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Original article

Cyclic GMP signaling in cardiomyocytes modulates fatty acid trafficking and prevents triglyceride accumulation

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Abstract

While the balance between carbohydrates and fatty acids for energy production appears to be crucial for cardiac homeostasis, much remains to be learned about the molecular mechanisms underlying this relationship. Given the reported benefits of cGMP signaling on the myocardium, we investigated the impact of its chronic activation on cardiac energy metabolism using mice overexpressing a constitutively active cytoplasmic guanylate cyclase ($GC^{+/0}$) in cardiomyocytes. *Ex vivo* working $GC^{+/0}$ heart perfusions with ^{13}C-labeled substrates revealed an altered pattern of exogenous substrate fuel selection compared to controls, namely a 38±9% lower contribution of exogenous fatty acids to acetyl-CoA formation, while that of carbohydrates remains unchanged despite a two-fold increase in glycolysis. The lower contribution of exogenous fatty acids to energy production is not associated with changes in energy demand or supply (contractile function, oxygen consumption, tissue acetyl-CoA or CoA levels, citric acid cycle flux rate) or in the regulation of β-oxidation (acetyl-CoA carboxylase activity, tissue malonyl-CoA levels). However, $GC^{+/0}$ hearts show a two-fold increase in the incorporation of exogenous oleate into triglycerides. Furthermore, the following molecular data are consistent with a concomitant increase in triglyceride hydrolysis: (i) increased abundance of hormone sensitive lipase (HSL) protein (24±11%) and mRNA (22±4%) as well as (ii) several phosphorylation events related to HSL inhibitory (AMPK) and activation (ERK 1/2) sites, which should contribute to enhance its activity. These changes in exogenous fatty acid trafficking in $GC^{+/0}$ hearts appear to be functionally relevant, as demonstrated by their resistance to fasting-induced triglyceride accumulation. While the documented metabolic profile of $GC^{+/0}$ mouse hearts is partly reminiscent of hypertrophied hearts, the observed changes in lipid trafficking have not been previously documented, and may be part of the molecular mechanism underlying the benefits of cGMP signaling on the myocardium.

Keywords: Hormone sensitive lipase; Energy metabolism; Guanylate cyclase; Perfusion; Isotopes

1. Introduction

In the healthy adult heart, the concerted regulation of long chain fatty acid and carbohydrate metabolism ensures optimal energy production and, hence, cardiac homeostasis. Likewise, alterations in cardiac substrate metabolism are considered as independent determining factors that contribute to contractile dysfunction, to the heart's susceptibility to injury, and to progression from compensated left ventricular hypertrophy to cardiac failure [1,2]. However, much remains to be learned about the (patho)physiological significance of specific alterations in cardiac substrate utilization beyond their effect on ATP production. For example, while a shift from fatty acid towards carbohydrate utilization for

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energy production (a characteristic of the hypertrophied heart [1,2]) has been shown to be beneficial for ischemic and failing hearts [1,2], decreased fatty acid oxidation may also lead to potentially detrimental consequences such as intracellular lipid accumulation and its associated lipotoxic sequelae [3,4].

Molecular mechanisms regulating the balance between carbohydrate and fatty acid utilization for energy production or storage involve the participation of signaling pathways. Among the latter, AMP kinase (AMPK) and protein kinase B (Akt) have been the subject of active research [5–7]. Recently, we became interested in cyclic GMP (cGMP), a downstream effector of the nitric oxide (NO) and natriuretic peptide pathways, since cGMP exerts a host of cardioprotective effects [8,9]. In this regard, our group has developed a mouse transgenic model overexpressing the constitutively active catalytic fragment of the guanylate cyclase domain of the atrial natriuretic factor (ANF) receptor in a cardiomyocyte-specific manner ($GC^{+/0}$) [10]. We have shown that expression of this transgene was accompanied by increased guanylate cyclase activity and cGMP concentration in isolated cardiomyocytes compared to non-transgenic littermates, and that it protects these mice against the hypertrophic effects of isoproterenol or abdominal aortic constriction [10]. Likewise, expression of the transgene improves the cardiac function of mice carrying *mdx* mutation of dystrophin, along with a increased of cGMP concentration in whole-heart extracts [11].

Interestingly, a number of studies have reported that NO or cGMP mimetics modulate energy metabolism in various tissues by influencing substrate selection for ATP production, expression of metabolic genes as well as genes of the nutrient signaling pathways [12–16]. However, there appears also to be a complex relationship between NO, the cGMP pathway and energy metabolism in the heart, which differs from that in the skeletal muscle and depends on many factors such as the level of myocardial activation of AMPK or contractility, as well as the (sub)cellular location of NO/cGMP production [17]. For example, myocardial glucose uptake or utilization are (i) enhanced following addition of NO synthase inhibitors [14] or in eNOS null mouse [18], and, conversely, (ii) decreased with addition of the cGMP analog 8-bromo-cGMP or of NO donors [15]. In contrast, a recent study shows that activation of the cGMP pathway contributes to the AMPK stimulation of glucose uptake in left ventricular papillary muscle [19]. Hence, much remains to be learned about the metabolic impact of enhanced cGMP signaling in cardiomyocytes.

To address this question, we used our previously described methodology of *ex vivo* working heart perfusion with ^{13}C-labeled substrates [20] to measure simultaneously various hemodynamic and metabolic flux parameters in our $GC^{+/0}$ transgenic mice. This approach allows for detailed and simultaneous measurements of the dynamics of cardiac energy substrate metabolism, information which is not accessible from static measurements of mRNA or protein expression. Our isotopic data demonstrate substantial differences in substrate selection for energy production as well as in lipid partitioning between β-oxidation and esterification between control and $GC^{+/0}$ mice hearts. Additional molecular data are consistent with a concomitant increase in triglyceride (TG) hydrolysis.

2. Experimental procedures

2.1. Materials and animal model

Sources of chemicals, biological products, and ^{13}C-substrates have been reported previously [20–26]. Antibodies against the phosphorylated forms of hormone-sensitive lipase (HSL) and extracellular-regulated kinase (ERK) forms 1 and 2 were purchased from Cell Signaling Technologies (Danvers, USA), and total HSL antibody was obtained from Cayman Chemicals (Ann Arbor, USA).

All procedures on the animals were approved by the local ethics committee in agreement with the guidelines of the Canadian Council on Animal Care. Our $GC^{+/0}$ transgenic mice [10] have been backcrossed for at least 12 generations into the C57Bl/6J mouse strain. We used male transgenic and age-matched wild-type (WT) littermates mice at 12 to 13 weeks of age, all of which had similar body weights (25.7±0.7 vs 26.3±0.6 g, respectively).

2.2. Working mouse heart perfusion

Mice were anesthetized (1 μL/g, i.p.) with a mixture of ketamine (100 mg/mL) and xylazine (20 mg/mL) and heparinized (5000 U/kg, i.p.) 15 min before surgery. The procedure for heart isolation and its *ex vivo* perfusion in the working mode has been previously described in detail [20]. The composition of the Krebs–Henseleit buffer (110 mM NaCl, 4.7 mM KCl, 2.1 mM $CaCl_2$, 0.24 mM KH_2PO_4, 0.48 mM K_2HPO_4, 0.48 mM Na_2HPO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 0.1 mM EDTA) was modified to adjust free calcium levels (1.55±0.02 mM) and sodium concentration to a physiological value. The preload and afterload pressures were set at 15 and 50 mmHg, respectively. Myocardial oxygen consumption (MVO_2; μmol/min), intracellular pH, rate pressure product (mm Hg·beats·min^{-1}·10^{-3}), cardiac power (mW), and cardiac efficiency (mW·μmol^{-1}·min^{-1}) were calculated from previously reported equations [20].

Working mouse hearts were perfused for 30 min with a semi-recirculating modified Krebs–Henseleit solution containing physiological concentrations of substrates (11 mM glucose, 0.8 nM insulin, 50 μM carnitine, 5 nM epinephrine, 1.5 mM lactate, 0.2 mM pyruvate, and 0.4 mM oleate bound to 3% albumin). For any given perfusion, one of the unlabeled substrates was replaced by its corresponding labeled substrate, i.e. either: [U-$^{13}C_{18}$]oleate (25% initial molar percent enrichment (MPE)), [U-$^{13}C_6$]glucose (25% initial MPE), and [U-$^{13}C_3$]lactate/[U-$^{13}C_3$]pyruvate (100% initial MPE).

Throughout the perfusion, influent and effluent perfusates were collected at regular intervals to document lactate dehydrogenase (LDH) release rates (every 5 min), the oxygen and carbon dioxide partial pressures (at 10 and 20 min) and the lactate and pyruvate efflux rates (at 30 min). Subsequent to each perfusion period, hearts were freeze-clamped with metal tongs chilled in liquid nitrogen and weighed. There were no significant differences in the wet weight of perfused hearts between groups (data not shown). All samples were stored at −80 °C until further analysis.