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Version 2

Measurement of Left Ventricular Performance in Langendorff Perfused Mouse Hearts V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

This protocol describes the procedure used by the DiaComp for measuring left ventricular performance in isolated retrogradely perfused mouse hearts.

Diabetic Complication:



Cardiovascular

Troubleshooting

Langendorff Heart Perfusion Protocol

- 1 Hearts are isolated and the aorta is cannulated using a 20g steel cannula. Hearts are perfused at a constant pressure of 60 mmHg by an aortic cannula delivering warm (37°C) Krebs buffer containing (in mM) 118 NaCl, 4.7 KCl, 25 NaHCO₃, 1.2 MgSO₄, 1.2 KH₂PO₄, 2 CaCl₂ gassed with 95% O₂, 5% CO₂. Hearts are perfused with glucose 11mM as sole substrate or in combination with 1 or 1.2 mM palmitate. The pulmonary artery is transected to facilitate coronary venous drainage. A left ventricular polyethylene apical drain is inserted through a left atrial incision to allow thebesian venous drainage. Left ventricular pressure is monitored from a water-filled balloon placed through the left atrial appendage and connected to a Millar transducer. The volume of the balloon is adjusted to obtain a left ventricular diastolic pressure of 7 mmHg. Heart rates are adjusted to 360 beats/min by pacing at 6 Hz at the level of the atria.

Inotropic stress protocol

- 2 After 30 min stabilization, data are acquired under baseline conditions (buffer calcium concentration = 2 mM). The hearts are then switched to a second buffer containing 4 mM CaCl₂. Contractile parameters are again measured after 20 min of stabilization. The langendorff protocols yield the following parameters:
 - (1) Left ventricle systolic pressure (LVSP): Units mmHg.
 - (2) Left ventricle developed pressure (LVDP or LVDevP), which is LVSP – LV Diastolic pressure: Units mmHg.
 - (3) Heart Rate (HR): Units beats per minute
 - (4) Rate Pressure Product (RPP), which is LVDP x HR. Units mmHg/sec
 - (5) dP/dtmin and dP/dtmax, which are the maximal rates of LV pressure decay and LV, pressure development respectively. Units mmHg/msec.
 - (6) Coronary Flow (ml/min). This is determined by measuring the coronary effluent from the perfused heart.

Myocardial oxygen consumption (MVO₂).

- 3 Coronary effluent is sampled from the pulmonary artery using a capillary tube. Oxygen content was measured using an Oxygen foxy probe (OceanOptics) and calculated using the formula:



$$MVO_2 = \% O_2 \text{ perfusate} - \% O_2 \text{ pulmonary artery} \times \text{Coronary Flow} \times \text{Atmospheric Pressure} / 760 \times O_2 \text{ Solubility} \times O_2 \text{ Density}$$

Where O₂ solubility and O₂ density are 23.9 µl/ml and 0.03933 µmol/µl respectively in a solution at 37°C respectively.

Cardiac efficiency is calculated as the ratio of RPP/MVO₂ expressed as a percentage.