector

Calibration kit for the BP-100

3-way stop cock if not using the BP-102

Other Materials

The requirements include adult Wistar rats/Sprague Dawley rats, heparinized saline, urethane/ketamine + xylazine/pentobarbital sodium, a 1 ml tuberculin syringe, 5 and 10 ml syringes, small (3″) and medium (5″) c, artery forceps (5″), small and medium forceps (with teeth, blunt and pointed), a bulldog clamp, dissecting forceps (toothed and non-toothed) (5″), 18 gauge needles, a surgical table, respiratory tubing (6″ infant feeding tube/ pediatric Ryle's tube may be used), a surgical lamp, an insertion needle, a surgical blade, thread, and adhesive tape and a sphygmomanometer.

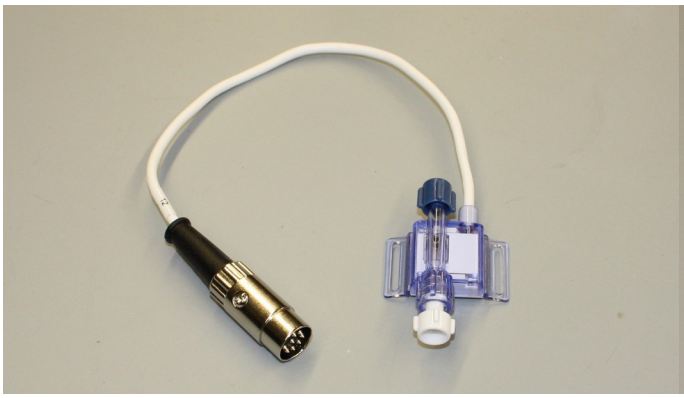
Drug solutions such as:

- normal saline,
- epinephrine (1 mg/ml),
- nor-epinephrine (1 mg/ml),
- acetylcholine (1 mg/ ml),

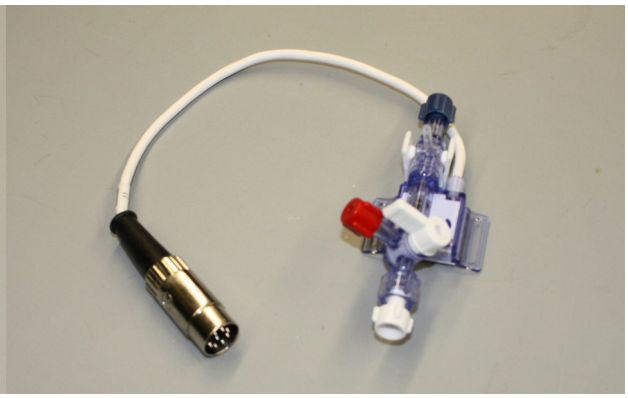
- histamine (1 mg/ml),
- dopamine (1 mg/ml),
- prazosin (1 mg/ml),
- isoprenaline (1 mg/ml),
- propranolol (1 mg/ml),

Other investigational products (required concentration) are prepared freshly to study and compare their effects on BP. A working standard of adequate concentration is prepared from each stock solution. The drug solutions are prepared using pyrogen-free distilled water.

BP-100/102 Pressure Transducer Calibration



BP-100 - Intravascular Blood Pressure Transducer



BP-102 - Intravascular Blood Pressure Transducer

The BP-100/102 Intravascular Blood Pressure Transducer is a clinical blood pressure transducer modified for use with digital recording equipment in research applications. It consists of an active element and a separate (included) molded 5 ft. extension cable. Although the elements are rugged and will stand up to repeated use, they are not permanent. Replacement elements can be purchased directly from iWorx Systems, Inc.

The BP-102 integrates a 3cc flush device and stopcock. The sensor employed in this blood pressure (BP) transducer, and all disposable BP transducers, is a strain gauge. The pressure output of the gauge is linear. This makes transducer calibration very straightforward.

- On the BP-100, the transducer element is open at both ends. One end is typically connected to a blood vessel with a piece of heparinized PE tubing. The other end of the element is connected through a 3-way stopcock to a syringe which is used for flushing the transducer and the lines.
- The BP-102 has a pinch valve on one end for flushing the device; the blood vessel tubing should be connected to the 3-way stopcock on the other end, To accurately report pressure, these transducers must be completely filled with fluid. Bubbles in the transducer or the lines will produce inaccurate results.

How to Use the BP-100/102

Equipment Setup

1. Plug the connector of a DIN8 extension cable into one of the DIN8 transducer inputs of an iWorx data acquisition unit or amplifier. Plug the DIN8 connector of the BP-100/102 into the DIN8 extension cable.
2. Attach the cannula from the subject's artery to either end of the BP-100 or to the 3-way stopcock on the BP-102. The cannula, stopcock, and transducer should be filled with heparinized saline solution to prevent clotting.

Start the Software

When using an iWorx data acquisition system with its own DIN8 transducer inputs, or an iWorx amplifier like the ETH-256 or the ETH-401, coupled to an iWorx data acquisition device:

1. Open LabScribe by double-clicking on the desktop shortcut.
2. When the program opens, select **Preferences** from the **Edit** menu (or from the **LabScribe** menu on a Macintosh). Click on the **Channel** button. In the **Channel** preferences dialog, name the channel to which the BP-100/102, or the amplifier supporting the BP-100/102, is connected.
3. Set the **Mode/Function** for this channel to **DIN8**. Set the sampling rate and display time. Click **OK**.

Calibration

Manometer Calibration

1. Turn the valve of the 3-way stopcock so that the line going to the animal is closed.
2. Connect a manometer to the open port on the 3-way stopcock.
3. Check that the pressure on the manometer is 0 (zero) mmHg. Start recording.
4. Pressurize the manometer to 100 mmHg and adjust the range displayed on the data acquisition device so that the change in the signal from 0 to 100 mmHg can be seen easily.
5. Mark the record to indicate 100mmHg.
6. Bleed pressure from the manometer until it drops back to 50mmHg. Mark the record again.
7. Stop recording the signal.
8. Position the section of the recording that contains the step from 100mmHg to 50mmHg in the center of the screen.
9. Position one of the cursors in the **Main window** in the 100mmHg section of the recording.
10. Position the second cursor in the 50mmHg section.
11. Right-click on the recording window of the channel to which the BP-100/102 is connected.
12. Select **Units**.from the right-click menu of the recording channel.
13. Select **2 point calibration** from the **Units Conversion** dialog. Notice that the voltages from the positions of the cursors are automatically entered into the left side data boxes.
14. Enter the two pressures, 50 and 100, used in the calibration recording in the corresponding boxes on the right side of the conversion equations.
15. Enter the name of the units, **mmHg**, in the box below the pressures.

16. Put a check mark in the box next to **Apply Units to all blocks**.
17. Click on the **OK** button in the lower right corner of the window to activate the units conversion.

Care

- After using the BP-100 transducer, flush out the element using a syringe that is attached to the transducer with a 3-way stopcock and filled with distilled water. Open the 3-way stopcock on the transducer to the air and allow all the parts of the element to dry. The BP-102 includes an integrated 3cc flush device and stopcock. Open the pinch valve at the far end while flushing water through the transducer.
- During testing or use, do not apply too much pressure to the sensor. Pressures of 500 mmHg or more can fracture the plastic enclosure and cause leaks.
- The removable caps supplied with the transducer element are only dust covers; these caps are not airtight.
- These transducers are nonlinear at pressures less than 7mmHg.

Technical Data and Specifications

SPECIFICATIONS

Operating Pressure	+0.50 to +300 mmHg
Over Pressure	-500 to +100 mmHg
Sensitivity	5 µV/V/mmHg at X1 Gain
Excitation Voltage	+5
Temperature Effect	+0.25 mmHg/oC
Impedance	<900 W

Animal Preparation and Methods for Recording

An overnight fasted (minimum period of 8–10 h) rat is used in the experiment. The animal is anesthetized with urethane (1200 mg/kg)/ketamine (80 mg/kg, i.p.) and xylazine (16 mg/ kg, i.p.) or pentobarbital sodium (60 mg/kg, i.p.).[4,5] The reflexes of the animal are checked, and it is placed on a suitable rodent surgical table or a flat movable surface. The surface must not be electrically conductive, and it is helpful to record the electrocardiogram of the animal. The skin on the ventral side of the neck, right hind leg, and chest is carefully shaved and disinfected.

Procedure for cannulation of the femoral vein

1. The femoral vein is cannulated for administration of saline as well as the drug to be tested.
2. Make a small incision(1–2 cm) in the epidermis (outer layer of the skin) of the right thigh, and carefully clean the matrix of collagen fibers interlaced with elastic fibers of the dermis.

   Deep in the dermis layer, the blood vessel (right femoral vein), and the nerve fiber are visible.
3. Differentiate the femoral vein from the nerve fiber, and the rodent femoral vein catheter or rat femoral vein cannula (PE catheter fabricated with 26 G × 1/2″ needle) is introduced for drug administration.

4. After cannulation, flush the cannulation line with normal or heparinized saline (0.1 ml) to prevent thrombosis.

NOTE: There are disadvantages to cannulating the femoral vein since there is a likelihood of clots and often the catheter comes out of the vein if it is not immobilized using adhesive tape.

Procedure for cannulation of the jugular vein

1. After making a small incision in the neck, locate the thick veins bilaterally in the incised region. These are visible just below the dermis, which can be separated from the underlying tissues using artery forceps or curved, blunt forceps.
2. Once a vein is isolated, tie the upper part (the part closer to the brain) with thread.
3. Make a small cut on the vein to insert a catheter up to 1″ towards the heart and tie the vein with thread along with the catheter.

Although cannulation of the jugular vein can be cumbersome, it is the best way of administering drugs.

NOTE: Jugular vein cannulation is not preferred for drug administration because tracheostomy and carotid cannulation are sometimes inconvenient to perform. After cannulation, avoid moving the leg. The cannula may be fastened to the surgical surface to avoid frequent movement. A small amount of any adhesive substance may be applied at the cannulation site to avoid removal of the cannula from the leg when injecting any investigational substance.

Helpful Hints

- The right leg is preferred for femoral vein cannulation because the left leg is used to as a reference electrode if recording an electrocardiogram.
- A shorter femoral cannula (10–15 cm) may be used for drug administration.

Procedure for the tracheostomy

1. Make a small incision (1.5–2 cm) in the neck of the rat for tracheostomy and carotid artery cannulation.
2. Carefully cut open the skin in the neck region and make a slit incision in the rat platysma muscles.
3. Avoid removal of any organs such as: the larynx, hyoid bone, thyroid cartilage, thyroid gland, and cricoid cartilage or muscles located in the neck region.
4. Once the trachea is identified, make a small incision on the cartilage tissue, and perform a tracheostomy using a small piece of pediatric Ryle's tube or rodent tracheal intubation tube.

NOTE: The pediatric Ryle's tube may be cut into small pieces (length 3–4 cm) and used for the tracheostomy. The tubing should be thick ex: an empty ball pen refill. After the tracheostomy, inject 0.1 ml of (0.5 IU/ml) heparinized saline intravenously through the femoral vein.

Continuous monitoring is essential to control the bronchial secretions. The secretions may be discharged slowly using a small PE tube without touching the wall of the trachea.

Procedure for cannulation of the carotid artery

1. Identify the carotid artery (red in color) along with the vagus nerve (white in color) on either side of the trachea.
2. Separate one side of the carotid artery, along with the vagus nerve, from the adjacent connective tissue.
3. Carefully clean the area without stimulating the vagus nerve.
4. Separate the blood vessel from the vagus nerve using a small needle, tie the cephalic end of the blood vessel and clamp the cardiac end with a bulldog clamp for cannulation.
5. Cannulate the blood vessel using a cannula pre-filled with heparinized normal saline (0.5 IU/ml).
6. Connect the other end of the cannula to a three-way stopcock/saline filled tuberculin syringe.
7. Tie the carotid artery cannulation site with a thread without obstructing the blood flow in the carotid cannula.
8. After cannulation, slowly release the bulldog clamp at the cardiac end of the blood vessel, ensuring that there is no bleeding at the cannulation site.
9. If there is no bleeding, remove the bulldog clamp. If there is any bleeding at the cannulation site, clamp the carotid end again to stop the bleeding.
10. An adhesive substance may be used at the cannulation site to avoid damaging the blood vessel and avoid expulsion of the cannula due and to ensure the free transmission of pressure in the carotid artery.

NOTE: Stimulation of the vagus nerve decreases the heart rate and increases the risk of various respiratory abnormalities, including respiratory arrest and may affect the subsequent part of the experimental procedure. If the cephalic end is not tied, the pressure is divided between the brain and carotid cannula, so that the actual BP is not transmitted to the pressure transducer. The three-way stopcock is connected to the pressure transducer and a syringe filled with heparinized saline. The heparinized saline helps apply a positive pressure and maintain it at the baseline value. Usually, the three-way stopcock works as a bridge connecting the carotid cannula and the pressure transducer. During the experiment, the positive pressure is to be blocked to avoid transmission of BP to the syringe.