Original article

MOLECULAR METABOLISM

Cardiomyocyte glucagon receptor signaling modulates outcomes in mice with experimental myocardial infarction

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ABSTRACT

Objective: Glucagon is a hormone with metabolic actions that maintains normoglycemia during the fasting state. Strategies enabling either inhibition or activation of glucagon receptor (Gcgr) signaling are being explored for the treatment of diabetes or obesity. However, the cardiovascular consequences of manipulating glucagon action are poorly understood.

Methods: We assessed infarct size and the following outcomes following left anterior descending (LAD) coronary artery ligation; cardiac gene and protein expression, acylcarnitine profiles, and cardiomyocyte survival in normoglycemic non-obese wildtype mice, and in newly generated mice with selective inactivation of the cardiomyocyte Gcgr. Complementary experiments analyzed Gcgr signaling and cell survival in cardiomyocyte cultures and cell lines, in the presence or absence of exogenous glucagon.

Results: Exogenous glucagon administration directly impaired recovery of ventricular pressure in ischemic mouse hearts *ex vivo*, and increased mortality from myocardial infarction after LAD coronary artery ligation in mice in a p38 MAPK-dependent manner. In contrast, cardiomyocyte-specific reduction of glucagon action in adult $Gcgr^{CM-/-}$ mice significantly improved survival, and reduced hypertrophy and infarct size following myocardial infarction. Metabolic profiling of hearts from $Gcgr^{CM-/-}$ mice revealed a marked reduction in long chain acylcarnitines in both aerobic and ischemic hearts, and following high fat feeding, consistent with an essential role for Gcgr signaling in the control of cardiac fatty acid utilization.

Conclusions: Activation or reduction of cardiac Gcgr signaling in the ischemic heart produces substantial cardiac phenotypes, findings with implications for therapeutic strategies designed to augment or inhibit Gcgr signaling for the treatment of metabolic disorders.

Keywords Glucagon; Myocardial infarction; Glucagon receptor; Cardiomyocytes; Heart; Diabetes; Fatty acid metabolism

1. INTRODUCTION

Glucagon is a 29 amino acid peptide hormone secreted from pancreatic islet α-cells that plays a critical role in maintenance of euglycemia, predominantly by increasing hepatic glucose output. Activation of glucagon receptor (Gcgr) signaling promotes glycogenolysis and enhanced gluconeogenesis, and regulates pathways controlling hepatic lipid oxidation and lipid secretion. Although the actions of glucagon are classically viewed as essential for prevention of hypoglycemia in the face of limited nutrient availability or excess insulin action [1], Gcgr signaling also controls cell survival pathways, as genetic interruption of Gcgr signaling increases the susceptibility to hepatic injury [2].

A single Gcgr is expressed not only in liver, but in extrahepatic tissues including the central and peripheral nervous system, pancreatic islets, adipose tissue, kidney, blood vessels and heart [3,4]. In the pancreas, glucagon potentiates glucose-dependent insulin secretion, whereas activation of Gcgr signaling in the brain regulates hepatic glucose production, control of appetite and body weight [5]. Glucagon actions in adipose tissue and kidney are less understood, but have been linked to control of fatty acid and glucose metabolism.

Although glucagon levels normally decrease during a meal, glucagon secretion is inappropriately increased in many subjects with type 2 diabetes (T2D) [1,6]. Over the last several decades, experimental studies attenuating glucagon action using glucagon immunoneutralizing antisera, Gcgr antagonists, antisense *Gcgr* oligonucleotides and

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Gcgr$^{-/-}$ mice have demonstrated amelioration of hyperglycemia in experimental models of diabetes [1,7]. Collectively, these findings have raised enthusiasm for glucagon antagonism as a potential therapeutic strategy for T2D. Indeed, GCGR antagonists and antisense oligonucleotides targeting hepatic GCGR expression robustly lower glucose in clinical trials of human subjects with T2D. However, mechanism-based toxicities noted in preclinical studies, including dyslipidemia, and transaminase elevations [2,8], have also been reported in clinical studies. Hence, the risk:benefit proposition for partial attenuation of GCGR signaling in diabetic humans requires further evaluation.

Complementary efforts are exploring whether partial enhancement of glucagon action, together with agonism of the glucagon-like peptide-1 receptor (GLP-1R), may be useful for the treatment of diabetes and/or obesity [9,10]. Oxyntomodulin, a naturally occurring proglucagon-derived peptide, contains the 29 amino acid sequence of glucagon plus a carboxyterminal extension and exerts potent glucoregulatory and anorectic actions in rodents and humans through activation of the GLP-1 and glucagon receptors [11,12]. More recent studies have demonstrated that simultaneous activation of the glucagon and GLP-1 receptors using synthetic balanced co-agonists produces potent glucoregulatory activity and greater weight loss than observed with GLP-1R agonists alone [13]. Hence there is also considerable interest in understanding the metabolic consequences and therapeutic potential arising from partial selective activation of GCGR signaling.

The increasing interest in development of drugs that reduce or activate GCGR signaling for the treatment of metabolic disorders such as diabetes and obesity raises important questions about the cardiovascular actions and safety of such agents. Current understanding of glucagon action in the heart is limited, and activation of Gcgr signaling in this organ has been reported to be either beneficial or harmful, depending on the experimental or clinical context [14–17]. We have now examined the consequences of manipulating Gcgr signaling in the non-diabetic ischemic mouse heart. Our findings reveal that exogenous glucagon impairs survival following ligation of the left anterior descending (LAD) coronary artery, actions requiring p38 MAP kinase. In contrast, *Gcgr*$^{CM-/-}$ mice with cardiac-specific inactivation of the *Gcgr* display a cardioprotective phenotype, associated with reduced accumulation of incompletely oxidized fatty acid metabolites in the heart. These findings have implications for pharmaceutical efforts directed at manipulating GCGR signaling for the treatment of human disease.

2. METHODS

2.1. Mice and reagents

Inducible αMHC^{Cre} (stock 005657) [18] and FLPe (stock 005703) transgenic mice in the C57BL/6 background were obtained from the Jackson Laboratory. *Gcgr*$^{CM-/-}$ mice were generated by crossing α*MHC*^{Cre} mice with *Gcgr*^{Flox} mice [19] in the C57BL/6 background. LAD coronary artery ligation was used to induce myocardial infarction (MI) in 12–14-week-old male mice as described in Ref. [20]. All mice were housed (5 per cage) under a light/dark cycle of 12 h in the Toronto Centre for Phenogenomics (TCP) animal facility, with free access to food and water except where noted. All procedures were conducted according to protocols and guidelines approved by the TCP Animal Care Committee. Genotypes were determined through analysis of genomic DNA prepared from tail snips. Tamoxifen (Sigma Aldrich, 50 mg/kg) dissolved in corn oil was administered for 5 consecutive days to 6- or 7-week-old male α*MHC*^{Cre} or *Gcgr*$^{CM-/-}$ mice to induce Cre expression. Before any cardiac assessment or procedure, all mice were allowed 6 weeks to recover after the last tamoxifen injection, as Cre expression in the heart often induces a transient cardiomyopathy that dissipates 5 weeks after tamoxifen-induced Cre expression [21]. Glucagon (Sigma) 30 ng/g body weight or saline in 10% gelatin was administered to C57BL/6 mice as described in Ref. [8], 3 injections daily, with or without 2 injections daily of 1 μmol/g body weight SB203580 (p38 MAPK inhibitor, Sigma) for 7 days. Blood pressure and heart rates were measured using a telemetry system (DSI technology) as described in Ref. [22]. The rat *Gcgr* adenovirus (Ad*Gcgr*) has been described previously in Ref. [2].

2.2. Ischemia/reperfusion

Global no-flow ischemia in Langendorff-perfused hearts was induced as described in Ref. [23]. Hearts underwent a 30 min aerobic perfusion with Krebs-Henseleit buffer, followed by a 30 min global no-flow ischemia, and either a 50 or 60 min reperfusion period during which left ventricular developed pressure (LVDP) was recorded (Biopac Systems Canada Inc.). In a separate set of hearts 1 μg/mL glucagon was administered 20 min prior to ischemia.

2.3. Myocardium metabolic profiling

Mass spectrometry-based metabolic profiling was performed to determine myocardial levels of acylcarnitines and organic acids [24]. Triacylglycerol (TAG) was extracted from frozen myocardial tissue (~20 mg) with a 2:1 chloroform-methanol solution and quantified with a commercially available enzymatic assay kit (Wako Pure Chemical Industry) as described in Ref. [25].

2.4. Heart histology

Animals were anesthetized using avertin (250 mg/kg body weight ip injection). The chest cavity was opened to expose the heart and 1 M KCl was injected into the apex to arrest the heart in diastole. The heart was perfusion-fixed with 4% buffered formalin at physiological pressure, post-fixed in formalin, embedded in paraffin, and sectioned at 6 μm, and stained with Masson's Trichrome or processed for Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). TUNEL staining was performed using the ApopTag peroxidase kit for apoptosis (EMD Millipore). Cardiac morphometry was performed on mid-ventricular cross-sections using Aperio ImageScope Viewer software (Aperio Technologies). The infarcted area was calculated as a % of total LV area. Cardiac hypertrophy was quantified as the heart weight-to-body weight ratio.

2.5. Glucose tolerance

12–14-week-old male mice were fasted overnight (16–18 h), and glucose (1.5 mg/g body weight) was administered orally (through a gavage tube) or via injection into the peritoneal cavity (intraperitoneal glucose tolerance test). Blood samples were drawn from the tail vein at 0, 15, 30, 60, 90, and 120 min post-glucose administration, and blood glucose and insulin levels were measured as described in Ref. [26].

2.6. Western blotting

Hearts were collected from fasted mice (5 h) 30 min following ischemia or sham surgery, washed in Krebs buffer containing 11 mM glucose and frozen. Frozen hearts were powdered and homogenized in buffer containing 50 mM Tris HCl, pH 8, 1 mM EDTA, 10% glycerol, 0.02% Brij-35. Western blotting was carried out as described in Refs. [27,28] and blots were visualized using an enhanced chemiluminescence Western blot detection kit (Perkin Elmer) and quantified with Carestream Molecular Imaging Software (Kodak).

