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Cardiac lipoprotein lipase activity in the hypertrophied heart may be regulated by fatty acid flux

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ABSTRACT

Cardiac hypertrophy is characterised by an imbalance between lipid uptake and fatty acid β-oxidation leading to an accumulation of lipids, particularly triacylglycerol (TAG). It is unclear whether uptake mechanisms such as lipoprotein lipase (LPL) can be attenuated to diminish this uptake. Rats were cold acclimated to induce cardiac hypertrophy and increase cardiac LPL. Lipid uptake and metabolism were altered by feeding a 'Western-style' high fat diet (WSD) or feeding oxfenicine (2 g/L) in the drinking water. Diastolic stiffness (increased volume change/unit pressure change) was induced in hypertrophied hearts for rats fed WSD ($P<0.05$) or WSD + oxfenicine ($P<0.01$), although absolute performance of cardiac muscle, estimated from stress–strain calculations was unchanged. Cold acclimation increased cardiac endothelial LPL ($P<0.05$) but this was diminished following oxfenicine. Following WSD LPL was further decreased below WSD-fed control hearts ($P<0.05$) with no further decrease by oxfenicine supplementation. A negative correlation was noted between plasma TAG and endothelial LPL (correlation coefficient = −0.654; $P<0.001$) but not cardiac TAG concentration. Transcript levels of angiopoietin-like protein-4 (ANGPTL4) were increased 6-fold by WSD ($P<0.05$) and increased 15-fold following WSD + oxfenicine ($P<0.001$). For CA-hearts fed WSD or WSD + oxfenicine ANGPTL4 mRNA levels were preserved at chow-fed levels. VLDLR protein levels were increased 10-fold ($P<0.01$) by CA. ANGPTL4 protein levels were increased 2-fold ($P<0.05$) by WSD, but restored following oxfenicine. For CA-hearts WSD increased ANGPTL4 protein levels 3-fold ($P<0.01$) with WSD + oxfenicine increasing ANGPTL4 protein 4-fold ($P<0.01$). These data suggest that endothelial LPL levels in the heart are altered to maintain FA flux and may exploit ANGPTL4.

Introduction

The heart has a high and unremitting demand for energy and relies on metabolic flexibility to derive this energy from the prevailing substrates present in plasma [1]. In the fed (postprandial) state the majority of energy is derived from lipids in the diet. These are principally transported in the form of chylomicrons and must be assimilated into the myocardium through lipoprotein lipase, an endothelium-expressed enzyme that controls the transport of triacylglycerol-derived fatty acids (FA) into the cardiomyocyte across the endothelium. Chylomicrons may provide the majority of FA-derived energy as measured for the *in vitro* perfused myocardium [2] and in the whole animal [3]. LPL is intricately coupled to the very-low-density lipoprotein receptor (VLDL-receptor) and investigations have demonstrated the potency of the VLDL-receptor to bind lipoproteins and act as an anchor [4], facilitating the lipolysis of lipoprotein particles to release NEFA and thus generate a high local concentration at the endothelium. The VLDL-receptor may also facilitate uptake of core lipids from lipoproteins [5]. Further studies have also demonstrated the ability of VLDL-receptor to bind LPL directly, and elegant experiments have proposed the exploitation of this mechanism for the translocation and subsequent re-expression of LPL from the cardiomyocyte, across the endothelium to the luminal surface of capillaries [6]. Indeed, the VLDL-receptor-null mouse has low herparin-releasable LPL (hrLPL) activity in muscle and heart [7]. Yet the steps controlling the presentation of LPL at the endothelial surface are unclear.

We have recently demonstrated that chronic activation of AMPK with metformin increased the endothelial localisation of LPL [8]. Given the assertion that the hypertrophied heart is relatively energy-depleted and the subsequent activation of AMPK increases the uptake of substrates (both glucose and fatty acids) to offset this ATP shortfall, suggests that chronic activation of AMPK, as occurs in cardiac hypertrophy [9], and may result in accumulation of lipid in the myocardium through increased translocation of LPL to the capillary endothelium. This may be exacerbated further by the decline in fatty acid oxidation noted for the hypertrophied heart [10]. Intracellular lipid content depends on the balance of uptake (both NEFA and LPL-mediated TAG uptake) and the rate of FA oxidation. Lipid accumulation is believed to contribute to lipotoxicity and alters the sensitivity of the myocardium to catecholamine-mediated inotropy [11], altered insulin signalling [12] and triggers apoptosis [13]. However, experimental models have tended to rely upon constitutive over-expression of LPL at the luminal

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surface of the capillary. What is unclear is whether a mechanism for altering the transfer of LPL to the capillary endothelium is functional in the intact myocardium to prevent the ectopic accumulation of lipid in the cardiomyocyte. Multiple sites are potential targets including the synthesis of LPL, the translation of mRNA to functional enzyme or the transfer of functional LPL from the cardiomyocyte to the endothelial surface of capillaries.

We have exploited the cold-acclimated rat as a model for physiological cardiac hypertrophy that does not show impaired β-oxidation of fatty acids [14] to investigate for the first time the influence of hypertrophy on the expression of LPL and VLDL-receptor proteins and quantify the activity of cardiac lipoprotein lipase following changes to the lipid milieu in the intact rat. Lipid accumulation was initiated using either a Western-style high fat diet and/or the chemical inhibitor of carnitine palmitoyl-transferase 1 (CPT1), oxfenicine. Cardiac performance was estimated and the expression and activity of LPL enzyme quantified to determine whether altering substrate availability can change the presentation of LPL at the cardiac capillary surface.

Materials and methods

Materials

^{3}H-[9,10]-triolein were purchased from Amersham Biosciences (Chalfont, UK). Fatty acid-free bovine albumin and all buffer salts were purchased from Sigma (Poole, UK). All solvents were ANALAR grade and purchased from Fisher Scientific (Loughborough, UK). Kits for the measurement of plasma and tissue triacylglycerol and cholesterol were obtained from Randox (Crumlin, Antrim UK). Ventricular balloons were constructed 'in house' using Saran Wrap polythene film. RT-PCR reagents were obtained from Applied Biosystems (Carlsbad, CA, USA) (assay on demand VLDLR – Rn01498163_m1: LPL – Rn01446981_m1: ANGPTL4 – Rn01528817_m1: Internal reference GAPDH – 4308313).

Methods

Animals

Animals were maintained in accordance with the UK Home Office, Animal Scientific Procedures Act (1986) and the experiments were approved by the University of Birmingham Ethical Review Committee. Animals were housed at 22 °C 12 h light/12 h dark with *ad libitum* access to food and water.

Cold acclimation

Animals were acclimated to cold as previously outlined [14,15]. Briefly rats (60gm) were housed in pairs in an environmental chamber with minimal wood-chip cage bedding and ad libitum access to both food and water. The chamber was cooled from 21 °C to 4 °C over a period of 4 weeks, with day length reduced from 12 h light/dark to 1 h light/23 h dark over the same period. After this 4 week period rats were randomised into separate groups and maintained on either a chow diet or a Western-style high fat/sucrose diet (WSD-824503, Special Diet Services, Lilico Biotechnology, UK) for a further 2 weeks. These two groups were further sub-divided into groups receiving either tap water to drink or water supplemented with 4-hydroxy-phenyl-glycine (Oxfenicine — 2 g/L) [16], giving a total of 4-experimental groups. These groups were duplicated using control animals maintained under standard conditions of temperature and day length, but supplemented with either chow diet or WSD and water or oxfenicine solution (2 weeks–2 g/L oxfenicine).

Tissue isolation and heart perfusion

Animals were prepared surgically following 6 weeks of diet/temperature manipulation as outlined previously [17]. Briefly, anaesthesia was induced with isoflurane (~4% isoflurane in oxygen). Blood was collected in a heparinised syringe from inferior vena cava and following thoracotomy, hearts were excised. Hearts were perfused in retrograde fashion, as outlined previously [17]. A small flexible non-elastic balloon was inserted into the left atrium through the mitral valve and into the left ventricle. This fluid-filled balloon was attached to a fine plastic catheter and connected to a pressure transducer (MEMSCAP, Skoppum, Norway) and a graduated syringe (0–1000 μl: Hamilton, Nevada, USA). Hearts were maintained at 37 °C and perfused at a constant pressure (100 cm H_2O) with a Krebs–Henseleit crystalloid medium supplemented with glucose (10 mM) and $CaCl_2$ (1.3 mM) gassed with oxygen/CO_2 (95:5). Developed pressure was measured following isovolumic contraction of the fluid-filled balloon and recorded to computer using a digital interface (AD Instruments, Chalgrove, Oxford, UK).

Ventricular performance

Ventricular performance was estimated, as outlined previously [17]. Balloon volume was increased in incremental steps (50 μl) and developed pressure was recorded in real time. Pressures were allowed to stabilise until diastolic pressure remained constant before initiating further increases in balloon volume. Incremental increases in balloon volume were performed until the peak systolic pressure developed exceeded 200 mmHg. The balloon was then deflated and the process repeated. Coronary flow was estimated from timed collections of a known volume of perfusate and expressed as volume/unit time/unit mass of cardiac tissue. Ventricular performance was calculated off-line following the experiment using computer analysis software (Chart Version 5.0, AD Instruments, Chalgrove, Oxford, UK). Heart rate, systolic pressure and diastolic pressure were measured and hence developed pressure calculated. Rate of change of pressure (+dP/dt) was calculated from the maxima of first order derivative of pressure trace. Rate pressure product (RPP) was calculated at each balloon volume as the product of heart rate (bpm) x developed pressure (mmHg).

Contractile reserve

For selected hearts, ventricular balloon volume was adjusted to give a stable end-diastolic pressure (20 mmHg). Estimates of cardiac performance were made prior to, and after, addition of the sympathomimetic inotrope isoprenaline (final concentration 10 μM) to the perfusate. Increases in developed pressure and rate of pressure development were estimated once stable cardiac performance was achieved.

Cardiac stress–strain calculations

Cardiac stress–strain calculations were undertaken as detailed in Woodiwiss and Norton [18] with modifications [19]. Briefly, LVEDV was estimated as detailed previously for perfused hearts (Cheng and Hauton 2008). LV wall volume was calculated from LV wall wet mass x 0.943 [18]. Data was represented as gradients of linearised stress–strain relationships, following linear regression analysis, for animals within the same treatment group.

Total lipid extraction

Total cholesterol, phospholipid and triglycerides were also extracted from liver and heart tissue as described previously [15]. Briefly, aliquots (100 mg) of tissue powder were extracted with methanol:chloroform (1:2). Extracts were evaporated to dryness and resuspended in absolute ethanol. Hepatic and cardiac TAG, phospholipid and cholesterol were measured using commercial kits.

Lipid infiltration

Lipid infiltration was estimated from tissue sections stained with Oil Red 'O' [20]. Briefly, frozen sections (10 μm) were air-dried and fixed in formaldehyde solution (3.7%w/v). Sections were rinsed in distilled water and stained with Oil Red 'O' (300 mg/ml in 36% v/v triethyl phosphate in distilled water). Sections were de-stained in distilled water before mounting. Lipid droplets were visualised by fluorescence microscopy with Texas red filter (×200 magnification). 3 non-