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Appraisal of state-of-the-art

Isolated heart perfusion according to Langendorff—Still viable in the new millennium

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Abstract

The isolated perfused mammalian heart preparation was established in 1897 by Oscar Langendorff. The method was developed on the basis of the isolated perfused frog heart established by Elias Cyon at the Carl Ludwig Institute of Physiology in Leipzig, Germany in 1866. Observations made using both methods at the end of the 19th and at the beginning of the 20th century led to important discoveries, forming the basis for our understanding of heart physiology. This included the role of temperature, oxygen and calcium ions for heart contractile function, the origin of cardiac electrical activity in the atrium, the negative chronotropic effect of vagus stimulation and the chemical transmission of impulses in the vagus nerve by acetylcholine. Langendorff himself demonstrated that the heart receives its nutrients and oxygen from blood via the coronary arteries and that cardiac mechanical function is reflected by changes in the coronary circulation.

The method underwent many modifications but its general principle remains the same today. Blood, or more commonly crystalloid perfusates, are delivered into the heart through a cannula inserted in the ascending aorta, either at constant pressure or constant flow. Retrograde flow in the aorta closes the leaflets of the aortic valve and as a consequence, the entire perfusate enters the coronary arteries via the ostia at the aortic root. After passing through the coronary circulation the perfusate drains into the right atrium via the coronary sinus.

The simplicity of the isolated mammalian heart preparation, the broad spectrum of measurements which can be done using this method, its high reproducibility and relatively low cost make it a very useful tool in modern cardiovascular and pharmacological research, in spite of a few shortcomings. In the last decade the method has brought many important advances in many areas including ischemia–reperfusion injury, cell-based therapy and donor heart preservation for transplant.

Keywords: Arrhythmia; Ischemia–reperfusion; Isolated heart; Matrix metalloproteinases; Method of Langendorff preparation; Perfusion; Rat

1. Introduction

Only few isolated organ experimental models have been used as extensively as the isolated heart preparation according to Langendorff. It was first applied by physiologists, biochemists and morphologists for the study of heart biology. It was further applied by pharmacologists to test the effect of different cardiovascular drugs on the coronary vasculature, muscle contraction and heart rate. Today a variety of cardiovascular researchers still use this vital technique in myriad ways to investigate the heart, from the study of the effect of a single gene alteration on heart physiology, to novel therapeutic means to protect the heart from ischemia and other insults.

Although there are some limitations of the method including the absence of normal humoral influences and neuronal regulation, as well as high coronary flow and oedema when using cell free perfusate, the impact of these shortcomings is limited by the very advantage that much important first hand information can be gained by virtue of the elegant simplicity of this technique. Moreover, methods of recording some biophysical and biochemical parameters have been improved over the decades and the number of parameters we can now obtain using this method, the accuracy of these measurements, their high reproducibility and the relatively low costs predominate over the shortcomings, making this method a very useful tool in modern cardiovascular and pharmacological research.

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This review will first provide a brief historical background of the method as well as details regarding organ isolation, perfusion solutions, set-ups for different modalities of perfusion, methods of recording various cardiovascular parameters and finally illustrate the utility of the isolated heart preparation according to Langendorff with a few select examples of recent research.

2. Historical background of the method

2.1. The isolated perfused frog heart

The isolated perfused mammalian heart preparation was developed on the basis of the isolated perfused frog heart. This method, widely used in the last part of the 19th century, was a starting point for the study of heart physiology. The scientist who deserves credit for first devising the isolated perfused frog heart preparation is Elias Cyon who established the method at the Carl Ludwig Institute of Physiology in Leipzig, Germany in 1866. For excellent reviews and original references in regards to historical aspects of the isolated frog heart preparation see Zimmer (1998), Zimmer (1999) and Zimmer (2000).

The model differs significantly from the one developed later by Oscar Langendorff for mammalian hearts as the frog is a cold-blooded species. Its heart consists of only three chambers (two atria and a single ventricle) and has no coronary vascular system, thus the exchange of metabolites and gases from blood to cardiac muscle tissue is achieved by diffusion. The underlying principle of the method originally established by Cyon was to pump serum obtained from rabbit blood into the heart through a cannula inserted into the vena cava (Cyon, 1866). After passing through the heart, the serum was ejected via the aorta and was circulated from the aorta through a glass tube to return via the vena cava. This circulatory system was surrounded by a glass cylinder filled with fluid, which could be adjusted to any desired temperature. Only three parameters were recorded from the isolated perfused frog heart: heart rate, circulatory pressure and the temperature of the circulating serum.

Several very important discoveries were made with the use of this experimental design. The influence of the hydrostatic preload pressure on heart contractions, the dependence of heart function on temperature and adequate supply of oxygen and the detrimental effect of carbon dioxide on the heart were all recognized with this model and became the basis for concepts to be elaborated later by scientists such as Coats, Schmiedeberg, Bodwitch, Luciani, Rossbach, Kronecker, Frank, Ringer, Dale and Loewi.

Coats observed that vagal stimulation reduced the ability of the heart to contract in response to electrical stimulation at the heart surface (Coats, 1869). This discovery was later elaborated by Webers who observed that electrical stimulation of parts of the brain from which the vagus nerve originated, as well as the vagus nerve itself, led to cardiac arrest, and by Schmiedeberg, who demonstrated that the negative chronotropic effect of vagus stimulation was prevented by nicotine or atropine.

Studies by Bodwitch with the isolated frog heart revealed the staircase phenomenon: following a brief arrest of the heart, the amplitude of muscle contraction gradually increases until reaching a plateau (Bowditch, 1871). In addition, the all or none law of the heart, the absolute refractory period and the origin of cardiac activity in the atrium were observed. Luciani developed his own modification of the Cyon heart preparation to observe the response of the heart to the pressure of a ligature placed in different locations around the atria and the bulbus aorticus. This resulted in irregular contractions, which occurred in periods, and with a positive or negative staircase, which are now known as Luciani periods. His discovery became one of the first steps toward creating models of arrhythmia. The mechanism of his observations was later explained by Rossbach who pointed out the crucial role of inadequate oxygenation of the perfusion solution in generating Luciani periods.

Kronecker was one of the first scientists who recognized the importance of adequate oxygen content in the perfusion medium (Zimmer, 1998). In his experiments he observed that adding blood or providing oxygen to a saline perfusion solution had a distinct positive effect on heart function. Another very important observation concerning perfusion medium was done by Ringer, who showed the dependence of contraction amplitude on the concentration of calcium in the perfusion solution. This discovery became the basis for understanding the role of calcium ions in heart muscle physiology.

Otto Loewi, using the isolated perfused frog heart, discovered chemical transmission of impulses in the vagus nerve by acetylcholine (Loewi, 1921). He filled a frog heart with the solution which bathed a vagus nerve during stimulation. The effect of this fluid mimicked the one achieved by vagus stimulation. For this discovery Otto Loewi was awarded the Nobel Prize in 1936.

2.2. The isolated perfused mammalian heart

The isolated frog heart preparation originally devised by Cyon and modified by the scientists mentioned above was the basis for the development of the isolated mammalian heart preparation. The method was established in 1897 by Oscar Langendorff (Langendorff, 1898; Taegtmeyer, 1995; Zimmer, 1998; Zimmer, 2000). His experiments were carried out mostly using the hearts from cats, but also those from dogs and rabbits. A general principle of the method was to deliver blood into the heart through a cannula inserted and fixed in the ascending aorta. Retrograde flow in the aorta closed the leaflets of the aortic valve, which did not permit the perfusion fluid to enter into the left ventricle. As a consequence, the entire perfusate entered the coronary arteries via the ostia at the aortic root. After passing through the coronary circulation the perfusate drained into the right atrium via the coronary sinus. The perfusion pressure during the experiment remained constant by using a constant hydrostatic pressure of the perfusate. The measurement of coronary flow was performed by measuring timed volumes of perfusate draining out of the right atrium (Langendorff, 1898). His technique of isolated mammalian heart perfusion thus became widely known as the Langendorff perfused heart. It was used to perfuse hearts from many warm-blooded animal species with a coronary vascular system and was subsequently subjected to various modifications (Doring & Dehnert, 1987; Zimmer, 2000).