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Review Article

Langendorff's isolated perfused rat heart technique: a review

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ABSTRACT

The Langendorff's isolated perfused small mammalian heart represents the optimal compromise in the conflict between the quantity and quality of data that can be acquired from an experimental model versus its clinical relevance, especially in relation to ischemia-reperfusion injury. We analyzed the important recent, as well as standard older articles to highlight the importance of Langendorff's isolated heart technique using the rat as an experimental animal model. The retrograde perfusion of isolated rat heart preparation is the most commonly used technique in cardiovascular research experiments with many advantages. The longevity of this preparation is one of the main advantages. From the moment an *ex vivo* preparation is established, it starts to deteriorate and the rate will depend on number of factors such as the skill of the operator, the species of animal, age, initial heart rate, choice and composition of the perfusion fluid, flow rate, presence or absence of various drugs, preload pressure, and the temperature at which the studies are carried out. Recently, various techniques and variables measured have undergone modifications. This review article has attempted to address many of the issues, developments, and applications which will assist investigators to make the best possible use of this experimental model using the rat.

Keywords: Langendorff's technique, Isolated perfused heart, Retrograde perfusion, Ischemia-reperfusion injury, Mammalian heart

INTRODUCTION

The isolated perfused mammalian heart preparation was established in 1895 by Oscar Langendorff. It is one of the most common and popular experimental models in cardiovascular research in the field of Pharmacology, Physiology, and Toxicology. A murine (mouse/rat) isolated perfused heart preparation has been studied since decades in comparison with larger hearts. For a century, the Langendorff's isolated perfused heart techniques have undergone marked development and provided us with important and intricate data on various parameters affected by novel molecules on murine hearts.[1]

At physiological conditions, during a normal cardiac contraction in a mammalian heart, the blood stored in the left ventricle is ejected at a pressure of about 80-100 mm Hg into the aorta and then into the peripheral circulation. At the base of the aorta is an ostium (hole), which diverts oxygenated blood under pressure into the coronary arteries supplying the heart muscles. This physiological information is the mainstay and backbone of the Langendorff method. All the isolated perfused heart preparations that have been developed are based on adaptations of the method originally described by Langendorff in 1895.[1,2]

Myocardial ischemia-reperfusion injury is one of the most prominent topics of current research in the field of drug development. The repeated failure of potential therapeutic agents and interventions which can enhance the clinical outcome of patients demonstrates the importance of using experimental animal models in the field of the cardiovascular system. Use of small laboratory animals in experiments has been recommended due to speed of development and ease of handling. Therefore, researchers in recent years have made use of murine models rather than larger animal models for experimental myocardial ischemia and reperfusion injury.[3,4]

In this review, we have explored the basic components and advancements in Langendorff's isolated heart preparation with the use of rat as an experimental model.

HISTORY

The historical method was first developed on the basis of the isolated perfused frog heart and was established by Elias Cyon in 1866, and further developed in 1895 by Langendorff. The model established by Langendorff was commonly used for research, and since then very few isolated organ experimental models have been used as extensively as the isolated heart preparation. Currently, it has become the mainstay of models for physiological, pathological, and pharmacological research. Today, it is used commonly to test the effect of different drugs on cardiovascular system including coronary vasculature, myocardial muscle contraction, and heart rate.[2]

The Langendorff's system allows the examination of cardiac inotropic, chronotropic, and vascular effects on the heart without the complications on an intact animal model. A major problem related to the confounding effects of other organs is avoided including physiological or structural factors such as the systemic circulation and a host of peripheral circulatory neurohormonal factors. These characteristics were considered as an investigational and confounding free advantage of the system. Today, this vital technique is extensively used to investigate the heart and circulatory system by a variety of cardiovascular researchers. It includes the study of the effect of single gene alteration on heart physiology, to novel therapeutic means to protect the heart from ischemia and other insults.[2,5,6]

METHOD OF LANGENDORFF'S ISOLATED HEART TECHNIQUE USING RAT AS A ANIMAL --MODEL

The basic principle of Langendorff's isolated heart perfusion still remains the same, and the current method is the adaptation of the original method described by Langendorrf in 1895. During a normal cardiac cycle, the stored blood from left ventricles is ejected into the aorta at a pressure of about 80-100 mm Hg, through the ostium (hole) present at the base of the aorta into the coronary arteries.

The perfusion of isolated heart is maintained through the use of a reservoir containing oxygenated perfusion fluid (Figure 1), with a pressure head that is connected via a tube to the cannula which is inserted and fixed in the ascending aorta, when the reservoir is opened, the perfusate is forced to flow into the aorta, this retrograde flow in the aorta closes the leaflets of aortic valve and prevents the perfusate from entering into the left ventricles, as a result, the entire perfusate solution enters the coronary arteries via the ostia at the base of aortic root.

After perfusing the entire ventricular mass of the heart, the perfusate exits the coronary venous circulation and drained in the right atrium via the coronary sinus. This perfusion is termed as a retrograde perfusion, in the sense that the perfusate flows down into the aorta rather than out of the left ventricle through the aorta, as blood does in situ, and during the entire procedure the ventricles remain empty.[2]

The perfusion solution is delivered in the retrograde direction down the aorta, either at a constant hydrostatic pressure or at a constant flow rate (with the use of a calibrated roller pump to maintain a preset flow of perfusate into the aortic cannula). Both systems, the constant hydrostatic pressure system and the constant flow system, do not normally permit the heart to generate pressure-volume work.

LANGENDORFF'S APPARATUS

The apparatus unit is a self-contained, comprehensive, and thermostatically insulated apparatus. It is available in different sizes specifically accommodating the perfusion of small or large animal hearts. Normally, most parts of the apparatus for Langendorff's heart perfusion are water-jacketed with warm circulating water to maintain the temperature of the perfusate and of the heart at 37°C. Perfusate reservoirs are constantly oxygenated and kept at a constant bath temperature of 37°C which is physiological for the heart. The solutions are pumped, which then pass through tubes around the pump to pressurize them at sufficient pressure. The pressurized perfusate fluids are warmed when they re-enter the bath unit in coiled tubes and feed into the heated junction block, from which the cannulated heart is attached. This provides an advantage of working *in vitro* suspension of heart in suitable warm water-jacketed organ bath, which maintains constant temperature and minimizes heat loss from the system.[7]

MODES OF PERFUSATE

Two modes of perfusate delivery can be applied depending on the requirements of the experiment, either at a constant hydrostatic pressure or at constant flow. A peristaltic pump which connects the perfusion reservoir and aortic cannulation are connected to a power source with a transducer which monitors the pressure. In studies using constant flow, the pump controller used to set the pump at the required speed, and the computer records the coronary flow and perfusion pressure. When constant pressure is used, the cannulation done under constant flow mode and after 1 min of cannulation, it can switch to constant pressure if required. Perfusion at constant pressure is given preference for studies of ischemia otherwise constant flow technique is preferred.[8,9] The expected flow rate differs and it depends on different species, and it ranges from 7 to 9 ml/min for rat. Switching between constant flow and constant pressure modes of perfusion is nowadays easily achieved by the use of commercially available STH pump controller systems. A further advantage of pump control system is the continuous measurement of coronary flow through calibration of the pump speed with known flow rate when studying coronary vascular tone/smooth

muscle/endothelial function. The normal value of systolic in situ perfusion pressure for rat hearts ranges from 70 to 90 mm Hg.[9-11]

PERFUSION SOLUTION

Different perfusion solutions are available for use which can be an asanguinous solution (Krebs Henseleit Buffer [KHB]), washed blood cells or whole blood. All media have to be aerated, oxygen is provided by gassing the perfusion fluid with high concentrations of oxygen, typically 95% O_2+5% CO_2. When bubbled with 95% O_2+5% CO_2, KHB gives a PH value of 7.4 at 37°C. KHB is composed of NaCl 118.5 mM, $NaHCO_3$ 25.0 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, glucose 11 mM, and a range of $CaCl_2$ 1.2-1.8 mM, which match with physiological calcium.[7] Many studies have demonstrated that using blood as a perfusion solution is more stable than the other isolated perfusion fluids. It causes less deterioration of left ventricular developed pressure (LVDP). Additional advantages are less edema of heart tissue since its protein content help to alleviate unwanted edema, together with the development of excellent pressure. It is generally in the range 130-180 mm Hg when end-diastolic pressure (EDP) is in the range 4-8 mm Hg. However, blood has few disadvantages as a perfusion fluid as it can undergo progressive hemolysis during the perfusion and such perfusion solutions are more difficult and time-consuming to prepare.[8-11] Washed erythrocyte perfusion solutions are found to be similar to those for the blood perfused preparation which uses a support animal. It enhances the stability when compared to crystalloid perfused hearts, with pressure development remaining stable for extended periods of perfusion. Other advantages are similar to blood perfusion fluid.[9,10]

PERFUSION TEMPERATURE

Normally, the perfusion needs to be carried out at a constant temperature close to the body temperature of the species under study. For rats, it is 35.9-37.5°C. The temperature of the air surrounding the heart and the heat provided to the heart with the perfusate can alter the temperature of heart in the experiment. The thermostatically controlled water-jacketed system can provide the required temperature. The delivery line tubes and the heart chamber is surrounded by re-circulating warm water at the desired temperature to maintain the temperature.[8]

SETUP

Langendorff's original method maintained the isolated heart through the use of a reservoir containing oxygenated perfusion fluid, with a pressure head that was connected via a tube to the aortic cannula. The perfusate was forced through the ostia into the coronary bed when the reservoir was opened. This retrograde perfusion flows down into the aorta rather than out the left ventricle through the aorta, as blood does in the living body. The perfusion solution is displaced via the ostia into the coronary arteries. After perfusing the entire ventricular mass of the heart, the perfusate leaves the coronary venous circulation via the coronary sinus and out of the open right atrium. The perfusion solution is delivered at a constant hydrostatic pressure or at a constant flow rate. Both systems, the constant hydrostatic pressure system and the constant flow system, do not normally permit the heart to generate pressure-volume work.[22] In both cases, this retrograde flow shuts the leaflets of the aortic valve so that the perfusion solution cannot enter the left ventricle. As mentioned above, most parts of the apparatus for Langendorff's heart perfusion are water-jacketed with warm circulating water to maintain the temperature of the perfusate and of the heart at constant temperature of 37°C. Figure 1 illustrates the various components of modern Langendorff's apparatus set up. Using this method recordings of many parameters are of high reproducibility and relatively low costs is possible. The accuracy of the measurements has been improved over decades with the introduction of computers and software. At present, this method is a very useful tool in translational cardiovascular and pharmacological research.[2,4,5,8]

EXPERIMENTAL ANIMALS

The Langendorff's heart preparation is used with all mammalian species, especially large animal hearts such as pigs, monkeys, sheep, dogs, and even man have been reported. These are not very commonly used due to on account of the high cost, greater physiological variability, the requirement of large volumes of perfusion fluids and cumbersome equipment and set up required. One of the most frequently studied and the best characterized isolated small heart model is that of the rat. Male species of the rat are more recommended and commonly used than females.[13] In order to avoid the effect of sex hormones during a different interval of female cycle.

ANESTHESIA

No anesthetic is ideal for Langendorff's technique. Each anesthetic has its advantages and disadvantages. The effects can vary from species to species. Removal and isolation of the heart require the donor animal to be rendered unconscious prior to excision. Different routes can be used including inhalation and injection. An alternative to anesthesia is cervical dislocation or concussion, but in both instances, there are major effects on catecholamine's and other circulating factors, so these are not recommended.[8-10,14,15]

Procedure

After surgical anesthesia, careful excision of aorta and cannulation are the first steps done. A set of sutures and instruments positioned close at hand and rat is placed in a

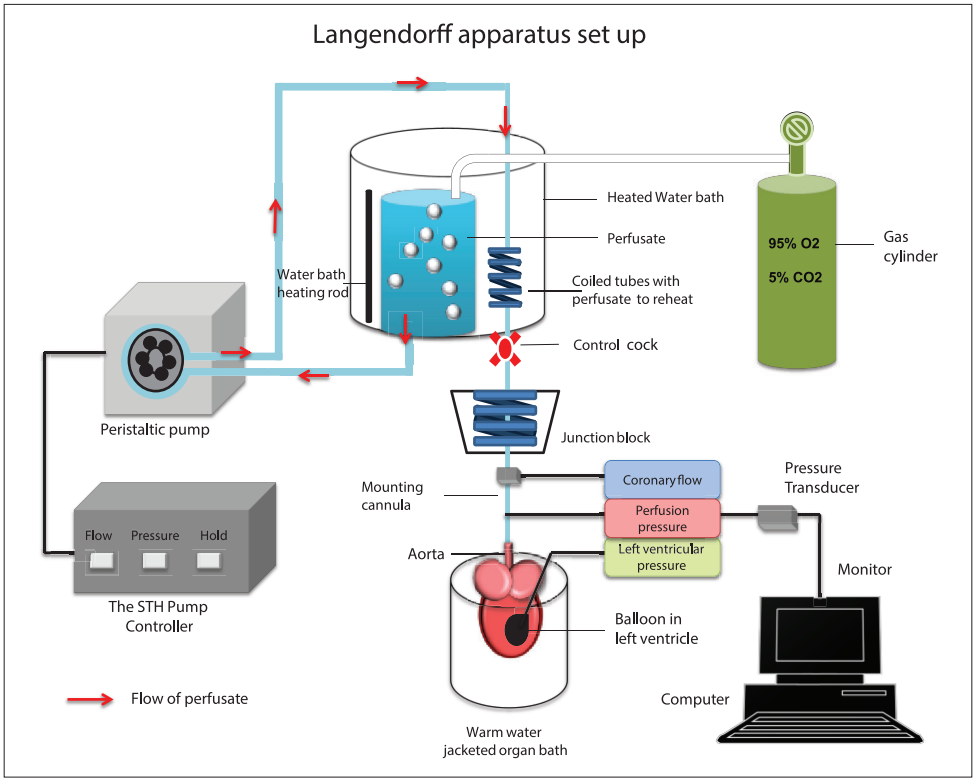

Figure 1: Langendorff apparatus set up

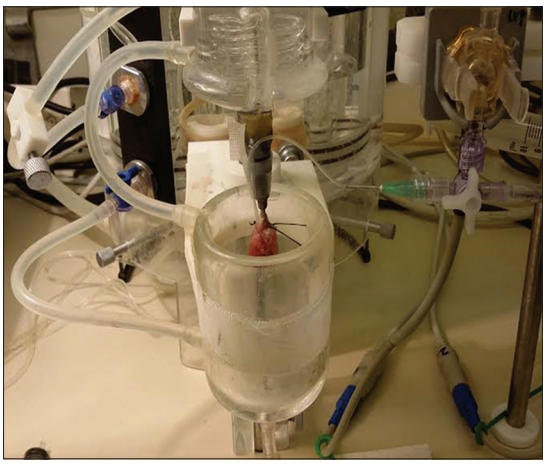

Figure 2: Real time preparation

dissecting tray near the isolated heart apparatus. Thoracic cavity is exposed after taking incision on diaphragm through the transabdominal incision. Using the blunt end of a pair of blunt-sharp pointed scissors the thorax is opened, by a bilateral incision along the lower margin of the last to first ribs (bilateral sternotomy). Few investigators support the heart between their fingers gently and then lift the heart slightly before incising across the arch of the aorta, vena cava, pulmonary vessels, and other structures. Adequate length of the aorta is kept for mounting on the cannula.[8,16] Hearts are usually immersed in cold perfusion solution (4°C) immediately to arrest its motion and to limit any ischemic injury during the period between excision and the restoration of vascular perfusion.[17]

Another way to this step is to cannulate the aorta in situ prior to excision of the heart by inserting an extension tube along the aortic arch. Prior to cannulation inspecting the cannula and tubes connecting the aortic cannula with the perfusate reservoir are essential to ensure that both are filled and free from air bubbles. The aortic valve is essential to maintain perfusion pressure at coronary ostia and in its absence reduced coronary pressure buffer lost via left ventricles. If the cannula is secured above brachiocephalic artery, buffer will be lost through drainage with a drop in coronary pressure.[2] The aorta is clamped to the cannula with a small blunt artery clip. The ligature is immediately tied around the aorta, looking into the grooves and the artery clip removed. The flow of perfusate needs to be initiated as soon as the heart is mounted on the cannula. The maximum time from excision of heart to mount on cannula is generally done under 5 mins of time.[8,17,18]

The heart can be paced or allowed to beat spontaneously. If allow to contract spontaneously, the isolated heart undergoes a small but progressive time-dependent decline in heart rate. Spontaneous heart rate in a perfused heart preparation is usually significantly below the physiologically normal values. For a rat, the heart rate *in vivo* is 350-400 beats per min. However, *in vitro* heart rates are 250-320 beats per min is expected for experimental purpose. Another method of pacing voltage is determined as a set percentage (normally 110-150%) above the voltage required to capture (pace) the heart. It should not exceed 3-5 v, with duration of 0.1-1 msec.[19]

One of the most commonly studied topics in cardiovascular research is ischemia and reperfusion injury. Global ischemia can be induced either by complete cessation of flow, a zero-flow ischemia or attenuation (low flow ischemia) of coronary flow lines. Regional ischemia is generally induced by the ligating left descending coronary artery. The size of the ischemic zone can be influenced by the positioning of the occlusion point. Precaution needs to be taken for preventing tearing of tissue. The occluding ligature is usually tied against a small length of plastic tubing to prevent tearing of tissue and also to facilitate reperfusion. Reperfusion process is initiated by re-establishing coronary flow to the region at risk of ischemia. In global ischemia model involve opening a tap or switching back on of a perfusion pump. In regional ischemia, flow is re-established by loosening or opening the snare. With the rat heart where traditionally 120 mins reperfusion were recommended. However, recent studies have shown clear infarct demarcation after 60 mins reperfusion with no detectable difference in infarct size after 60 or 120 mins of reperfusion.[3-5,35]

MEASUREMENTS

Langendorff's preparation provides the measurement and evaluation of a very wide spectrum of highly reproducible data in a rapid and cost-effective manner. A variety of physiographs can be used to record the data. Digital and computer based recording devices allow better data storage and analysis without human error and personal bias.[8]

The preparation measures contractility of tissue *in vitro*. The technique is important while using with heart isolation, type of perfusion solution, perfusion chamber conditions, temperature, calcium concentration in perfusate, flow rate, coronary pressure, and stability of measured parameters over extended time period. Applications of the technique and ischemia and reperfusion over a fixed period of time. The Langendorff's-perfused isolated mouse heart offers a high throughput and potentially reliable model for the analysis of contractile function and responses to ischemic insult.

The temperature inside the heart can be monitored by placing retrograde a thermocouple into the right ventricles attached to a digital thermometer, and temperature is displayed by the computer.

Heart rate and LVDP is measured by using intra-ventricular balloon. Balloons can be made from thin silicon rubber, domestic plastic food wrap, condoms, or latex. Varying sizes of balloons are commercially available depending on different species, such as 3-4 mm for rat, 5-6 mm for guinea pig, and 8-10 mm for rabbit and even bigger for big animal hearts are available. The deflated balloon should be inserted gently into the left ventricle via the left atrium appendage through the mitral valve. Once positioned the balloon is inflated through a water filled syringe attached to a catheter to achieve ventricular preload pressure or LVEDP of 5-10 mm Hg. According to the Frank–Starling principle, the force of contraction increases with increased filling load (preload) of the ventricle (Frank, 1895). In an isovolumically working condition, the cardiac contractile function increases as the LVEDP (preload) increases once the preload pressure is established, Balloon volume should not be altered during entire measurements. The other end of the catheter which is attached to pressure transducer measures the systolic and diastolic pressure. All pressure transducers are connected to digital and computer recording devices that record continuously throughout the experiment.[7]

Electrocardiogram monitoring of murine heart can be recorded by use of special electrodes, (monopolar or bipolar construction) attached on the surface of the isolated perfused heart. The isolated heart preparation allows studying the effect of proarrhythmic and anti-arrhythmic mechanism of various drugs using rat as an experimental animal, and its applicability to human disease and drug development. It is a well-established model for testing the actions of drugs on ventricular fibrillation induced by regional or global myocardial ischemia. The analysis of the available data suggests that the Langendorff's rat heart preparation subjected to regional ischemia is ideal for first line use.[7,20]

Analysis of contractile function and responses to ischemic insult can also be studied. Measurement of contractile force is made using various methods. Commonly used ones are a force transducer, or a strain gauge, tied to the apex of the heart with a pulley in between the heart and the transducer in a three point mount. Using wire which can be a fine gauge teflon-coated stainless steel, silver or platinum, used as hook, for recording electrode on the ventricle with a reference electrode attached to a stainless steel aortic cannula as a ground. Various other electrodes which can be used include a suction, wick, and sewn-on contacts.[7,21,22]

Cardiac contractile function and coronary flow are generally studied simultaneously. Any change in coronary flow also affects the contractility of the heart. The relation is directly proportional; that is to say, if the coronary flow increases, the metabolites will be washed out and so the cardiac function will increase. It is manifested by an increase in both EDP and peak systolic pressures. It is important to keep coronary flow constant so as to measure the contractility.[23,24]

Infract size and cellular viability are checked by triphenyltetrazolium chloride (TTC) staining. Tetrazolium salts are hydrogen acceptors and are reduced by succinate dehydrogenase to form formazan pigments. TTC staining is a reliable technique for the assessment of infarct size analysis and delineation of tissue necrosis.[34] Infracted tissue size after ischemia and reperfusion is variable and usually incomplete and shows three distinct areas: (1) uneffected myocardium that is normal tissue, (2) area at risk, and

(3) area of necrosis. The final infract size is expressed as the ratio of the area of necrosis to area at risk. To achieve the former result double staining is regarded as the gold standard by many studies[35] prior to TTC staining, re-occlude the coronary artery, and inject the heart with 1% Evans blue dye which stains the normal myocardium blue, and leaves the area at risk unstained.[36] Excised the heart and kept in a freezer at −20°C for 30 mins and cut into fine slices of 2 mm, and incubated by 1% TTC solution (15 mins at 37°C) and fixed by formalin. This method allows to delineate nonviable tissue (necrotic) appears pale (uncolored) while viable tissue cells are stained by tetrazolium colored (pink). The area at risk and infarct size is determined by digital tracings or by computerized planimetry. TTC enters in to cell membranes, and binds to intracellular dehydrogenases of alive cells with reducing potential (preserved NADPH) are colored brick-red. Other dead cells with ruptured sarcolemmal membranes and enzyme wash out, appear pale. TTC is one of the best ways to assess the correct infracts size.[11,12]

Detailed cardiac morphology can be analyzed using multiple sequential microscopic visualizations. The heart can also be perfused with a variety of gels, particles, and resins that allow the specific visualization of vascular perfusion beds.[25] Apart from a structural examination of the heart, various biochemical parameters can also be analyzed. Various metabolites of myocites such as lactate, oxygen, and a host of other markers of normal, and abnormal metabolism can be made. The ability to perfuse the hearts in a nuclear magnetic resonance (NMR) spectrophotometer allows continuous on-line measurement of metabolites and intracellular ions such as calcium, protons, or sodium.[8]

POST EXPERIMENT CLEANING AND PRECAUTIONS

After the experiment is complete, all the equipment needs to be carefully cleaned to reduce the accumulation of biological deposits, debris, and precipitate within the tubing and glassware. Glass wares can be cleaned with soap, dilute hydrochloric acid or other cleaning solvents. Great care is required when handling non-glass ware apparatus. Non-glass portions are treated with aqueous soap solutions. The entire equipment and system are rinsed multiple times in distilled water after cleaning. Areas which should be extremely clean include, the aerator, tubing, syringe ports, cannula, pressure transducer fittings, as well as balloon catheters and electrodes inserted in the heart.[4,5]

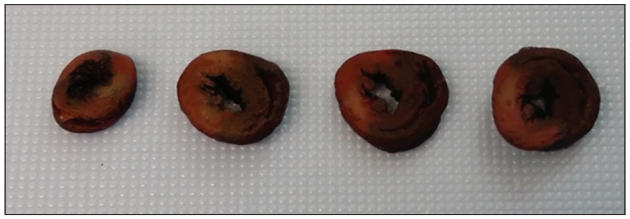

Figure 3: one percent ttc staining slides

DISCUSSION

In the late 19th century, a number of investigators were working on improving isolated heart model, but it was Oscar Langendorff who, in 1895, pioneered the isolated perfused mammalian heart. Since then, the Langendorff's preparation has evolved and provided a wealth of data reinforcing our understanding of the fundamental physiology of the heart, its contractile function, coronary flow regulation, and cardiac metabolism.[1]

Experiments related to viable tissue, done *in vitro* or *ex vivo* have many challenges regarding survival outside living body. It is recognized that, as an *ex vivo* preparation, the isolated living and beating heart is a constantly deteriorating preparation. However, nonetheless, the rat heart is viable for study for several hours when using the Langendorff method. The preparation also allows the induction of whole heart (global) or regional ischemia, and this can be achieved at various levels of flow. Similarly, experimental insults such as anoxia or hypoxia at various degrees of oxygen deprivation can be easily imposed. While it should be taken in account that the isolated heart is denervated this can be an advantage in this method as it allows the separation of cardiac, from sympathetic, and vagal stimulation.[15,16]

Many recent studies such as Skrzypiec-Spring et al., 2007, described the general principle of Langendorff's heart preparation to involve the cannulation of the aorta, over a fixed cannula on the perfusion apparatus. This cannula is attached to the outflow of a reservoir containing an oxygenated perfusion solution maintained at 37°C and continuously gassed with a gas mixture containing 5% CO_2 and 95% oxygen for normal aerobic perfusion studies, perfusing with a physiological salt solution (KHB) containing bicarbonate which mimics the ionic content of plasma, and delivered at 37°C at a physiological pH of 7.4.[4,5,7]

Selecting between two methods is based on the physiological system. From a physiological point of view, when perfusing at constant flow rate, autoregulatory mechanisms are overridden, and the amount of perfusate delivered to the whole heart is not altered physiologically in response to changes in heart rate or contraction, or as a result of pathological conditions such as ischemia. Therefore, perfusion at constant pressure should be given preference in particular for studies of ischemia.[8]

Previously the content of $CaCl_2$ in KHB was 2.5 mM, which is well in excess of physiologic bioavailable ionized calcium in whole blood (around 1.3 mM in rat heart calcium binding to circulating protein). When it was known and recognized, most researchers reduced the calcium concentration in their KHB buffer to match with physiologic calcium, a range between 1.2 and 1.8 mM.[26] Furthermore, the glucose concentration in KHB (11 mmol/l) is very high compared to physiological levels.

It is regarded as "diabetic range," which is far greater than normal range present in blood (5-6 mmol/l). The reason for using a higher than physiologic glucose level is that there is a lack of an alternate energy substrate in the perfusion buffer. Thus, it is compensated by use of more glucose. The excess glucose may be replaced with an alternate substrate as pyruvate or free fatty acids.[27,28]

The use of blood as a perfusion fluid is not recommended for many reasons despite its physiological advantages. The volume of blood needed (perfusing one rat heart would require the blood of several rats), the inability to use of blood from some (but not all) larger species because they have larger erythrocytes than the rat and cannot traverse rat heart capillaries and the difficulty in oxygenating the blood since conventional gassing creates extensive foaming and damage to blood cells.[7,29,30]

Temperature plays very important role in isolated perfused heart experiment. There is a strong relationship in between cardiac contractile function and temperature. One of the effects is; changes in temperature causes change in heart rate and contractile force. Therefore, perfusion should be carried out at constant temperature close to the normal body temperature of the species under study.[7-10]

This technique can be performed using many mammalian species; however, there are multiple advantages for using murine animals over others. Rats are commonly used and, male species are recommended to avoid the effect of sex hormones. The literature contains an increasing number of studies which use mouse hearts, which have smaller hearts, making intra-ventricular pressure recordings more difficult, having high heart rate, the miniaturization of equipment.[31]

One of most commonly studied parameter in the Langendorff's method is the electrical activity of the heart. Electrical activity of perfused heart has been used in studies of cardiac arrhythmias, action potentials and T-wave alternans, ischemic preconditioning, and action potential propagation.[32,33]

In recent studies, Langendorff's preparation has been utilized to isolate single cardiac myocytes, by perfusing the heart with a solution containing collagenases. Such modifications required a new set of skills and materials to be used. Thus, allowing an ever evolving system for understanding the patho-physiology, and drug development related to cardio-vascular system.

ADVANTAGES AND LIMITATIONS

The method has the advantages of long viability duration, simple preparation, low cost, reliable data collection in quality and quantity. Furthermore, it permits the measurement of inotropic, chronotropic, and coronary vascular effects, without the interference of hormones or nervous system. The effects of drugs directly on the heart can also be studied easily. Cardiac contractile function (in terms of LVDP by the balloon (isovolumic) method measurement is the best-employed technique for measuring LV pressure. The balloon is inflated with degassed water to adjust preload pressure (end diastolic) of 5-10 mm Hg, and LV pressure is recorded via pressure transducer.[36] Electrical activity, heart rate, ischemia/reperfusion injury, infract size, myocyte isolation. Measurements of metabolites using NMR and electron paramagnetic resonance and recent discovery is optical technologies in the isolated heart[36] Langendorff's apparatus and set up preparations require high skill and care while harvesting the heart from animal to cannulation within an appropriate time frame to ensure good results. The cannulation step is important in terms of the viability of heart. The maximum time from excision of heart to mount on cannula should be less 5 mins time to avoid damage to the heart. In comparison with the crystalloid buffer, the rate of flow is very high compare to oxygenated whole blood or RBC enriched solution. The major disadvantage is that 5-10% deterioration per hour in contractility and chronotropic function needs special attention.[2,3,6]

Currently, KHB is the preferred perfusate despite its limitations including, the low oxygen carrying capacity, development of edema, and a decrease in contractility over a period of time. Nevertheless, KHB remains a practical and useful method of maintaining the Langendorff's heart for several hours Langendorff or ejecting heart, constant perfusion or constant flow, isometric force or isovolumetric pressure, and buffer perfused or erythrocyte perfused: each has its own advantages and limitations that must be taken into account.[36]

CONCLUSION

Having selected the isolated perfused heart as a model to investigate a cardiovascular phenomenon, the choice of preparation is very wide. Blood perfused or crystalloid solution perfused. Modifications in Langendorff's can be established to pursuit a better understanding of cardiac function and malfunction. Although the investigative power of the preparation for physiological and pharmacological research is great, they are fraught with potential pitfalls, which must be recognized and addressed. However, the disadvantages of the model are outweighed by its benefits, and the technique possesses an optimal balance of both quality and quantity of data with clear clinical relevance. This review article has attempted to address many of these issues in the hope that it will assist researchers to make the best possible use of this experimental method using the rat as a model.

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