Aug 12, 2019

Phenotypic Characterization of the Working Heart

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1 Works for me dx.doi.org/10.17504/protocols.io.3ucgnsw

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ABSTRACT

Summary:

This protocol describes the procedure used by the DiaComp for cardiac performance, oxygen consumption and substrate metabolic rates in isolated working mouse hearts.

Diabetic Complication:

Cardiovascular

References:

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EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=41&docType=Protocol

1 **Protocol for Determining Substrate Metabolism in Isolated Working Mouse Hearts.**

Cardiac metabolism is measured in hearts isolated from mice as early as four weeks of age. Mice are heparinized by intraperitoneal injection of 200µl of heparin (1000USP units/ml) and then subjected to deep anesthesia by injecting chloral hydrate at a dose of 0.7mg/g body weight. The hearts are then removed from the mouse, dissected and trimmed of fat and extraneous pulmonary tissue. The aorta is cannulated first using an 18G steel cannula and the heart is initially perfused in the retrograde mode with Krebs Henseleit Buffer (KHB). The pulmonary vein is then cannulated and the perfusate flow reversed so that buffer enters the left side of the heart via the pulmonary vein and is ejected via the aorta. Details of these protocols have been extensively described in the literature (1-6).

The working heart buffer is KHB containing (in mM) (118.5 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 0.5 EDTA, (5 or 11) glucose and gassed with 95% O_2-5% CO_2, supplemented with (0.4, 1.0 or 1.2) mM palmitate bound to 3% BSA and 5 or 11 mM glucose. The final concentrations of palmitate and glucose are determined by the specific model that is being evaluated. For example in studies of db/db mice, hearts are perfused at 11mM glucose and 1.2 mM palmitate as these concentrations more closely reflect the in vivo milieu of the animals. In contrast, in animals with cardiac restricted changes in gene expression in which systemic concentrations of glucose and fatty acids are normal, hearts are perfused with 5mM glucose and 0.4 mM palmitate. Hearts are also perfused in the presence or absence of 1nM insulin, which measures the degree of insulin responsiveness of the perfused hearts. For determination of metabolism; rates of glycolysis for exogenous glucose, glucose oxidation, and palmitate oxidation are measured over a 60-min period. Hearts are perfused at a preload of 15 mmHg and aortic pressures and cardiac performance determined as described in (1-6).

Throughout the 60-min perfusion, pressure and flow measurements are obtained every 10-min. At 20-min intervals (starting at 0 min), a 2.5-ml sample of buffer is withdrawn for determinations of metabolite content. All determinations of substrate metabolism for each time point are made in duplicate. At the end of the experiment, the hearts are quickly frozen between metal blocks cooled to -80°C, rapidly weighed, and stored at - 80°C. Hearts are subsequently pulverized under liquid nitrogen using a mortar and pestle. A sample of heart tissue (~20 mg) is also weighed (wet wt), and then dried to constant weight (dry wt). The ratio of this sample (dry-to-wet wt) is used to calculate the total dry mass of the heart.

Glycolysis and glucose oxidation are measured simultaneously in one set of hearts, while palmitate oxidation is

measured in a separate set of hearts. Glycolytic flux is determined by measuring the amount of ^3H_2O released from the metabolism of exogenous [5-^{3}H] glucose (specific activity = 400 Mbq/mol). Glucose oxidation is determined by trapping and measuring $^{14}CO_2$released by the metabolism of [U-^{14}C] glucose (specific activity = 400 Mbq/mol). Palmitate oxidation is determined in separate perfused hearts by measuring the amount of ^3H_2O released from [9,10-^{3}H] palmitate (specific activity = 18.5 Gbq/mol); calculation of palmitate oxidation rates takes into consideration the endogenous fatty acid content of the BSA in the perfusate. Metabolic rates are calculated using the total dry mass of the heart to correct for variations in heart size.

2 **Determination of myocardial oxygen consumption:** Oxygen consumption (MVO_2) is measured at 20 min intervals in hearts that are used for the determination of palmitate oxidation, as the glucose oxidation protocols require that the perfusion apparatus be totally sealed in order to prevent loss of CO_2. Oxygen concentration is measured in pulmonary artery effluent that is collected using a capillary tube (PvO_2). The oxygen content of freshly oxygenated buffer is also determined (PaO_2). Oxygen concentration in the sample is measured using a fiber-optic oxygen sensor (Ocean Optics Inc, Orlando FL). The sensor consists of a probe that is coated with the fluorescent dye, ruthinium red, which emits fluorescence at 600 nm, when excited with a light source that is also integrated with a spectrophotometer. In the presence of oxygen the fluorescence is quenched in proportion to the oxygen content of the sample, and the data are analyzed using accompanying software (Ocean Optics). The following formulae are used to determine myocardial oxygen consumption, cardiac hydraulic work and cardiac efficiency:

MVO_2 (ml/min/g WHW) = ((PaO_2-PvO_2)/100) x (Coronary flow/WHW) x (725/760) x (1000 x C); where C= Bunsen Coefficient for plasma i.e. 0.0212, PaO_2= Arterial partial pressure of oxygen and PvO_2 = Venous partial pressure of oxygen (both in mmHg), 725 and 760 are atmospheric pressures at the University of Utah and at sea level respectively (mmHg) and WHW is the wet heart weight in grams (g).

Cardiac hydraulic work [J/min/g WHW] = CO (ml/Min) x DevP (mm Hg) x 1.33 x 10^{-4} /g WHW where CO = Cardiac output and DevP= Developed pressure

Cardiac efficiency (%) = Hydraulic work /MVO_2 x 100.

MVO_2 (ml/min) was converted to µmol/min by multiplying by the conversion factor 0.0393, and then to Joules (J/min) using the conversion of 1µmol 0_2 = 0.4478 J as described by Suga (7).