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Insulin down-regulates cardioprotective SUR2A in the heart-derived H9c2 cells: A possible explanation for some adverse effects of insulin therapy

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ABSTRACT

Some recent studies associated insulin therapy with negative cardiovascular events and shorter lifespan. SUR2A, a K_{ATP} channel subunit, regulate cardioprotection and cardiac ageing. Here, we have tested whether glucose and insulin regulate expression of SUR2A/K_{ATP} channel subunits and resistance to metabolic stress in heart H9c2 cells. Absence of glucose in culture media decreased SUR2A mRNA, while mRNAs of Kir6.2, Kir6.1, SUR1 and IES SUR2B were increased. 2-deoxyglucose (50 mM) decreased mRNAs of SUR2A, SUR2B and SUR1, did not affect IES SUR2A and IES SUR2B mRNAs and increased Kir6.2 mRNA. No glucose and 2-deoxyglucose (50 mM) decreased resistance to an inhibitor of oxidative phosphorylation, DNP (10 mM). 50 mM glucose did not alter K_{ATP} channel subunits nor cellular resistance to DNP (10 mM). Insulin (20 ng/ml) in both physiological and high glucose (50 mM) down-regulated SUR2A while upregulating Kir6.1 and Kir6.2 (in high glucose only). Insulin (20 ng/ml) in physiological and high glucose decreased cell survival in DNP (10 mM). As opposed to Kir6.2, infection with SUR2A resulted in titre-dependent cytoprotection. We conclude that insulin decreases resistance to metabolic stress in H9c2 cells by decreasing SUR2A expression. Lower cardiac SUR2A levels underlie increased myocardial susceptibility to metabolic stress and shorter lifespan.

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterised by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Insulin is a peptide hormone produced by pancreatic β-cells that regulates metabolism of carbohydrates and fats [2]. Insulin is the main therapy for type 1 diabetes (diabetes characterised by absolute insulin deficiency) and it is also sometimes used for therapy of type 2 diabetes (diabetes characterised by relative insulin deficiency and/or insulin resistance) [3]. In cardiac muscle, insulin promotes glucose uptake and its utilization via glycolysis and also participates in the regulation of long-chain fatty acid uptake and protein synthesis [4]. Traditionally, insulin has been considered to be cardioprotective [5–8]. However, some more recent studies reported that insulin have cardiac effects that would not be expected from a cardioprotective hormone. In experimental animals, it has been demonstrated that insulin inhibits cardioprotection afforded by ischaemic preconditioning [9] while in patients with type 2 diabetes, concerns about negative cardiac events when insulin is used as a therapeutic have been raised [10]. A large meta-analysis suggested that insulin treatment is associated with a significantly higher short and long-term adverse cardiovascular outcomes after percutaneous coronary intervention compared to diabetic patients not treated by insulin therapy [11].

SUR2A belongs to a group of "atypical" ABC proteins as, although possessing a structure of an ABC protein, it does not seem to mediate transport [12]. In fact, SUR2A binds to inward rectifier Kir6.2 to form cardiac ATP-sensitive K^+ (K_{ATP}) channels. Increased level of SUR2A in the heart is demonstrated to 1) Protect myocardium against ischaemia-reperfusion [13], 2) Protect cardiomyocytes against hypoxia and other types of metabolic stresses [13–15], 3) Increase physical endurance [15], 4) Counteract ageing-induced increase in myocardial susceptibility towards hypoxia [16], 5) Counteract ageing-induced decrease in physical endurance (this effect could involve SUR2A effect on skeletal muscle as well, 9) and 6) Reprogram embryonic cardiomyocytes towards less differentiated stem cells [17]. Recently, we have uncovered that PI3K/Akt signalling pathway regulate SUR2A, ie. activation of PI3K/Akt up-regulates SUR2A and confers cardioprotection [23]. In addition to that, SUR2A expression seems to be regulated by intracellular ATP [24]. As insulin activates PI3K/Akt and regulates intracellular ATP by regulating metabolism of carbohydrates and fats [5],

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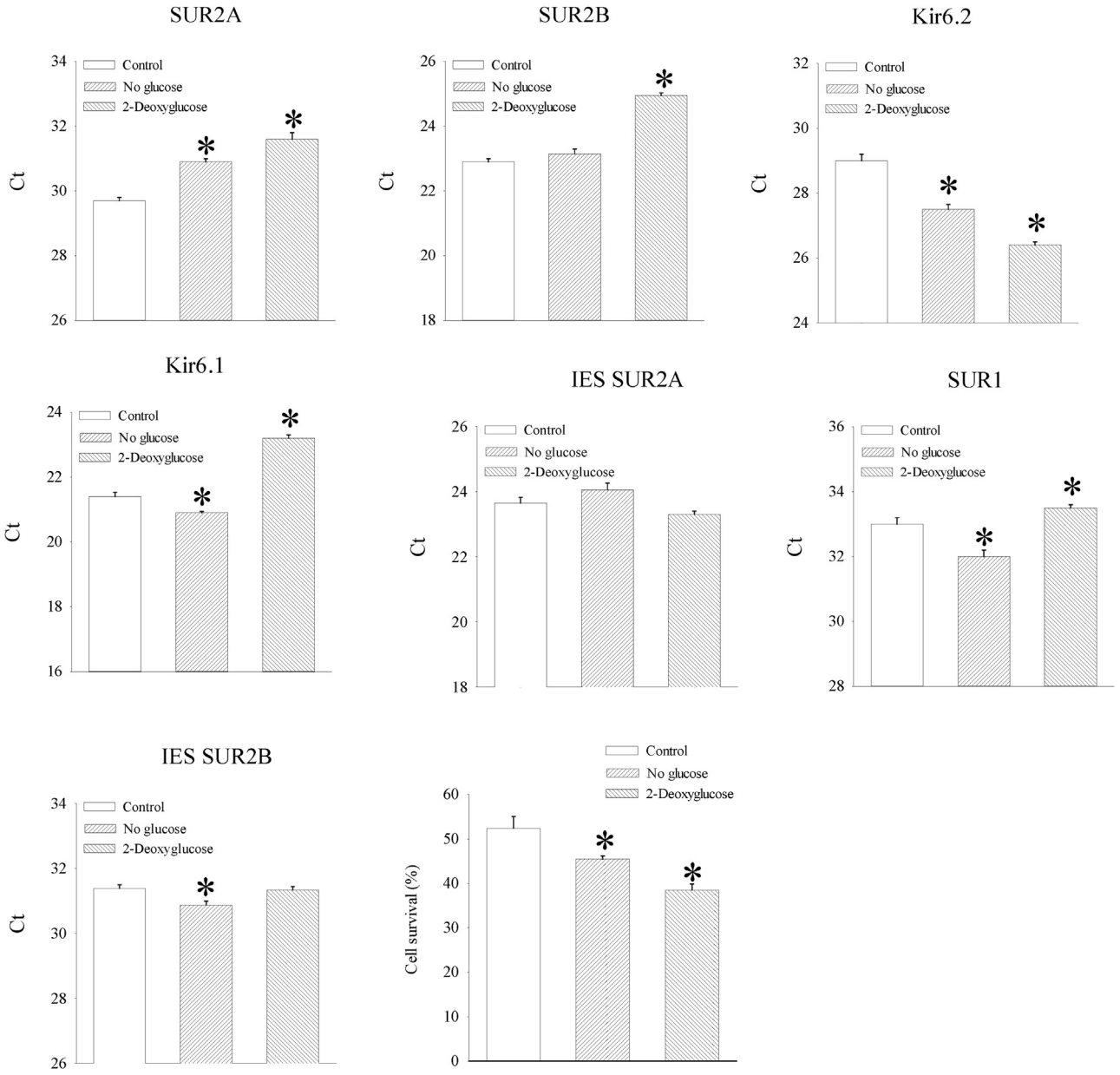

Fig. 1. No glucose and 2-deoxyglucose down-regulate SUR2A in H9c2 cells and decrease cellular resistance to metabolic stress. Bar graphs represent cycling thresholds of the real time RT-PCR progress curves of K_{ATP} channel subunits as labelled and a bar graph (a graph on the right third row) showing a percentage of survival in control cells and cells cultured without glucose (no glucose) or cells cultured without glucose in the presence of 50 mM 2- deoxyglucose exposed to DNP (10 mM). Each bar represent mean ± SEM (n = 6–7). *P < 0.05 when compared to control.

it is quite possible that this hormone could regulate SUR2A and, consequently, cardiac resistance to stress.

H9c2 cells are well-established experimental model that is similar to adult cardiomyocytes in crucial aspects of K_{ATP} channels structure, regulation and function; in both cell types express all seven K_{ATP} channel subunits [13–25] and increase in SUR2A increase numbers of fully functional K_{ATP} channels generating cellular phenotype more resistant to stress [13,14]. Signalling pathway regulating K_{ATP} channel levels and mediating preconditioning and cardioprotection are similar between adult cardiomyocytes and H9c2 cells [23–29]. Therefore, we used this experimental model to examine whether glucose and insulin regulate SUR2A levels of cellular resistance to stress.

2. Methods

2.1. H9C2 cells and treatments with viral constructs

H9C2 cells Rat embryonic heart H9c2 cells (ECACC, Salisbury, UK) were cultured in a tissue flask (at 5% CO_2) containing Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 2 mM glutamine and 1) 5 mM glucose added (control experimental group), 2) 20 ng/ml insulin and 5 mM glucose added (insulin experimental group), 3) 20 ng/ml insulin and 50 mM glucose added (insulin in high glucose experimental group). The cells were cultured in incubators (Galaxy, oxygen control model, RS Biotech, Irvine, UK) under those conditions for 24 h before experiments on them were performed. For some experiments H9C2 cells were infected with adenoviral constructs containing either green fluorescent protein (Ad-GFP), luciferase (Ad-luciferase), SUR2A (Ad-SUR2A) and Kir6.2 (Ad-Kir6.2). To infect