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Activation of Akt kinase accompanies increased cardiac resistance to ischemia/reperfusion in rats after short-term feeding with lard-based high-fat diet and increased sucrose intake

Monika Ivanova [a,*], Pavol Janega [b,c], Jana Matejikova [a], Petra Simoncikova [a], Dezider Pancza [a], Tanya Ravingerova [a], Miroslav Barancik [a]

[a] Institute for Heart Research, Slovak Academy of Sciences, PO Box 104, 840 05, Bratislava, Slovakia
[b] Department of Pathology, Faculty of Medicine, Comenius University, 813 72, Bratislava, Slovakia
[c] Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, 813 71, Bratislava, Slovakia

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Abstract

High-fat or high-carbohydrate food consumption contributes to changes in myocardial tolerance to ischemia. However, with respect to experimental models, most studies used diets with very high doses of cholesterol, saturated fatty acids, or fructose. In our study, we fed rats a high-fat diet based on lard in combination with administration of a sweet beverage (30% sucrose solution) (high-fat sucrose diet [HFS]). This diet was used to simulate the unhealthy dietary habit typical for developed countries. We hypothesized that the application of HFS diet for 48 days might initiate progression of pathologic changes in the heart associated with myocardial remodeling and activation of adaptive mechanisms. We investigated the influence of HFS diet on cardiac function and vulnerability to ischemia-reperfusion (I/R) injury in Langendorff-perfused rat hearts subjected to 30-minute global ischemia and 120-minute reperfusion as well as on Akt kinase and matrix metalloproteinases. We found lower food consumption in HFS group compared with controls, but a significant increase in visceral fat mass and concentrations of triacylglycerol, low-density lipoprotein, and very low-density lipoprotein cholesterol. Baseline heart functional parameters and their postischemic recovery were not affected by HFS diet. On the other hand, hearts of HFS group were more resistant to lethal I/R injury manifested by significantly smaller infarct size. In addition, there was lower content of collagen I and III in the left ventricle associated with Akt kinase activation and matrix metalloproteinase 9 up-regulation. In conclusion, feeding rats with HFS diet resulted in heart remodeling associated with activation of some adaptive mechanisms, which can contribute to modulation of myocardial resistance to I/R injury.

Keywords: High-fat and sucrose diet; Rat heart; Ischemia-reperfusion; Akt kinase; Physiologic adaptation

Abbreviations: AR, area at risk; BW, body weight; CF, coronary flow; FA, fatty acids; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HFS, high-fat sucrose diet; HR, heart rate; I/R, ischemia-reperfusion; IR, insulin resistance; IS, infarct size; ITT, insulin tolerance test; LV, left ventricle; LVDiP, LV diastolic pressure; LVDP, LV developed pressure; LVSP, LV systolic pressure; MMP, matrix metalloproteinase; RV, right ventricle; SBP, systolic blood pressure; SC, standard rat chow; TAG, triacylglycerols.

1. Introduction

In Western countries, both dietary sugars and saturated fatty acids represent important components of human diets.

* Corresponding author. Tel.: +421 2 5477 4405; fax: +421 2 5477 6637.
E-mail address: usrdmost@savba.sk (M. Ivanova).

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Research has shown that high-fat or high-carbohydrate food consumption contributes to high blood cholesterol levels and a greater risk of coronary artery disease. It is often associated with the development of insulin resistance, hypertension, and dyslipidemia [1,2].

Several experimental studies have investigated the effects of dietary fat or sugar consumption on cardiovascular morbidity or susceptibility of rodent hearts to ischemia-reperfusion (I/R) injury. However, the results revealed some controversies—increased [3-5], decreased [6-12], and unchanged [13] cardiac tolerance to myocardial I/R injury has been demonstrated. Some of these studies indicated that feeding the animals with high-fat or high-sugar experimental diets led to the development of insulin resistance (IR). Insulin resistance may be a primary etiologic factor for various cardiomyopathies and lead to an injury by increasing lipotoxicity, inflammation, oxidative stress, and cardiac fibrosis. Insulin resistance is characterized by an alteration of the hepatic response to insulin and a decrease in glucose uptake in insulin-sensitive organs, including the heart, which may contribute to an increase in cardiac sensitivity to ischemia. The mechanisms involved in the development of IR are not fully clear, but one of the proteins that are believed to be influenced during this state is Akt kinase (also termed *protein kinase B*). Akt kinase appears to be a common mediator of the metabolic effects of insulin in several physiologically important target tissues [14]. Thus, Akt kinase is involved in insulin-mediated regulation of glucose transport and mediates the facilitating effect of insulin on lipogenesis.

With respect to the insulin action on I/R injury, it is well recognized that application of insulin during ischemia and reperfusion exerts a cardioprotective effect accompanied by elevation in Akt kinase phosphorylation (activation) [15,16]. A positive role of Akt kinase activation in the attenuation of myocardial ischemic injury was also demonstrated in several other studies [17-21]. Our previous studies also pointed out the positive role of this kinase on the rat hearts exposed to both short- and long-term adaptation associated with cardioprotection [22-24]. In addition, Du Toit et al [9] have shown a cardioprotective effect of insulin against I/R injury in a prediabetic model of dietary-induced obesity.

Within the context of IR, some studies performed in rats, mice, and humans have demonstrated significantly impaired Akt kinase activation. In the myocardium, Lee et al [25] found that myocardial IR develops along with the systemic resistance to insulin and is associated with blunted Akt kinase phosphorylation in response to insulin in a porcine model of diet-induced obesity. Deng et al [26] have also shown reduced Akt kinase–mediated signaling in insulin-resistant rat hearts after feeding a high-cholesterol diet combined with 10% fructose solution. This was associated with impaired myocardial contractile performance. On the other hand, Axelsen et al [27] were unable to reproduce these findings. Moreover, insulin-mediated Akt kinase phosphorylation was not significantly altered in fat cells of diabetic rats or in skeletal muscle from humans with type II diabetes in other studies [28-30].

In most of the mentioned studies, diets abundant in fat, cholesterol, or carbohydrate (sucrose or fructose) were used to generate obese rodent models and mimic metabolic syndrome. In our study, we used a standard diet enriched by lard and accompanied by administration of a sweet beverage (30% sucrose solution) 4 times a week. This diet simulated unhealthy dietary habits in developed countries (ie, eating mainly lard and drinking sweet beverages) and, therefore, could better mimic the phenotype of human metabolic syndrome. We hypothesized that application of this diet for 48 days might induce the initial stage of progression of pathologic changes in the heart, which result in heart remodeling and are connected with activation of adaptive mechanisms, including intracellular signaling. The purpose of the present study was to investigate the effects of this type of diet on heart function and vulnerability to I/R injury. The understanding of the early mechanisms is critical for the future prevention of the negative effects of high energy intake. To determine the possible molecular mechanisms involved in these processes, the role of Akt kinase signaling was investigated. Because changes in Akt kinase activation may influence the enzymes involved in extracellular matrix remodeling, our study was also focused on the evaluation of changes in matrix metalloproteinases (MMPs), a possible target of the Akt kinase action.

Matrix metalloproteinases are proteolytical enzymes well recognized for their action on extracellular matrix proteins and their involvement in long-term remodeling processes. These occur in physiologic as well as pathologic events such as ischemic heart disease and atherosclerosis. Moreover, recent data implicate MMPs in acute processes, and it was shown that MMP-2 is a primary modulator of the acute mechanical dysfunction of the heart after ischemia/reperfusion and inhibition of its activity significantly improves cardiac function [31,32]. It is suggested that the role of MMPs in these processes is realized through proteolysis of substrates unrelated to the extracellular matrix, such as troponin I [33], myosin light chain [34], and α-actinin [35]. Regarding the relationship between MMPs and Akt kinase during ischemia/reperfusion, we found that specific inhibitor of Akt kinase pathway modulated changes in MMP-2 activation induced by cycle of short ischemia and reperfusion in rat hearts [23].

2. Methods and materials

2.1. Animals

Twelve-week-old male Wistar rats were purchased from Velaz (Prague, Czech Republic). All animals had free access to liquids and diets. The study was performed in accordance with the Guide for Care and Use of laboratory animals published by the US National Institutes of Health (NIH publication No 85-23, revised 1996) and approved by the Animal Care and Use Committee of the Slovak Republic.