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Niacin-induced hyperglycemia is partially mediated via niacin receptor GPR109a in pancreatic islets

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ABSTRACT

The widely used lipid-lowering drug niacin is reported to induce hyperglycemia during chronic and high-dose treatments, but the mechanism is poorly understood. Recently, the niacin receptor [G-protein-coupled receptor, (GPR) 109a], has been localized to islet cells while its potential role therein remains unclear. We, therefore, aimed at investigating how GPR109a regulates islet beta-cell function and its downstream signaling using high-fat diet-induced obese mice and INS-1E beta cells. Eight-week niacin treatment elevated blood glucose concentration in obese mice with increased areas under the curve at oral glucose and intraperitoneal insulin tolerance tests. Additionally, niacin treatment significantly decreased glucose-stimulated insulin secretion (GSIS) but induced peroxisome proliferator-activated receptor gamma (*Pparg*) and GPR109a expression in isolated pancreatic islets; concomitantly, reactive oxygen species (ROS) were transiently increased, with decreases in GSIS, intracellular cyclic adenosine monophosphate (cAMP) accumulation and mitochondrial membrane potential ($\Delta\Psi m$), but with increased expression of uncoupling protein 2 (*Ucp2*), *Pparg* and *Gpr109a* in INS-1E cells. Corroborating these findings, the decreases in GSIS, $\Delta\Psi m$ and cAMP production and increases in ROS, *Pparg* and GPR109a expression were abolished in INS-1E cells by GPR109a knockdown. Our data indicate that niacin-induced pancreatic islet dysfunction is probably modulated through activation of the islet beta-cell GPR109a-induced ROS-PPARγ-UCP2 pathways.

1. Introduction

Hyperglycemia and diabetes mellitus are leading causes of mortality and morbidity worldwide, and it is estimated 347 million people have diabetes globally, mostly type 2 DM (Clement et al., 2004; Danaei et al., 2011). In addition to hyperglycemia, abnormal lipid profiles with increased circulating total and LDL-cholesterol and triglycerides, or hyperlipidemia, are frequently found in severe diabetic patients (Abbate and Brunzell, 1990). The major clinical outcome of hyperlipidemia is atherosclerosis, which is the leading cause of death in the United States and other Western countries (Murray and Lopez, 1997).

Niacin (nicotinic acid) is widely used to regulate abnormalities in plasma lipid and lipoprotein metabolism, as evidenced by its potential for reducing atherosclerotic cardiovascular disease risks (Guyton, 1998), due to its ability to reduce circulating triglycerides and apolipoprotein-B containing lipoproteins (Kamanna and Kashyap, 2008). In this regard, the discovery of niacin receptor GPR109a (PUMA-G in mouse and HM74 in human) in adipose tissue has brought greater understanding of its anti-lipolytic effects. GPR109a is Gi-coupled, binding to niacin and related compounds, and widely expressed in other tissues, including skin, macrophages, spleen, lung and pancreas (Benyo et al., 2006; Li et al., 2011; Lukasova et al., 2011; Tunaru et al., 2003). A substantial body of evidence has shown that the effects of niacin on target tissues are mediated via this receptor (Benyo et al., 2006; Lukasova et al., 2011).

Following GPR109a activation, niacin has direct pleiotropic effects influencing various events in the body, such as activation of PPARs (peroxisome proliferator-activated receptor) which may affect glucose metabolism and induction of prostanoid synthesis (Kamanna and Kashyap, 2007; Vosper, 2009). Furthermore, a recent study has demonstrated the existence of the GPR109a receptor in almost all pancreatic islet beta cells and in ~40% of alpha cells, and the niacin-induced decreases in glucose-induced insulin release was blunted by G-protein receptor blockade (Li et al., 2011). It has been thought that niacin could reduce insulin resistance due to its beneficial effects on lipid profiles and induction of adiponectin secretion through the

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GPR109a receptor (Linke et al., 2009; Plaisance et al., 2009). There is, however, a growing body of evidence from basic science and clinical studies that this medication results in deterioration of glycemic control with prolonged, high dose (and even short-term) treatment (Garg and Grundy, 1990; Goldberg and Jacobson, 2008) and these increases appear rather greater than would be expected due to ageing of study subjects (Chang et al., 2006; Rasouli et al., 2005). Furthermore, a recent study has revealed that the consequences of GPR109A receptor signaling are varied, depending on the ligand, and on cellular context (Gaidarov et al., 2013).

Of great interest in this context, any increase in glycemia, even within the normal range, increases the risk of overt cardiovascular disease, as well as increasing the risks of complications of type 2 DM, such as renal failure and cardiovascular disease (Stratton et al., 2000; Nathan et al., 2009). Thus, the mechanisms for niacin-induced reduction in islet beta-cell response to glucose are of interest and it is clearly relevant to examine regulation and function of the GPR109a in pancreatic beta-cells as potential therapeutic modalities. Though the presence of GPR109a in pancreatic islet cells and the preliminary data on the effects of niacin on insulin secretion have been reported (Li et al., 2011), how GPR109a modulates insulin secretion and which signaling pathways may be involved remain to be elucidated. In this regard, we aimed to investigate the hyperglycemic effect of niacin in a diet-induced obese mouse model and to elucidate the mechanism(s) by which GPR109a-regulated islet function, and energy, metabolism are modulated, hypothesizing that they are mediated via ROS and/or PPARs.

2. Materials and methods

2.1. Animal model and experimental design

C57BL/6J mice were obtained from the Laboratory Animal Services Center of the Chinese University of Hong Kong. Prior to this study, mice were housed at 22 ± 2 °C with a 12-hour light/dark cycle and provided with *ad libitum* rodent chow (Teklad 7001, 4.4%; Harlan Teklad Global Diets). Five-week old mice were then fed either chow or a high-fat diet (TD.08811, 45%; Teklad, Harlan Laboratories, Madison, WI, USA) in order to establish high-fat diet-induced obese mice. After 16 weeks on these diets, mice were treated with 30 mg/kg niacin (Sigma-Aldrich, St. Louis, MO, USA), or Mk1903 (specific GPR109a agonist) at 1 or 5 mg/kg (Tocris Bioscience, Ellisville, MO, USA) daily in their drinking water for 8 weeks.

2.2. In vivo glucose homeostasis

Blood was withdrawn from the tail vein for measurements of fasting blood glucose at the end of treatments, using a glucometer (Bayer Corporation, Robinson Township, PA, USA); serum was separated and insulin content determined using commercial ELISA kits (Mercodia, Uppsala, Sweden). Briefly, for oral glucose tolerance test (OGTT), after a 16-h overnight fast, mice were challenged with glucose (2 g/kg B.W.), and blood glucose was measured at 0, 15, 30, 60, 90, and 120 minutes thereafter; for intraperitoneal insulin tolerance test (ITT), mice were allowed to eat for a 6-hour period following a 14-hour overnight fast, and then challenged with an intraperitoneal injection of insulin (0.75 U/kg B.W.); blood glucose was then measured at 0, 15, 30 and 60 minutes thereafter. Areas under the curve of OGTT and ITT were then calculated. Degrees of insulin resistance and beta-cell function was estimated by 'homeostasis model assessment' (HOMA-IR and HOMA-beta) using the following equations: HOMA-IR = fasting serum insulin (mU/L) × fasting serum glucose (mM)/22.4; HOMA-B = fasting serum insulin (mU/L) × 20/(fasting serum glucose (mM)-3.5), as previously described (Cheng et al., 2013).

2.3. Pancreas and islet studies

Pancreatic islets were isolated by intraductal injection of collagenase P (Roche, Kaiseraugst, Switzerland) as described previously (Cheng et al., 2008). GSIS was performed by incubating isolated islets with KRB containing low glucose (1.7 mM), followed by high glucose (16.7 mM) concentrations. Supernatants were collected and insulin concentrations measured using the ELISA kit (see above). Pancreata were fixed and embedded in paraffin. Sections were then cut and mounted on glass slides. For measurement of alpha-cell and beta-cell areas, slides were incubated overnight at 4 °C with rabbit anti-insulin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and mouse anti-glucagon (Abcam, Cambridge, UK), and then incubated with goat anti-rabbit 568, and anti-rabbit 488 (Invitrogen, Waltham, MA, USA). Immunolabeling was visualized using fluorescent microscopy. Islet alpha-cell and beta-cell areas were assessed by determining the proportion of area occupied by the red and green fluorescent signals within each islet, using Leica Qwin image analysis software (Leica), and calculating the ratios of the two areas.

2.4. INS-1E cell culture and treatment

INS-1E cells were cultured in a humidified atmosphere containing 5% CO_2 in complete medium composed of RPMI 1640 (11.1 mM glucose) supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 10 mM HEPES, 100 U/mL penicillin, and 100 μg/mL streptomycin (all materials from Invitrogen, Waltham, MA, USA). Maintenance cultures were passaged once a week using gentle trypsinization.

2.5. Construction of small interfering RNA for GPR109a

Small interfering RNAs (siRNAs) for rat GPR109a were synthesized (Invitrogen, Hong Kong) and transfected according to the manufacturer's instructions. INS-1 cells were transfected with 50 nM of siRNA-GPR109a oligos or negative control (random sequence) in Lipofectamine RNAi Max transfection reagent (Invitrogen, Waltham, MA, USA) for 24 hours and then recovered in fresh culture medium overnight. Cells were then treated with niacin for the indicated time and doses. The efficiency of siRNA-GPR109a in reducing endogenous G*pr109a* mRNA expression/secretion was measured by real-time PCR and Western blotting (see Fig. S3). The siRNA and negative control siRNA sequences are listed in Table S1.

2.6. Real-time PCR analyses

Trizol reagent (Invitrogen, Waltham, MA, USA) was used for total RNA extraction from pancreatic islets and cells according to the manufacturer's instructions. Real-time quantitative PCR was performed using SYBR Green labeling (Bio-Rad) and specific primers (Table S1). Relative expression was normalized as a percentage of *Gapdh*/*β-actin* and calculated using the comparative threshold cycle method ($2^{-\Delta\Delta CT}$).

2.7. Western blotting assay

Pancreatic islets or INS-1E cells were lysed by RIPA (Thermo Scientific, Waltham, MA, USA). Proteins were fractionated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore Corp., Billerica, MA, USA). After blocking, membranes were incubated with rabbit anti-GPR109a (Novus, St. Louis, MO, USA) or mouse anti-actin (Santa Cruz Biotechnology, Inc.) at 4 °C overnight and then with horseradish peroxidase-conjugated donkey anti-rabbit, sheep anti-mouse IgG (Amersham Pharmacia Biotech). The proteins were visualized with Fuji medical film (FUJIFILM Corp., Tokyo, Japan) using a chemiluminescence system (ECL, Amersham