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# Isolated hind limb preparation to study the peripheral vascular resistance

**NEETU PRINCE**
Department of Physiology, CHRISTIAN MEDICAL COLLEGE

**Abstract :** A denervated, isolated rat hind limb model was used to study vascular resistance, one of the major determinants of blood pressure. The aim of this work was to assess the potential usefulness of this model to study vascular properties namely, arteriolar resistance, arterial compliance and impedance. Rat abdominal aorta was cannulated proximal to its bifurcation, pressure transducer was connected and hind limb vasculature was perfused at a constant flow rate with a peristaltic pump. Vascular resistance can be calculated as Mean pressureflow rate as flow is set by the experimenter and pressure is measured. If flow is kept constant, vascular resistance and compliance are reflected by changes in mean arterial pressure and pulse pressure respectively. Norepinephrine increased mean pressure, but not pulse pressure therefore it increased resistance, but did not change compliance. High potassium increased mean pressure and pulse pressure Therefore it increases resistance, as well as decreases compliance. The ease of this technique makes this preparation an ideal tool for research and teaching. In conclusion, this will aid in unravelling mechanisms by which blood vessels helps in regulating blood pressure.

**Keyword :**Hind limb preparation, vascular resistance, arterial pressure, impedance, compliance, CMC daq, Lab Chart Reader.

**BACKGROUND:**

The interrelationship between blood pressure, cardiac output and peripheral resistance are well known concepts in physiology. Blood pressure refers to the pressure exerted by the blood column on the walls of the arteries. The term “Blood pressure” always refers to the arterial pressure. Arterial pressure depends on the compliance of the arteries, inflow to the arteries (cardiac output) and the outflow from the arteries which depends on the arteriolar diameter (arteriolar resistance or total peripheral resistance). The relationship of blood pressure to cardiac output and total peripheral resistance can be described by a mathematical equation: Mean arterial pressure (MAP)= Cardiac output (CO) x Total peripheral resistance (TPR)

Short term regulation of blood pressure mainly happens through autonomic reflexes by altering cardiac output and total peripheral resistance. It is a known fact that cardiac output is regulated by intrinsic mechanisms of the heart which modulate stroke volume and heart rate. Peripheral resistance is by virtue of the resistance vessels. It is important to understand that TPR can be *calculated from* MAP and CO, but it is *not determined by* either of these variables. A more precise way to understand this relationship is that at a given CO, if the MAP is very high, it is due to high vascular resistance. Main mechanism causing alterations in systemic vascular resistance involve changes in vessel diameter, especially at the arteriolar level. The Poiseuille relationship shows that resistance is inversely related to the fourth power of vessel radius. Due to this, changes in arteriolar diameter can cause a resultant increase or decrease in peripheral resistance at a larger magnitude. Vasoactive molecules (NO, endothelins), intrinsic myogenic mechanisms and tissue factors (histamine, pH) are some factors which modify vascular tone to adjust arterial pressure and maintain local blood flow. Sympathetic nerve fibres supplying vascular smooth muscle release norepinephrine and epinephrine which act on alpha and beta receptors to cause either vasoconstriction or vasodilation. It is understood that regulation of systemic vascular resistance is crucial to maintain normal mean arterial pressure. So much so that therpeutic effect of many antihypertensives is based primarily on the reduction of total peripheral resistance.

**Isolated vessel model**

Blood vessels are generally studied in isolation to define their mechanical and biological properties under controlled conditions (1),(2), (3), (4). Isolated vessel models help to differentiate between venous and arteriolar effect of different classes of drugs. A drawback with this model is the slow reaction of vessel to drugs. Also it being an *in vitro* model, it does not successfully mimic the normal physiological environment. An important consideration while using this preparation is that it does not offer any insight into the dynamic or transient response exhibited by the tissue (5).Moreover, all preparations differ from each other depending on the extent to which the tissue was damaged during isolation, causing the pharmacodynamics to vary each time (6), (7).

**Isolated hind limb model**

Isolated hind limb preparation model has been used to study various cardiovascular parameters and other physiological aspects (8), (9), (10),(11),(12), (13). Isolated hindlimb preparation used in this study has several advantages over the isolated vessel model and *in vivo* animal model. It allows us to study the effect of drugs and toxins on the target organ itself ie. the peripheral vasculature without overwhelming effect of

autonomic reflexes as seen in whole animal model. Also dose dependent effect as well as multiple drug effect may be studied in a single experiment conveniently. The exact replication of normal circulatory circuit (large artery- medium artery-small artery-arteriole-capillaries-venules-veins) and minimal structural deformation (when compared to isolated vessel preparation) prevent interference with vessel properties and give us an insight about the actual role of blood vessels in regulating pressure variations. Since flow is regulated and may even be kept constant, pressure changes are easily measured and mean arterial pressure serve as a surrogate for change in peripheral resistance. Therefore calculation of resistance and observation of transient response is made easier during normal recording as well as following intervention. This preparation may serve as a valuable research and study tool for both undergraduate and post graduate teaching in order to further knowledge regarding physiological concepts.

**OBJECTIVE:**

The aim of our study was to assess vascular resistance using isolated hindlimb preparation.

**METHODOLOGY:**

Albino Wistar rats weighing between 200gms to 300gms were used in this study. In the current study, the hind limbs of rats used for other experiments were used after obtaining the clearance from IAEC. An independent experiment involving isolation of the rat heart was carried out after anaesthetizing the animal (intraperitoneal ketamine, 100mg/kg body weight), following which it was handed over. The skin over the abdomen was wiped with distilled water. A vertical incision extending from xiphisternum to pubic area was made to expose the abdominal cavity. Skin and muscle layer were divided and retracted laterally. Bowel was removed from the abdominal cavity for proper visualization of the vessels. To remove the bowel without spillage of fecal matter, ligatures were applied at the proximal and the distal ends of the bowel. Abdominal aorta and the inferior vena cava were identified anterior to lower part of the vertebral column. Perivascular fat and connective tissues surrounding the vessels were separated to free the vessels. A spatula was placed under the chosen aortic segment (distal to origin of renal artery and proximal to the bifurcation of abdominal aorta). Two ligatures were placed, one cranially and other caudally. Cranial ligature was applied as far cranially as possible to get an enough length of the aortic segment to repeat the cannulation, if it fails.

The caudal ligature was tied after cannulation to fix the cannula. Inferior venacava was ligated to avoid spillage of blood during transection of the trunk. This ligature was removed following the cannulation of aorta. Another set of ligatures was applied to both the vessels above the cranial ligatures (above the transection plane) to avoid bleed from upper half. Once the vessels were ligated, trunk was transected above the cranial ligatures and the upper half of the body was separated. Heparinized 24 G cannula was inserted gently into the aorta and the needle withdrawn. The caudal ligature was tied to fix the cannula. The preparation was gently flushed with heparinized ringer from a 20 ml syringe attached to the cannula, until the effluent was clear. Cannula was then connected to the flow line from the peristaltic pump. Pressure transducer was connected to the flow line. Preparation was perfused with ringer solution and allowed to equilibrate. After baseline values had been obtained, interventions in the form of norepinephrine 10 micromolar was given followed by a wash with ringer. Change in pressure were also noted using high potassium ringer (80Mm). These interventions also served to demonstrate the viability of vascular system. Pressure transducer was purchased from the pharmacy (iPeX Pressure Monitoring Kit). Flow rate was adjusted using peristaltic pump and set at 2.5ml/min.

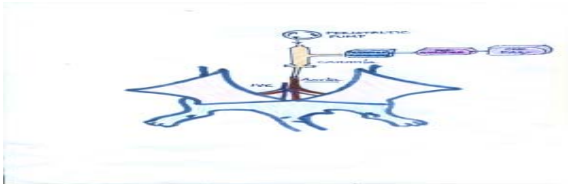
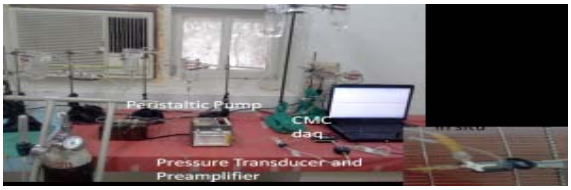


**Figure 1:Isolated hindlimb preparation set up.**




| Chemical compound | Concentration (mmol/L) |
|---|---|
| NaCl | 135 |
| KCl | 5.4 |
| $NaH_2PO_4$ | 0.4 |
| $MgCl_2$ | 3 |
| $CaCl_2$ | 1 |
| HEPES | 10 |
| Glucose | 10 |

**Table 1: Composition of ringer solution**

**CMCdaq Set up :**

Calibration of pressure transducer and amplifier: A given combination of transducer and amplifier were calibrated using known pressures and the values were entered in CMC daq software in the computer that was used for acquisition. A Y tube is connected to the pressure transducer, such that the stem of the Y tube is connected to the bulb and cuff of BP apparatus, one end to the dial of the BP apparatus and the other end to the pressure transducer. Ensure the pressure dial is 0 and press the offset button on the preamplifier so that the recording is 0 upto three decimal values. Inflate the cuff to a fixed pressure, say 10 mmHg. Maintain the pressure at 100 mm Hg and note the amplitude in the recording. Inflate the cuff again to 20 mmHg and note the amplitude. Now calculate the pressure rise of the unit increase in voltage. For example, if values at 0, 10 and 20 mmHg were 0, 71 and 112 millivolts respectively then,

| | | |
|---|---|---|
| 1 | 0mmHg | 0v |
| 2 | 50mmHg | .183v |
| 3 | 100mmHg | 369v |

Difference between row 1 and row 2: 50 mmHg = .183V
Difference between row 3 and row 2: 50 mmHg = .186 V
So the mean value is .184 V for 50mmHg.
The rise in pressure for 1V will be 50/0.184=271.73 mmHg. Since the voltage range of DAQ is 1.5V, the maximum pressure that can be measured within the system is 271.73 * 1.5= 407mm Hg. CMC daq pressure transducer with pre-amplifier was connected to a computer through the CMC daq data acquisition system. The analog data was acquired and stored in the computer using the above system. Data was analysed with both CMC daq as well as Lab Chart Reader software. Sampling rate for recording was kept at 1000Hz and the filters were set between 0-500Hz (high pass and low pass filters). Arterial pressure changes were continously recorded during the experiment and mean pressures were calculated. Resistance value was derived from the mean pressure and flow rate.

**RESULTS:**

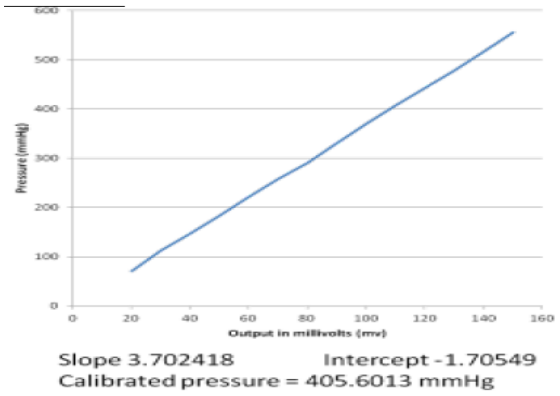



**Figure 2: Pressure calibration graph (x axis- output in mv, y axis- pressures in mmHg)**

Norepinephrine showed considerable increase in mean arterial pressure and a slight increase in pulse pressure when compared to baseline pressures (normal ringer).

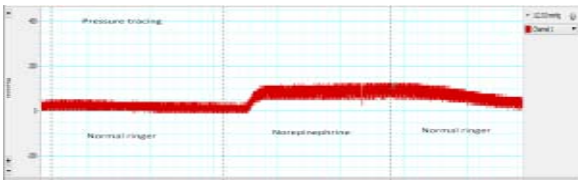


**Fig 3: Representative pressure tracing with norepinephrine (10 micromolar), Lab chart reader software.**

High potassium increased mean arterial pressure and pulse pressure considerably.

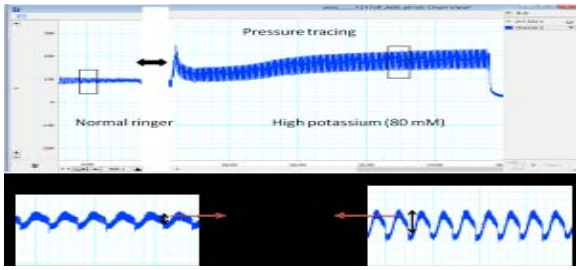



**Fig 4: Representative pressure tracing with high potassium (80mM), Lab chart reader software.**

**DISCUSSION:**

In our study we used wistar rats to prepare and standardize isolated hind limb model in order to assess the effect of various drugs and similar interventions on hind limb vasculature. Flow is set by the experimenter, pressure is measured and therefore vascular resistance can be calculated from mean arterial pressure and flow as Mean pressure/flow rate. Flow, if set with a peristaltic pump is pulsatile and therefore it simulates the in vivo situation. Pulse pressure is dependant on compliance of the arteries and the mean arterial pressure is dependant on resistance of arterioles. Since norepinephrine increased mean arterial pressure, it can be said that it increased resistance by constricting arterioles. The slight increase in pulse pressure is due to increase in impedance as a result of increase in resistance. High potassium on the other hand increased mean arterial pressure and pulse pressure considerably. Since mean arterial pressure has increased, it is concluded that potassium constricts arterioles, thereby increasing peripheral resistance. Since pulse pressure also has increased, potassium has decreased compliance probably by constricting smooth muscles in arteries. These results demonstrate that this preparation will help to dissect the various component of the vascular system namely impedance, compliance and resistance. The ease of isolation and recording make this preparation ideal for postgraduate and undergraduate research and teaching. In conclusion, we recommend use of this preparation to unlock numerous questions related to vascular physiology.

**REFERENCES:**

1.Peiper U, Griebel L, Wende W. Activation of vascular smooth muscle of rat aorta by noradrenaline and depolarization: Two different mechanisms. Pflüg Arch. 1971 Mar 1;330(1):74–89.

2.Bolton TB, Lang RJ, Takewaki T. Mechanisms of action of noradrenaline and carbachol on smooth muscle of guinea-pig anterior mesenteric artery. J Physiol. 1984 Jun;351:549–72.

3.Jerius H BC, Beall A, Karolyi D, Brophy C. Vascular smooth muscle mechanics in isolated perfused segments of carotid arteries. Surg 2000 1272148-54. 2000;

4.Marra SP, Kennedy FE, Kinkaid JN, Fillinger MF. Elastic and rupture properties of porcine aortic tissue measured using inflation testing. Cardiovasc Eng 20066125-33. 2006;

5. M. Rosic SP, M. Colic1 , V. Ilic2 , Z. Tepic. Transport and biomechanical studies in the isolated blood vessel segment. J Serbian Soc Comput Mech Vol 5 No 2 2011 Pp 51-58. 2011;

6. Monson KL, Mathur V, Powell DA. Deformations and end effects in isolated blood vessel testing. J Biomech Eng. 2011 Jan;133(1):011005.

7. Angus JA, Wright CE. Techniques to study the pharmacodynamics of isolated large and small blood vessels. J Pharmacol Toxicol Methods. 2000 Oct;44(2): 395–407.

8. Cox RH, Bagshaw RJ. Reflex control of canine hindlimb resistance under different modes of perfusion. Am J Physiol. 1981 Dec;241(6):H789–94.

9. Hainsworth R, Karim F, McGregor KH, Wood LM. Hind-limb vascular-capacitance responses in anaesthetized dogs. J Physiol. 1983 Apr;337:417–28.

10. Reitan JA, Kien ND, Martucci RW, Thorup SJ, Dennis PJ. Development of a near anesthetic-free isolated canine hindlimb model. The effects of halothane and atropine sulfate on vascular resistance. J Pharmacol Methods. 1991 Nov;26 (3):223–32.

11. Wu ZY, Rivory LP, Roberts MS. Physiological pharmacokinetics of solutes in the isolated perfused rat hindlimb: characterization of the physiology with changing perfusate flow, protein content, and temperature using statistical moment analysis. J Pharmacokinet Biopharm. 1993 Dec;21(6):653–88.

12.Bonen A, Clark MG, Henriksen EJ. Experimental approaches in muscle metabolism: hindlimb perfusion and isolated muscle incubations. Am J Physiol. 1994 Jan; 266(1 Pt 1):E1–16.

13. Casquero-Dorado AC, Sánchez-Navarro A. The isolated hind limb preparation as a drug distribution model. Methods Find Exp Clin Pharmacol. 1996 Sep;18(7):453–8.