

Measurement of invasive blood pressure in rats

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ABSTRACT

Blood pressure (BP) is one of the vital parameters used to assess the cardiovascular functions of a mammal. BP is commonly recorded using invasive, noninvasive, and radio telemetry methods, but invasive blood pressure (IBP) recording is considered the gold standard. IBP provides a direct indication of the effect of the investigational products on the circulatory system. Recording the IBP in rodents is an essential part of the preliminary screening of any product to determine its effect on the cardiovascular system. The present article describes the measurement of the IBP in Wistar rats/Sprague Dawley rats.

Key words: Carotid artery, invasive blood pressure, tracheostomy

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: (i) tail cuff plethysmography (noninvasive), (ii) intra-arterial catheters (invasive), and (iii) radio telemetry. Intra-arterial catheters yield the most precise values, and surgery is required to use them.^[1] Most of our physiological and pharmacological knowledge related to BP, and its regulation has been derived from acutely prepared, anesthetized, or immobilized laboratory animals.^[2] 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 cannula (saline-filled catheter).^[3]

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 BP measurements is urethane or pentobarbitone.^[4] The rat BP model is more suitable since the use of dogs and cats is restricted in India by the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

MATERIALS AND METHODS

The requirements include an adult Wistar rats/Sprague Dawley rats, heparin, urethane/ketamine + xylazine/pentobarbital sodium, an intravenous cannula, a 1 ml tuberculin syringe, 5 and 10 ml syringes, small (3") and medium (5") pairs of scissors, artery forceps (5"), small and medium forceps (with teeth, blunt and pointed), a bulldog clamp [Figure 1a], Adson dissecting forceps (toothed and non-toothed) (5"), 18 G needles, a surgical table, respiratory tubing (6" infant feeding tube/ pediatric Ryle's tube may be used) [Figure 1b], normal saline, a surgical lamp, an insertion needle, a surgical blade, a thread, and adhesive tape.

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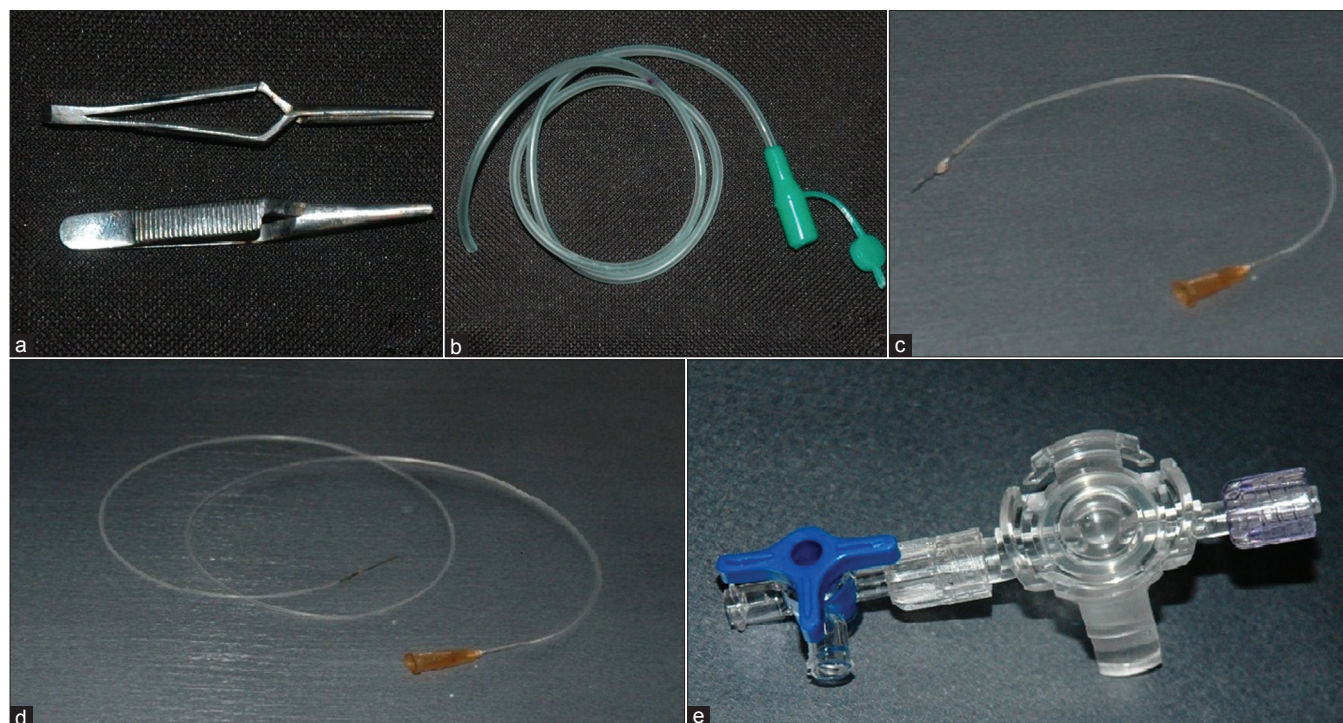


Figure 1: The materials used for recording the rat blood pressure. (a) bulldog clamp; (b) 6" infant feeding tube [part of it used as respiratory tubing] for tracheostomy; (c) PE tube [15–20 cm length] with a 26 G \times 1/2" needle for femoral vein cannulation; (d) PE tube [15–20 cm length] with a 26 G \times 1/2" needle for carotid artery cannulation; and (e) disposable clip-on BP domes

Drug solutions (stock) 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), heparin (5 IU/ml), and 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.

Intravenous cannula

The cannula consists of sterile polyethylene (PE) tubing with an internal diameter (ID) of 0.5 mm and an outer diameter (OD) of 0.9 mm (AD Instruments, Australia, catalog no. SP0109) provided with a 26 G \times 1/2" needle [Figures 1c and d].

Instruments

The instruments required include a sphygmomanometer and a student physiograph/data acquisition system with a pressure transducer.

Student physiograph

This is used to record bio-electrical potentials, e.g. those associated with BP, respiration, and electrocardiogram, in mammals. The major components of the instrument include a main console, couplers (biopotential ECG, and pulse-respiration), transducers (pressure, pulse and force), a chart

drive, a writing pen, and electrodes.

Data acquisition system

This device acquires external analog signals and amplifies them and converts them into digital numerical values. The major components of this instrument (only for IBP experiments) include an analog input unit, a pressure transducer with disposable clip-on BP domes (MLT844 physiological pressure transducer with clip-on BP domes, AD instruments, Australia) [Figure 1e], bipolar electrocardiogram leads, and a spirometer with a suitable recording device (computer).

Animals

Healthy adult male Wistar rats/Sprague Dawley rats (body weight 150–200 g) are preferred for the experiment. The experimental protocol and the maintenance of the experimental animals should be in accordance with the regulations of the local government. In India, animal experiment protocols should be approved by the Institute Animals Ethics Committee (IAEC), and all animal experiments should be carried out in accordance with the guidelines of the CPCSEA, India.

Pressure transducer calibration

Calibration is one of the important steps in the experiment, and it will reflect in the experimental results. Calibration is performed using a known pressure levels with the help of a sphygmomanometer. The pressure cuff of the sphygmomanometer is removed and connected to the pressure

transducer of the student physiograph/data acquisition system. Then, the pressure transducer is checked by inflating to a known pressure level. The conversion factor for calculating BP is determined. In the case of the data acquisition system, the calibration between the voltage (millivolts) and the pressure is performed previously, and the results are automatically calculated based on the calibration value.

Methods

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/jugular vein

The femoral vein is cannulated for administration of saline as well as the drug to be tested. A small incision (1–2 cm) is made in the epidermis (outer layer of the skin) of the right thigh, and the matrix of collagen fibers interlaced with elastic fibers of the dermis is cleaned carefully. Deep in the dermis layer, the blood vessel (right femoral vein), and the nerve fiber are visible. The femoral vein is differentiated 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. After cannulation, the cannulation line is flushed with normal saline (0.1 ml) to prevent thrombosis.^[6-8] 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 [Figure 2].

Under experimental conditions, the jugular vein may also be cannulated and used for drug administration. After making a small incision in the neck, one can locate 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. Once a vein is isolated, the upper part (the part closer to the brain) should be tied with the thread. A small cut should be made on the vein to insert a catheter up to 1" towards the heart, and the vein should be tied with a thread along with the catheter.^[9] Although cannulation of the jugular vein is 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.

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

Procedure for tracheostomy

A small incision (1.5–2 cm) is made in the neck of the rat for tracheostomy and carotid artery cannulation. The skin in the neck region is carefully cut open, and a slit incision is made in the rat platysma muscles. Avoid removal of any organ such as the larynx, hyoid bone, thyroid cartilage, thyroid gland, and cricoid cartilage or muscles located in the neck region. The trachea is identified, small incision is made on the cartilage tissue, and the tracheostomy is performed using a small piece of pediatric Ryle's tube or rodent tracheal intubation tube [Figure 3].

Note:

- The pediatric Ryle's tube may be cut into small pieces (length 3–4 cm) and used for tracheostomy.
- For tracheostomy, the tubing should be thick. One can use an empty (used) ball pen refill.
- After tracheostomy 0.1 ml of (0.5 IU/ml) heparinized saline is injected 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 carotid artery

The carotid artery (red in color) is identified along with the vagus nerve (white in color) on either side of the trachea. One side of the carotid artery, along with the vagus nerve, is separated from the adjacent connective tissue and cleaned carefully without stimulating the vagus nerve. The blood vessel is separated from the vagus using a small needle, and the cephalic end of the blood vessel is tied and the cardiac end is clamped with a bulldog clamp for cannulation. The blood vessel is cannulated using a cannula pre-filled with heparinized normal saline (0.5 IU/ml). The other end of the cannula connected to a three-way stopcock/saline filled tuberculin syringe. Then the carotid artery cannulation site is tied with a thread without obstructing the blood flow in the carotid cannula. After cannulation, the bulldog clamp at the cardiac end of the blood vessel is released slowly, ensuring that there is no bleeding at the cannulation site. If there is no bleeding, the bulldog clamp is removed. If there is any bleeding at the cannulation site, the carotid end is clamped again to stop the bleeding. If possible, adhesive substance may be used at the cannulation site to avoid damaging the blood vessel and

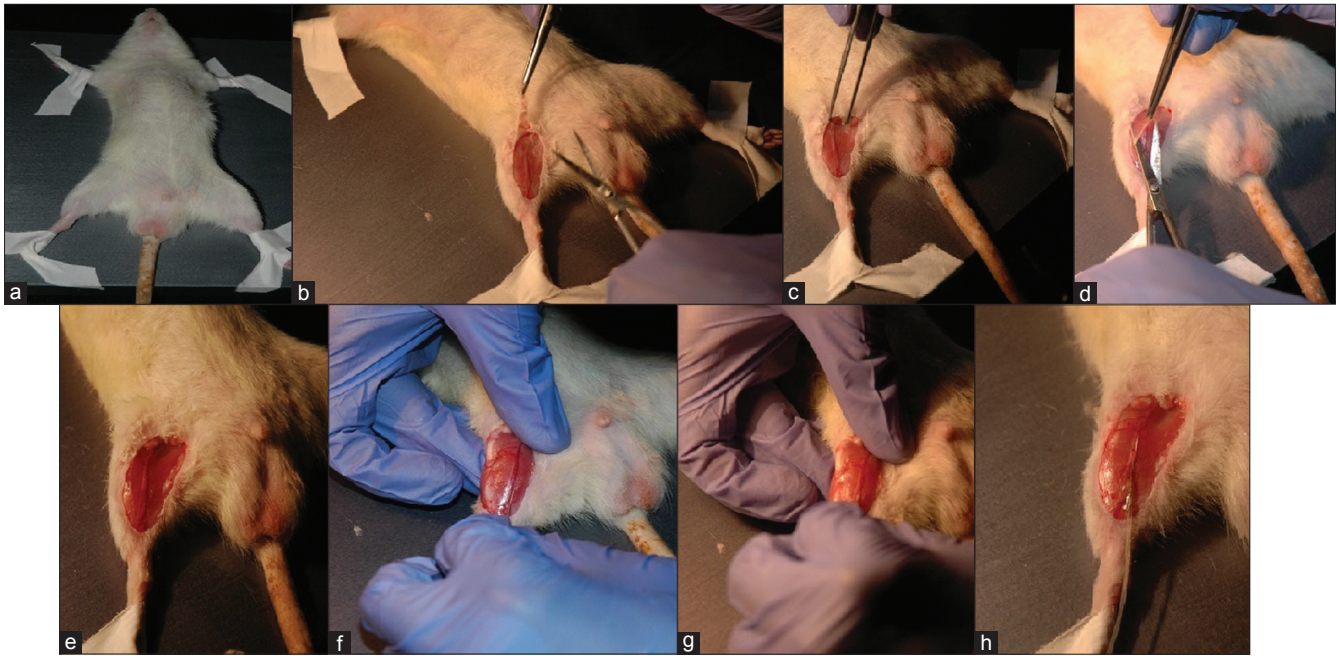


Figure 2: Cannulation of femoral vein. (a) experimental animal on the surgical platform; (b-e) a small incision (1–2 cm) is made in the epidermis of the right thigh, and the matrix of collagen fibers interlaced with elastic fibers of the dermis is cleaned and femoral vein is identified; (f–h) femoral vein is cannulated using a femoral vein cannula

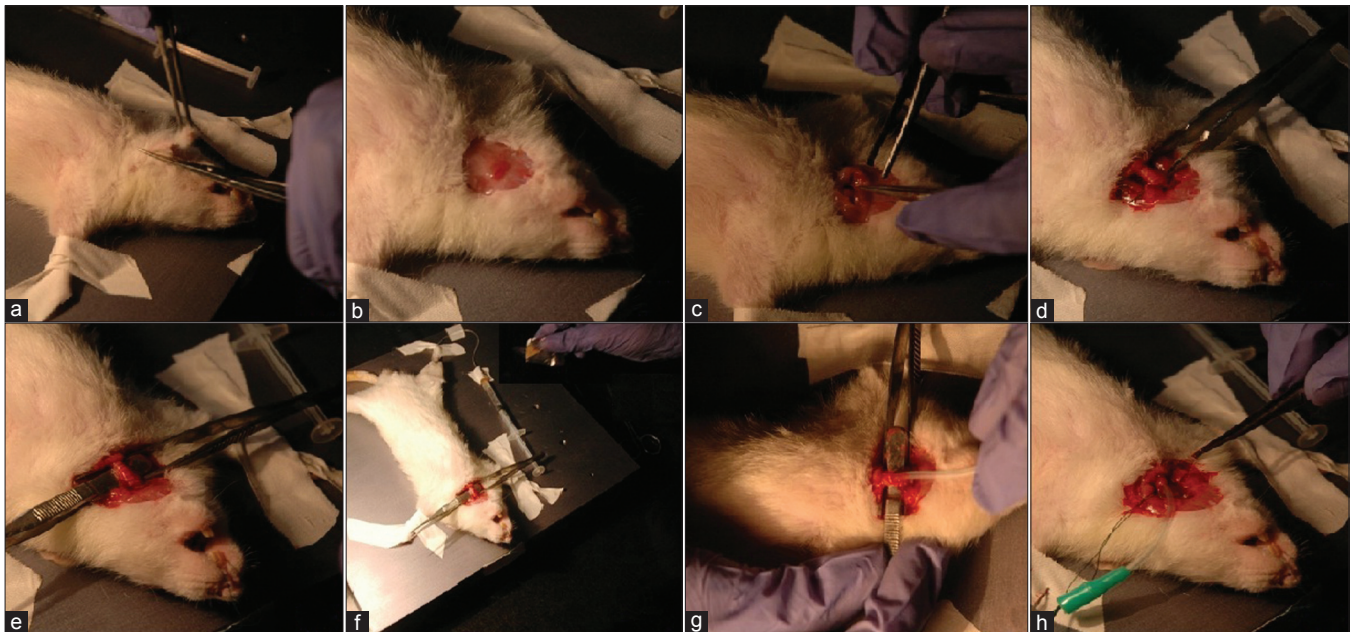


Figure 3: Procedure for tracheostomy. (a and b) a small incision (1.5–2 cm) is made in the neck of the rat for tracheostomy and carotid artery cannulation; (c) slit incision is made on platysma to identify the trachea; (d–f) trachea is identified and small incision made on the cartilage tissue; (g and h) tracheostomy is performed using a piece of pediatric Ryle's tube

expulsion of the cannula due to the BP and to ensure the free transmission of pressure in the carotid artery [Figure 4].^[6,10,11]

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.^[12,13]

- 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.

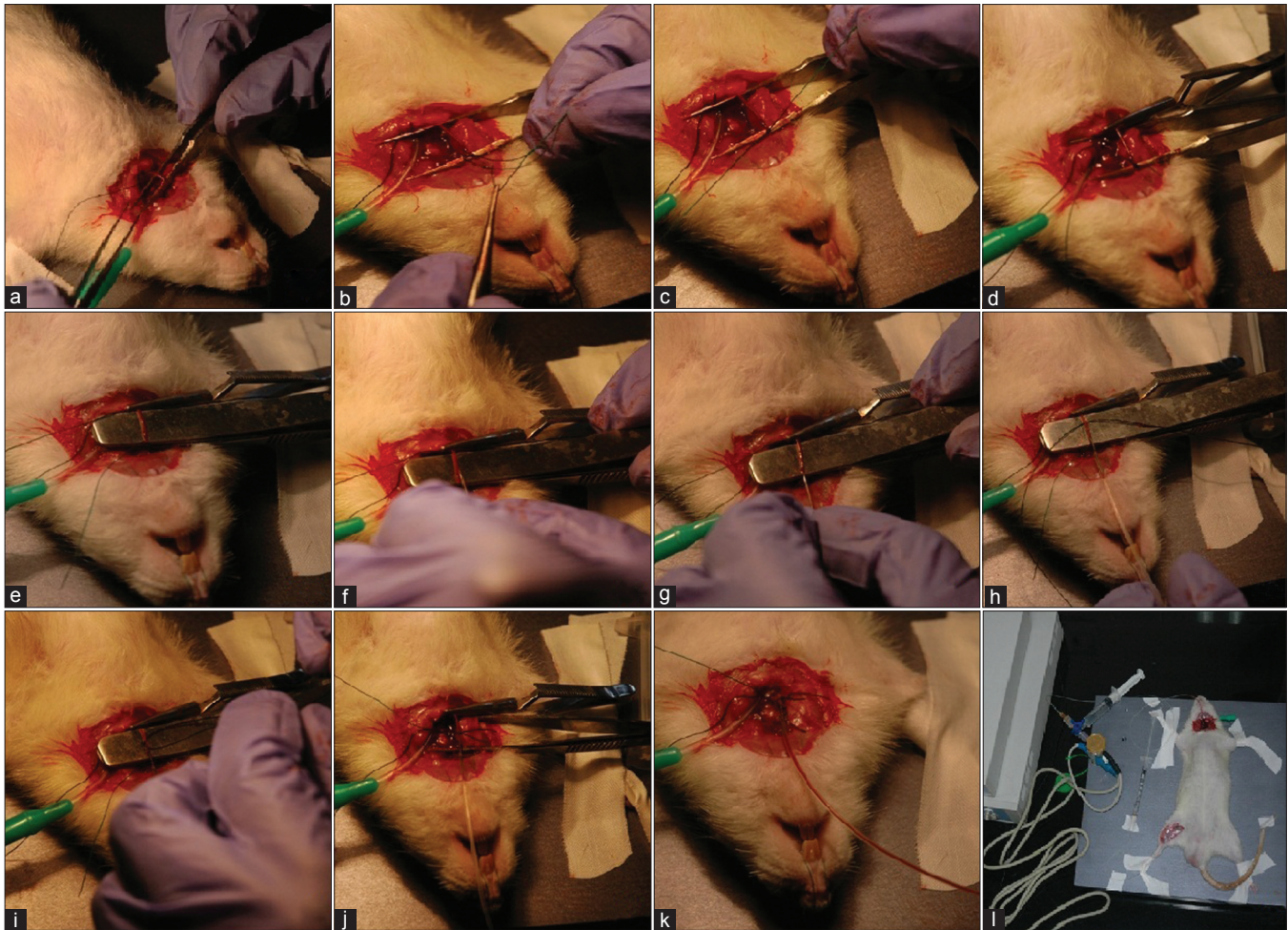


Figure 4: Cannulation of carotid artery. (a) right side of the carotid artery is separated from vagus nerve; (b and c) cephalic end of the blood vessel is tied to avoid the bifurcation of blood pressure; (d) cardiac end of the blood vessel is clamped with a bulldog clamp for cannulation; (e) small platform is placed in the canulation site; (f–i) carotid artery is cannulated and tied; (j, k) The support platform and bulldog clamp are removed and ensure there is no leakage in the cannulation site; and (l) the carotid cannula is connected to pressure transducer through disposable clip-on BP domes

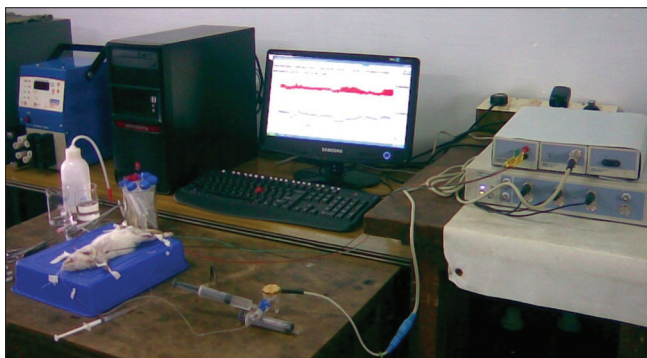


Figure 5: Complete rat blood pressure experimental setup

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

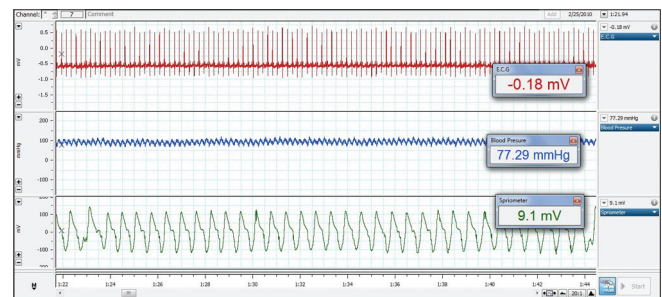


Figure 6: Baseline recording of the rat ECG (Red), blood pressure (blue), and respiratory rate (green)

BP to the syringe.

The pressure transducer unit of the student physiograph/data acquisition system converts BP into an electrical signal. Using suitable devices, the effects of the investigational product on the ECG and respiration may be recorded along with the BP.

Three-lead bipolar ECG is preferred in electrocardiography of animals. Positive, negative, and reference electrocardiogram electrodes are placed at the left fore leg, right fore leg, and left thigh, respectively, to record electrocardiogram. The spirometer is connected to cannula in the trachea to monitor the respiration rate.^[6,14,15]

Note:

- The location of the electrode may be changed if an animal has dextrocardia.
- The length of the spirometer tube may be reduced to avoid the dead space of the respiratory air.
- The cannulation site may be closed with wet cotton to avoid drying of the surgical site.
- The length of the carotid cannula may be greater (25–30 cm) to avoid contact with blood in the pressure transducer unit.

Recording blood pressure

After cannulation, the animal is connected to the student physiograph/data acquisition system to record the BP, ECG, and respiratory rate. The whole setup [Figure 5] is allowed to stabilize for 10–20 min, during this time the animal is also monitored for any kind of bleeding. If there is bleeding, it should be controlled and the cause of the problem should be addressed. If the problem is not identified, there will be a loss of BP which will give false negative results. The animal may die due to continuous bleeding.

Baseline recording [Figure 6] may be carried out for 10–15 min to ensure the stability of the preparation. Sympathomimetic, sympatholytic, dopaminergic, and histaminergic agents are administered in the desired dose of 0.1 ml, after which 0.1 ml of normal saline is injected through the femoral vein to complete the drug administration.

The effect and duration of the action of the drug are observed. After administration of the drug, the BP of the animal is allowed to return to the normal baseline value. Otherwise the responses to the other drugs may interfere with the result.

DISCUSSION

The IBP is quantifiable and accurate. Individual variations may be observed, but the action is similar for a given compound. The effect of the investigational product is compared with that of a standard drug, and the pharmacological class of the investigational product is inferred. The compound's action on the autonomic nervous system (drugs/investigational products acting on the cholinergic and sympathetic nervous system) may be monitored using the IBP of the rat to determine the pharmacological action and the mechanism of the action.

Points to be remembered

- The animal should be completely anesthetized.
- Experimental skill is required to cannulate small blood vessels.
- Do not panic when bleeding occurs at a cannulated site.
- Do not damage the carotid artery when separating it from the vagus.
- Tracheal cannulation should be monitored continually. Sometimes the bronchial secretion is increased due to reflex.
- Maintain the body temperature of the animal at the desired value to avoid temperature effects on BP.
- When terminating the experiment, ensure the death of the animal. The animal may be killed using a recommended anesthetic agent.

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