Journal of Molecular and Cellular Cardiology 76 (2014) 208–217

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

Protein kinase-D1 overexpression prevents lipid-induced cardiac insulin resistance

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ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form 19 August 2014
Accepted 21 August 2014
Available online 28 August 2014

Keywords:
Cardiomyopathy
Fatty acid transport
Glucose transport
Insulin resistance
Protein kinase D

ABSTRACT

In the insulin resistant heart, energy fuel selection shifts away from glucose utilization towards almost complete dependence on long-chain fatty acids (LCFA). This shift results in excessive cardiac lipid accumulation and eventually heart failure. Lipid-induced cardiomyopathy may be averted by strategies that increase glucose uptake without elevating LCFA uptake. Protein kinase-D1 (PKD1) is involved in contraction-induced glucose, but not LCFA, uptake allowing to hypothesize that this kinase is an attractive target to treat lipid-induced cardiomyopathy. For this, cardiospecific constitutively active PKD1 overexpression (caPKD1)-mice were subjected to an insulin resistance-inducing high fat-diet for 20-weeks. Substrate utilization was assessed by microPET and cardiac function by echocardiography. Cardiomyocytes were isolated for measurement of substrate uptake, lipid accumulation and insulin sensitivity. Wild-type mice on a high fat-diet displayed increased basal myocellular LCFA uptake, increased lipid deposition, greatly impaired insulin signaling, and loss of insulin-stimulated glucose and LCFA uptake, which was associated with concentric hypertrophic remodeling. The caPKD1 mice on high-fat diet showed none of these characteristics, whereas on low-fat diet a shift towards cardiac glucose utilization in combination with hypertrophy and ventricular dilation was observed. In conclusion, these data suggest that PKD pathway activation may be an attractive therapeutic strategy to mitigate lipid accumulation, insulin resistance and maladaptive remodeling in the lipid-overloaded heart, but this requires further investigation.
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1. Introduction

Heart disease, often presenting as cardiomyopathy, is the leading cause of death among patients with type-2 diabetes [1,2]. It is the metabolic component of diabetes that has been increasingly implicated as the primary factor leading to cardiac dysfunction in these patients [3,4]. In particular, altered substrate utilization by the heart has been recently positioned as causative to the development of diabetic cardiomyopathy [3,5].

GLUT4 and CD36 are the main cardiac glucose and long-chain fatty acid (LCFA) transporters, respectively, and are critical regulatory sites in substrate utilization [5–7]. In the healthy heart, both GLUT4 and CD36 are stored in endosomal compartments, from where these transporters can translocate to the sarcolemma to increase glucose and LCFA uptake. GLUT4 and CD36 translocation appear to be similarly regulated, since the same physiological stimuli, i.e., circulating insulin levels and increased contractile activity, induce their simultaneous translocation to the sarcolemma [8].

The diabetic heart displays increased LCFA utilization at the expense of glucose. The greater influx of LCFA is due to a permanent CD36 relocation from intracellular stores to the sarcolemma [5], and results in the gradual myocellular build-up of triacylglycerols (TAG), diacylglycerols (DAG) and other lipid species. Especially, DAG activate Ser/

Abbreviations: AMPK, AMP-activated protein kinase; AW, anterior wall; BNP, B-type natriuretic peptide; caPKD1, cardiospecific constitutively active PKD1 overexpression; DAG, diacylglycerol; EFS, electric field stimulation; [^{18}F]FDG, [^{18}F]fluoro-deoxyglucose; [^{18}F]FTHA, 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid; GSK3, glycogen synthase kinase-3; HDAC5, histone deacetylase-5; LCFA, long-chain fatty acids; LFD, low fat diet; LV, left ventricular; microPET, micro-positron emission tomography; PKD1, protein kinase-D1; SUV, standardized uptake value; TAG, triacylglycerol; WD, Western diet; WT, wild-type.

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http://dx.doi.org/10.1016/j.yjmcc.2014.08.017
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Thr-kinase cascades, which impair upstream insulin signaling, and subsequently reduce insulin-stimulated glucose uptake [9]. Over time, both excessive lipid accumulation and progressive insulin resistance will gradually initiate a remodeling process in the heart, which at first appears to be a compensatory adaptation, but which thereafter turns maladaptive [10,11].

Strategies to stimulate glucose uptake in the diabetic heart, without increasing LCFA uptake, may be well suited to restore the substrate balance and combat the diabetic state. Insulin-induced GLUT4 translocation is defective in the type-2 diabetic heart [12]. Therefore, activation of signaling mechanisms involved in contraction-induced GLUT4 translocation may be excellent targets to increase cardiac glucose utilization during insulin resistance [13]. We expect that greater glucose influx into the diabetic heart would counterbalance excessive lipid usage, and eventually recuperate contractile function [5].

One of the best-studied proteins in the signaling events induced by contraction is AMP-activated protein kinase (AMPK). Unfortunately, AMPK stimulates not only the translocation of GLUT4 to the sarcolemma but simultaneously also that of CD36 [14]. Thus, AMPK activation would not be capable of shifting the excessive LCFA utilization in the insulin resistant heart towards a more balanced utilization of glucose and LCFA.

Another kinase involved in contraction-induced GLUT4 translocation is protein kinase-D1 (PKD1). This kinase is the defining member of a novel class of Ser/Thr kinases belonging to the PKD family [15]. Recently, we have shown that PKD1 is activated by contraction [16]. Importantly, silencing of PKD1 in HL1-cardiomyocytes resulted in loss of contraction-induced glucose uptake whereas contraction-induced LCFA uptake was retained [17]. Hence, we hypothesized that activation of this kinase would shift cardiac substrate utilization towards glucose.

Cardiospecific overexpression of PKD1 in mice on a normal diet leads to hypertrophy, ventricular dilatation and impaired systolic function [18]. This maladaptive hypertrophic remodeling is associated with and thought to be due to increased phosphorylation of HDAC5, a direct substrate of PKD [19]. Subsequently, this phosphorylation disattaches HDAC5 from the hypertrophic transcription factor MEF2, thereby inducing the transcription of MEF2-regulated hypertrophic genes [20]. Notwithstanding these adverse effects of PKD1 upregulation in hearts of mice under a normal diet regime, PKD1 overexpression might still be beneficial in the lipid-overloaded insulin resistant heart to counterbalance the impairment in glucose uptake. Therefore, we subjected mice cardiospecifically overexpressing PKD1 to a Western diet (WD) in order to investigate whether increased expression/activity of this kinase would be protective against lipid-induced insulin resistance and lipid-induced cardiac remodeling.

2. Materials and methods

2.1. Animals

Cardiac-specific constitutively active PKD1 overexpression (caPKD1) mice were a kind gift from Prof. Dr. Eric Olson (University of Texas Southwestern Medical Center, Dallas, USA). Breeding was performed with founder pairs of heterozygous mice. Both male and female mice were taken from a restricted number of nests, containing wild type as well as transgenic animals. From earlier studies with cardiomyocytes or hearts from wild-type (WT) mice and various transgenic mouse models, we found that metabolic and signaling parameters were not different between both sexes [14,16,17,21]. From the age of 12 weeks, caPKD1 mice were administered with a low fat diet (LFD) containing 10 en% fat (D12450B, Research Diets) or WD containing 45 en% fat (D12451, Research Diets). For this, male and female mice were divided over the different experimental groups as unpaired littermates. In all experiments in this study, the number of male and female mice was about equal. Animals were maintained at the Experimental Animal Facility of Maastricht University, and had free access to food and water. All study protocols involving the animal experiments were approved by the Animal Care and Use Committee of Maastricht University and were performed according to the official rules formulated in the Dutch law on care and use of experimental animals, highly similar to those of the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Blood glucose and insulin levels

After 4 h of fasting, blood was collected via the tail vein. To measure blood glucose levels, a blood glucose monitor was used (OneTouch Ultra, Milpitas, CA, USA). Insulin plasma levels were measured by performing an insulin ELISA (Millipore, Billerica, MA, USA).

2.3. Isolation and treatment of adult mouse cardiomyocytes

Cardiomyocytes were isolated from male and female WT (C57BL/6) or transgenic mice as previously described [21]. Briefly, mice were anesthetized via one intraperitoneal injection with pentobarbital (120 mg/kg body mass; pharmacy of the Faculty of Veterinary Medicine, Utrecht, Netherlands). Then, these mice were examined on the adequacy of the anesthesia via monitoring eye and leg reflexes. Hearts were excised and perfused using a Langendorff perfusion system and Liberase blendzyme-1 (Roche Diagnostics, Indianapolis, IN) for isolation of cardiomyocytes. Upon 90 min of recovery after the isolation procedure, cardiomyocytes were subjected to electric field stimulation (EFS)[1] for 4 min at 37 °C (2 Hz, 40 V, pulse duration 10 msec) or incubated with 100 nM insulin for 15 min. Cellular uptake of [^{3}H]deoxyglucose and [^{14}C]palmitate was measured as previously described [21]. In short, 0.5 ml of a mixture of [1-^{14}C]palmitate/BSA complex and [1-^{3}H] deoxyglucose was added at the start of the incubations so that the final concentration of both substrates amounted to 100 μM with a corresponding palmitate/BSA ratio of 0.3. Cellular uptake was determined after 5 min, which time point is within the initial uptake phase (i.e., the phase in which uptake of both substrates proceeds linearly with time). Within this phase there is no release of CO_2, which production starts at >10 min after [1-^{14}C]palmitate addition [22]. For assessment of substrate uptake, cells were washed three times in an ice-cold stop solution containing 0.2 mM phloretin, and subsequently lysed in 5 ml Opti-Fluor scintillation fluid (PerkinElmer, Waltham, USA).

2.4. Immunoblotting

Proteins were separated by SDS-PAGE (4–12% Bis-Tris Criterion XT precast gels; Biorad, Hercules, USA) and transferred to nitrocellulose membranes for Western blotting. The protein bands were visualized as previously described [16]. Antibodies against Akt (phospho-Ser473), caveolin-3, ERK1/2 (phospho-Thr202/Tyr204), glycogen synthase kinase-3β (phospho-Ser9), mammalian target of rapamycin (phopho-Ser2448), p38 (phospho-Thr180/Tyr182), p70 S6 kinase (phospho-Thr389), PKD1 and PKD1 (phospho-Ser744/748) were purchased from Cell Signalling Technologies (Danvers, USA). The antibody directed against GLUT4 was obtained from Santa Cruz Biotechnology (Santa Cruz, USA), the antibody against mouse CD36 was from Chemicon (Billerica, USA), and the antibodies against phospho-Ser498-histone deacetylase-5 and the OXPHOS complexes were from Abcam (Cambridge, UK).

2.5. RT-PCR

Total RNA was isolated from snap frozen heart tissue using the mirVana Isolation Kit (Ambion) and treated with DNase I (DNA-free, Ambion) to remove residual DNA. 700 ng of the total RNA was reverse transcribed using the miScript II RT Kit (Qiagen). RT-qPCR was performed using SybrGreen (Bio-Rad) and the following primer sequences (5′ → 3′): GTTTGGGCTGTAACGCACTGA (Bnp-F), GAAAGAGACCCAGG CAGAGTCA (Bnp-R), CGCTTCGGCAGCACATATAC (U6-F) and TTCACG AATTTGCGTGTCAT (U6-R).

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