PACING THE HEART: IS IT A MUST IN STUDYING MYOCARDIAL CONTRACTILE FUNCTION IN ISOLATED HEARTS PERFUSED USING THE LANGENDORFF SYSTEM

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Background: Myocardial contractile function has been studied extensively in isolated hearts using the Langendorff System. The effects of changing the heart rate on myocardial contractility have not been described. The aim of the present study was to examine the effects of changing the heart rate on studying myocardial contractility in isolated heart models using the Langendorff System, and to determine whether pacing the hearts is required.
Methods: Male Sprague Dawley rats were used. Hearts were harvested and perfused using the Langendorff System. Hearts were assigned to either paced or unpaced groups. Hearts were paced at 5 Hz that is 300 beats per minute using an electrical stimulator using a 6020 Stimulator from Harvard Apparatus. Myocardial contractility was measured with a balloon-tipped catheter inserted into the left ventricle via the mitral valve and at constant coronary flow. Indices of left ventricular function were measured
Results: The hearts paced showed an increase in left ventricular end-diastolic pressure at 45 and 60 minutes of perfusion ($p<0.05$) compared to the unpaced hearts. Left ventricular peak systolic pressure was significantly higher in the unpaced group compared to the paced group throughout the one-hour of experimental protocol ($p<0.05$). In the paced hearts there was a decrease in the generated pressure at the 45 and 60 minute perfusion time ($p<0.05$) compared to the unpaced group.
Conclusion: The cardiac contractile function was affected by changes in the heart rate in the isolated perfused hearts. In other words, to study the contractility in the isolated perfused hearts, the hearts should be paced at a constant heart rate.
Keywords: Langendorff, isolated heart, heart rate,rat, cardiac contractility.

Introduction

MYOCARDIAL CONTRACTILE FUNCTION has been extensively studied using isolated perfused hearts on the Langendorff System. The Langendorff system is a unit for perfusion of isolated hearts. Prefusion is the flow of the solution through vascular systems of intact organs (coronary arteries in the heart, kidney, lung, liver) and tubular organs (trachea) or tissues. The Langendorff system was first discovered by Oscar Langendorff in 1895[1]. Oscar Langendorff has made a number of discoveries in the field of cardiac physiology. The method has become increasingly popular in recent times, providing researchers with a stable environment to examine the function and metabolism of isolated organs in vitro away from the neuroendocrine, metabolic and hemodynamic changes[2, 3].

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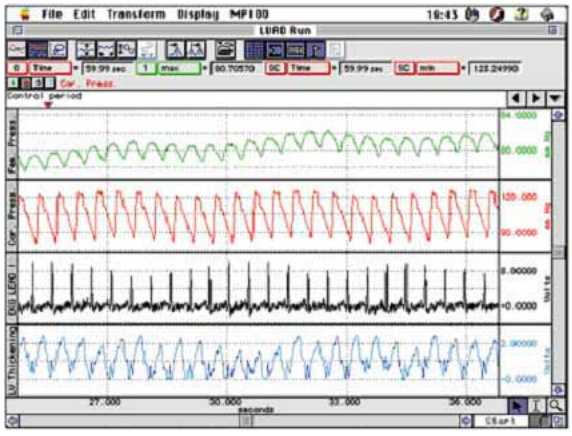
The BIOPAC MP100 system is a data acquisition and analysis system that can be used both in human and animal studies. The MP100 system offers many options for recording different physiological events.

The MP100 data acquisition system uses the AcqKnowledge software (shown below).



The software performs data collection, display and analysis in a simple and efficient way. The software

was applied to Windows operating system. AcqKnowledge software can be used to study different cardiovascular hemodynamic data, such as: ECG, blood pressure, blood flow, cardiac output, left ventricular pressure, temperature, etc, as well as other physiologic measurements.

To study the contractility of the heart, the affecting parameters are: 1) The coronary flow, 2) The heart rate and 3) The fiber length. Any change in coronary flow will affect myocardial contractility. An increase in arterial perfusion pressure results in a simultaneous increase in myocardial oxygen consumption, a phenomenon known thereafter as the "Gregg Effect"[4].

So, in order to study the contractility of the heart, the coronary flow should be kept constant (10 ml/min)[5, 6] . The effects of changing heart rate on myocardial contractility have not been studied.

If the hearts were allowed to beat spontaneously, the isolated hearts undergoes a progressive decline in the heart rate. For rat hearts, which have an in vivo rate of 300-400 beats/minute, heart rates of 250-320 beats/minute can be expected. This changing heart rate will add additional variability to the data.

In order to keep the heart rate constant, an electrical stimulator was used to pace the hearts (6020 Stimulator from Harvard Apparatus).

The frequency of the pulses was adjusted to 5 Hz, the pulse width, which is the duration of the stimulation pulses was 1 ms and the pulse amplitude was 5-7 Volts. Pacing voltage was determined as a set percentage (normally 110-150%) above the voltage required for the hearts to capture (pace).

The stimulator was connected to the heart via a double stranded copper wire which was embedded into the tissue of the right atrial appendage was considered as the recording electrode. A reference electrode was attached to a stainless steel aortic cannula as a ground.

The aim of this study was to show the effect of changing heart rate due to pacing and unpacing on the cardiac contractility using the Langendorff System.

The experiments were divided into two groups:

(a) The isolated perfused paced hearts

In this group, the hearts were isolated from the rats and perfused on the Langendorff system.

The hearts were paced at 5 Hz and perfused with KHB for 60 minutes.

(b) The isolated perfused unpaced hearts:

In the unpaced group the hearts were isolated from the rats and perfused on the Langendorff system for 60 minutes as described previously, but the hearts were not paced. This was the only unpaced group in the present study.

Methods

Preparation of the Animal

In the present study, male Sprague-Dawley rats weighing 300-400gms were injected intra-peritonealy (i.p.) with heparin sodium 2000 I.U in 1ml of 0.9% NaCl.

Rats were anesthetized using urethane 125mg/kg i.p., providing complete anesthesia for the experimental period. Animals were randomly assigned to either paced or unpaced groups (n=6 per group).

Ex vivo Perfusion of the Isolated Hearts

A Sub-xiphoid transverse incision was made in the abdomen and extended superiorly along both mid axillary lines. The diaphragm was carefully transected and the ventral rib cage was lifted . Hearts were then excised quickly and placed on ice cold physiological solution. Hearts were attached to the perfusion cannula of the Langendorff System by the aorta.

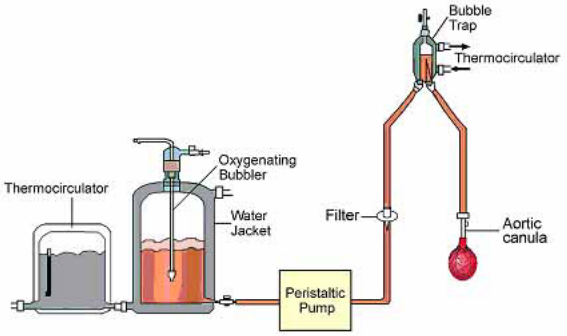


Figure 1. *Illustration of the Langendorff System. The water jacket contains the perfusate fluid that is heated by the thermocirculator and oxygenated through the oxygenator bubbler. The perfusate is pumped by a peristaltic pump, then passes through a filter to the bubble trap were perfusate (also connected to the thermocirculator to maintain constant temperature) perfuse the heart via the aortic cannula.*

The Langendorff system contains (Figure 1) a 5 liter water jacketed reservoir, where the perfusate is inside the reservoir and surrounded by a heated water circuit that is connected to a thermocirculator. The perfusate reservoir is of a heavy duty

borosilicate glass. The reservoir has two outlets, one for the water circuit to enter the thermocirculator and the second is for perfusing the isolated heart. At the top of the perfusate reservoir, is an opening that fits a barosilicate glass oxygenator bubbler connected to the laboratory oxygen supply, permitting the perfusate to be gassed effectively.

The perfusate temperatute is kept constant by the surrounding heated watrer that come from the thermocirculator. The perfusate is gassed via the oxygenator bubbler connected to a gas cylinder.

The thermocirculator provides a source of temperature controlled water for use with water warming jacketed glassware The thermocirculator contains a 2 liter water chamber that has a safety device in the form of a float switch that will not allow power to be supplied to either the heater or the circulating pump unless the chamber is adequately filled with water. The large volume ensures minimum temperature gradient in baths. On the front panel, there is a stopcock for filling and drainig the system. There is also a valve so that air can be bled from the system as it is being filled. The temperature of the water within the thermocirculator is shown on the front panel. The temperature was adjusted to 42-45 degrees Celscius, to permit a margin for loosing some heat in the tubings as the perfusate reaches the heart. The outgoing and return tubing for the water circulating system are mounted on the front panel.

As the perfusate leaves the reservoir, it passes through tubes that go to a bubble trapThe bubble trap removes gas bubbles introduced during aeration or an addition of perfusate or drugs. This bubble trap is used to prevent air embolism and reaching the heart. The bubble trap is also made of barosilicate glass. The chamber contains perfusate and is water-jacketed to prevent cooling of the perfusate. There are three connections at the bubble trap- two at the bottom for fluid entry and exit, and a third at the top serving for fluid level adjustment. If the valve is open, fluid will rise in the air bubble trap. The valve is then kept closed to allow trapping of air. When air is trapped, the perfusate level will fall. When the perfusate level is too low, it can be adjusted by opening the valve to fill up the chamber.

After the perfusate passes through the bubble trap, it flows to the peristaltic pump that will pump it at a constant flow

This peristaltic pump is a pump that will transfer the fluid by a peristaltic action. The speed of the pump is adjusted to maintain the flow rate of 10 ml/min. The flow was maintained constant throughout the experimental protocol.

The perfusate then passes to the aortic cannula through which it will enter the aorta to perfuse the heart. The aortic cannula is a thin walled stainless steel as glass cannula. The external diameter is typically similar to or slightly larger than that of the aorta (about 3 mm for a heart from a 250 g rat). A small circumferential groove is present at the distal end of the cannula to prevent the aorta from slipping off, after a ligature is placed around the aorta.

This type of perfusion of the isolated hearts is called the "retrograde perfusion", since perfusate flows or is pumped retrogradely into the aorta, where it then enters the coronary arteries.

The isolated hearts were perfused with a physiological solution: Krebs Hanseleit Bicarbonate (KHB)[7] consisting of the following (in mM/L): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 21, glucose 11, EDTA 0.5.

The solution was gassed with a mixture of oxygen 95% plus 5% CO_2. The pH of the perfusate was checked and maintained at physiological level 7.4.

The perfusate was prepared fresh daily. A sample of the solution was sent to the central biochemistry laboratory in the King Khalid University Hospital, where it was analyzed for electrolytes and glucose concentrations (DADE -Dimention clinical chemistry system, RxL).

Astab incision was made in the left ventricle using a # 15 scalple blade. A saline-filled cellophane balloon-tipped catheter was placed into the left ventricle via the mitral valve and used to measure LV pressure. The coronary perfusion pressure was measured using a pressure transducer that was connected to the side arm of the aortic perfusion catheter and positioned at the level of the aorta.

The paced group was paced electrically at 300 beats per minute using a 6020 Stimulator from Harvard Apparatus. The flow rate was adjusted to 10ml/min. Perfusate temperature was maintained at 37°C.

Hemodynamic and Cardiodynamic Measurements

Left ventricular end diastolic pressure (LVEDP) and the left ventricular peak systolic pressure (LVPSP) were measured continuously. Coronary vascular resistance (CVR) was calculated as coronary perfusion pressure (CPP) divided by the coronary flow (Q) , ($CVR = \Delta CPP / \Delta Q$). Since

coronary flow was maintained constant, any change in perfusion pressure was directly proportional to changes in coronary vascular resistance.

Left ventricular generated pressure (LVGP) was calculated as the difference between the left ventricular peak systolic pressure (LVPSP) and left ventricular end diastolic pressure(LVEDP); (LVGP=LVPSP-LVEDP).

Left ventricular compliance (C) was calculated by dividing the change in balloon volume (BV) by the change in the left ventricular end diastolic pressure (LVEDP), ($C = \Delta BV / \Delta LVEDP$).

The maximum ± dP/dt, which is the first derivative of pressure over the first derivative of time. The maximum ±dP/dt is used as an index to study the changes in the cardiac contractility. The maximum ±dP/dt was also calculated by the AcKnowledge software as the first derivative of pressure over time.

Statistical Aanalysis:

All data were initially analyzed with Bartlett's test for homogeneity. Data found not to be homogeneous were transformed and reanalyzed. Data were analyzed with MANOVA. Means were analyzed using Duncan's test and were considered significant when yielding a "p" value less than or equal to 0.05. Data are expressed as means ± SEM .

Results

Figure 2 shows the effects of electrical pacing on the changes in left ventricular end diastolic pressure in the isolated perfused hearts during one hour of non-recirculating perfusion of the heart using the Langendorff. In the hearts paced at 5 Hz, there was an increase in left ventricular end-diastolic pressure at 45 and 60 minutes of perfusion ($p<0.05$). In the unpaced hearts, left ventricular end diastolic pressure remained constant during the one hour perfusion period.

Figure 3. shows the effects of electrical pacing on the changes in the left ventricular peak systolic pressure in the isolated perfused hearts during one hour of perfusion. In the hearts paced at 5 Hz, and the unpaced hearts, left ventricular peak systolic pressures were unchanged during the one hour experimental protocol ($p>0.05$). However, left ventricular peak systolic pressure was significantly higher in the unpaced group compared to the paced group throughout the one-hour of experimental

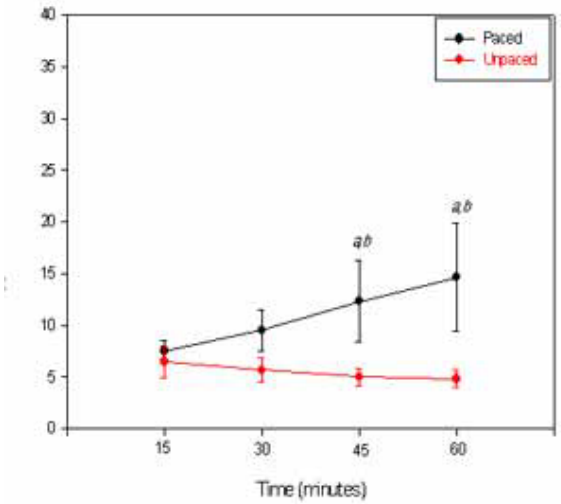


Figure 2. *Left ventricular end diastolic pressure in the isolated perfused hearts over one hour of in vitro perfusion of the hearts (n=6).*
a = p < 0.05 compared to within group 15 minute time peroid
b = p < 0.05 unpaced time peroid

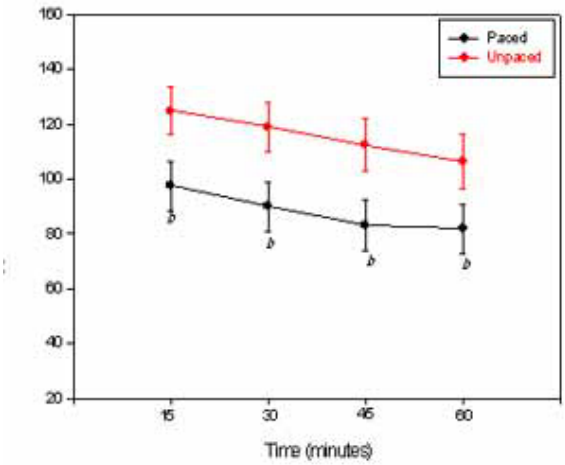


Figure 3. *Left ventricular peak systolic pressure in the isolated perfused hearts over one hour of in vitro perfusion of the hearts (n=6)*

protocol ($p<0.05$).

Figure 4. shows the effects of electrical pacing on the changes in left ventricular generated pressure in the isolated perfused hearts. In the paced hearts there was a decrease in the generated pressure at the 45 and 60 minute perfusion time ($p<0.05$). Generated pressure was higher in the unpaced group when compared to the paced group throughout the one-hour experimental protocol ($p<0.05$). Unlike the paced hearts, left ventricular generated pressure in the unpaced hearts did not change significantly throughout the sixty minute experimental protocol

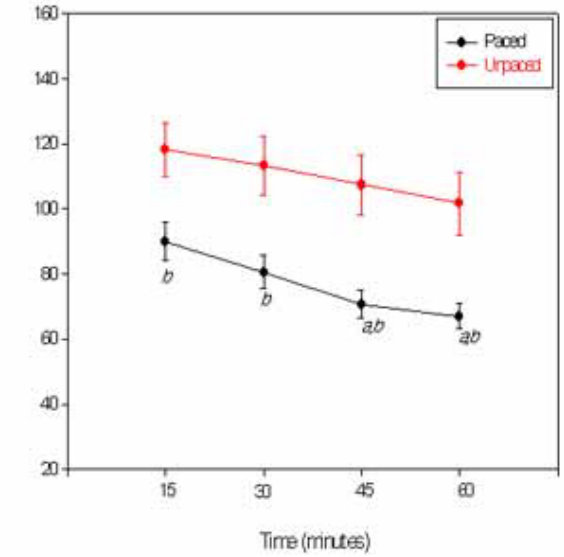


Figure 4. *Left ventricular generated pressure in the isolated perfused hearts.*
over one hour of in vitro perfusion of the hearts (n=6).
a = p < 0.05 compared to within group 15 minute time peroid
b = p < 0.05 unpaced time period

(p>0.05).

Table 1. shows the heart rate of the isolated perfused hearts in the paced and unpaced group at 15, 30, 45 and 60 minutes. The paced hearts were paced at 5 Hz, that is 300 bpm, while the unpaced group ranged between 192.17 ± 26.15 to 201 ± 26.19bpm at 15 and 60 minutes respectively.

Table 1. *Heart rate of the isolated perfused paced and unpaced hearts at 15, 30, 45 and 60 minutes respectively.*

	15 Minutes	30 Minutes	45 Minutes	60 Minutes
Paced Group (beats/min)	300 ± 0.00	300 ± 0.00	300 ± 0.00	300 ± 0.00
Unpaced Group (beats/min)	201 ± 26.19	200.50 ± 19.82	197.17 ± 23.76	192.17 ± 26.15

Discussion

The present study also showed that studying the contractility of the isolated perfused hearts in the Langendorff System without pacing the hearts affected the cardiac contractile activity. In other words, to study the cardiac contractile function, the hearts should be paced.

If the heart rate is slow, there will be more time for the heart to relax. In other words, there will be more time for the Ca^{+2} to be taken up by the sarcoplasmic reticulum (SR)[2]. As a result the left ventricular end diastolic pressure will decrease. In next contraction, there will be more calcium available at the SR to be released, so the next contraction will stronger[5, 8-10]. In other words, the left ventricular peak systolic pressure will increase. As a net result, the decrease in the heart rate increased the contractility of the heart (potential beats).

On the other hand, when the heart rate is increased, there will be reduced time for the Ca+2 to be taken up by the SR, so the heart will not fully relax and the left ventricular end diastolic pressure will increase. In the next contraction, decreased calcium will be released from the SR, so the peak systolic pressure will decrease. In other wards, the increase in the heart rate decreased the contractility. Accordingly, in order to study the contractility, the heart rate should be constant to avoid any effect of changing the heart rate on the contractility.

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