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Epigallocatechin-3-gallate (EGCG) activates AMPK through the inhibition of glutamate dehydrogenase in muscle and pancreatic ß-cells: A potential beneficial effect in the pre-diabetic state?

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ABSTRACT

Glucose homeostasis is determined by insulin secretion from the ß-cells in pancreatic islets and by glucose uptake in skeletal muscle and other insulin target tissues. While glutamate dehydrogenase (GDH) senses mitochondrial energy supply and regulates insulin secretion, its role in the muscle has not been elucidated. Here we investigated the possible interplay between GDH and the cytosolic energy sensing enzyme 5′-AMP kinase (AMPK), in both isolated islets and myotubes from mice and humans. The green tea polyphenol epigallocatechin-3-gallate (EGCG) was used to inhibit GDH. Insulin secretion was reduced by EGCG upon glucose stimulation and blocked in response to glutamine combined with the allosteric GDH activator BCH (2-aminobicyclo-[2,2,1] heptane-2-carboxylic acid). Insulin secretion was similarly decreased in islets of mice with ß-cell-targeted deletion of GDH (ß*Glud1*$^{-/-}$). EGCG did not further reduce insulin secretion in the mutant islets, validating its specificity. In human islets, EGCG attenuated both basal and nutrient-stimulated insulin secretion. Glutamine/BCH-induced lowering of AMPK phosphorylation did not operate in ß*Glud1*$^{-/-}$ islets and was similarly prevented by EGCG in control islets, while high glucose systematically inactivated AMPK. In mouse C2C12 myotubes, like in islets, the inhibition of AMPK following GDH activation with glutamine/BCH was reversed by EGCG. Stimulation of GDH in primary human myotubes caused lowering of insulin-induced 2-deoxy-glucose uptake, partially counteracted by EGCG. Thus, mitochondrial energy provision through anaplerotic input via GDH influences the activity of the cytosolic energy sensor AMPK. EGCG may be useful in obesity by resensitizing insulin-resistant muscle while blunting hypersecretion of insulin in hypermetabolic states.

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1. Introduction

In obese subjects, the pre-diabetic state is characterized by hyperinsulinemia secondary to insulin resistance (Kasuga, 2006), which may lead to diabetes in case of subsequent ß-cell failure (Prentki and Nolan, 2006). However, ß-cell-targeted genetic intervention in mice protects against obesity, either by the reduction of insulin gene dosage (Mehran et al., 2012) or by the ablation of the amplifying pathway of glucose-stimulated insulin secretion (Vetterli et al., 2016a). Clinical data have shown that pharmacological inhibition of insulin secretion in obese subjects can promote weight loss (Lustig et al., 2006; van Boekel et al., 2008), although drugs used in these studies are associated with undesired side effects, such as hyperglycemia. Weight control by lifestyle modification has a low success rate, therefore calling for alternative therapy. In this context, green tea has been widely investigated. It is rich in polyphenols including epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG), which cause weight reduction in various animal models (Sae-tan et al., 2011; Wang et al., 2014) and obese subjects (Basu et al., 2010). However, human trials have

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shown some inconsistencies in this regard probably due to the heterogeneity of both protocols and populations used (for review see (Wang et al., 2014; Hursel et al., 2009; Phung et al., 2010)).

The mechanism of action of EGCG remains obscure, although its antioxidant effect is regularly emphasized. In addition, inhibition of the mitochondrial enzyme glutamate dehydrogenase (GDH) by EGCG in the μmolar range has been reported (Li et al., 2012). GDH catalyses the following reaction: α-ketoglutarate + NH_3 + NADH ↔ glutamate + NAD^+. Its direct link to the TCA cycle makes GDH the pivotal regulator of, on the one hand, carbohydrate-induced carbon efflux (cataplerosis) and, on the other hand, amino acid-mediated influx into the TCA cycle (anaplerosis). Moreover, GDH is allosterically regulated by leucine as well as pyridine, adenine and guanine nucleotides; being inhibited by GTP and activated by ADP (Fisher, 1985; Smith et al., 2001). In pancreatic ß-cells, cataplerosis participates in the amplifying pathway of insulin secretion (Vetterli et al., 2016b). Conversely, GDH-mediated anaplerosis, evoked for instance by glutamine, is not favoured in ß-cells but can be prompted by the allosteric activator BCH (2-aminobicyclo-[2,2,1] heptane-2-carboxylic acid) resulting in insulin release (Carobbio et al., 2004; Sener et al., 1981) or by activating mutations causing a syndrome of hyperinsulinism (Stanley et al., 1998).

Glucose-stimulated insulin secretion from the pancreatic ß-cell comprises two modalities, the obligatory calcium-mediated signalling and the more long-lasting amplifying pathway. The latter depends on the cataplerotic activity of GDH (Vetterli et al., 2012) and its genetic abrogation protects against obesity (Vetterli et al., 2016a). In the present study, we tested the pharmacological inhibition of GDH on insulin secretion in mouse and human islets. Moreover, the putative implication of GDH in insulin action was monitored through glucose uptake in human myotubes as well as the activity of the energy sensor AMPK, shown to be stimulated by EGCG (Wu et al., 2014).

2. Materials and methods

2.1. Pancreatic islet and cell preparations

Mouse pancreatic islets were obtained from either C57BL/6J wild type mice or ß-cell-specific GDH knockout mice (ß*Glud1*$^{-/-}$) described previously (Carobbio et al., 2009). Islets were isolated by collagenase digestion (collagenase P, Roche) as detailed elsewhere (Carobbio et al., 2004) and cultured overnight free floating in RPMI-1640 medium (Gibco) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 2 mM glutamine, 10 mM Hepes, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate and 50 μM 2-mercaptoethanol. Human islets were isolated from pancreases of deceased multiorgan donors, who had provided written informed consent (ECIT consortium), and maintained in CMRL-1066 before experiments (Vetterli et al., 2011). Rat insulinoma INS-1E ß-cells were cultured in RPMI-1640 as detailed previously (Merglen et al., 2004).

2.2. Epigallocatechin-3-gallate (EGCG) preparation

EGCG stock solution (4 mM, Sigma-Aldrich) was prepared in 1 mM ascorbic acid and stored at −20 °C. Since EGCG is unstable and its concentration decreases rapidly in cell culture medium, with a half-life of about 30 min (Sang et al., 2005), 0.5 mM ascorbic acid was added freshly in all media and buffers used for treatment of islets, INS-1E cells, and myotubes (Lambert et al., 2006).

2.3. Insulin secretion, calcium measurements, ATP and amino acid measurements

Mouse islets were maintained for 2 h in glucose- and glutamine-free RPMI-1640 medium in the absence or presence of 20 μM EGCG and then washed in Krebs Ringer bicarbonate HEPES buffer (KRBH, containing in mM: 135 NaCl, 3.6 KCl, 10 HEPES (pH 7.4), 5 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $MgCl_2$, 1.5 $CaCl_2$, and 0.1% bovine serum albumin, BSA) containing basal (2.8 mM) glucose concentration. Then, islets were handpicked and incubated (10 islets per tube) for 1 h at basal glucose, 22.8 mM glucose, and the combination of 5 mM glutamine plus 10 mM BCH (glutamine + BCH) in the absence or presence of 20 μM EGCG. At the end of the incubation, islets were put on ice before collection of supernatant for insulin measurements by radioimmunoassay (Linco), and finally re-suspended in acid-EtOH to determine their insulin content. Values were expressed as percent of total islet insulin content (Vetterli et al., 2012).

Cytosolic calcium concentration was monitored in INS-1E cells by ratiometric measurements of Fura-2 fluorescence with filters set at 340/380 nm for excitation and 510 nm for emission in multiwell mode as detailed previously (Rubi et al., 2005). ATP levels were assessed in INS-1E cells using a Seahorse XF^{e}24 Flux Analyzer (Seahorse Biosciences) as described (Vanderperre et al., 2016). Amino acid measurements were done by reverse-phase high-performance liquid chromatography (HPLC) after derivatization with O-phthalaldehyde (Vetterli et al., 2012).

2.4. Immunoblotting

About 200 mouse or human islets were treated as mentioned above for insulin secretion. At the end of incubation islet lysates were prepared using lysis buffer (20 mM Tris-HCl, 1 mM EGTA, 1 mM EDTA, 50 mM NaCl, 25 mM NaF, 2 mM orthovanadate, 1% glycerol, 1% Triton X100 and a protease inhibitor cocktail from Roche). Proteins were separated by SDS-PAGE, then blotted onto nitrocellulose membrane Hybond-ECL (Amersham Bioscience), and blocked with 5% nonfat milk in TBST (10 mM Tris-HCl, 150 mM NaCl, 0.02% Tween 20), finally probed with antibodies against phospho-AMPKα Thr172, AMPKα (Cell Signaling Technology), and actin (Sigma-Aldrich); then incubated with secondary horseradish peroxidase-coupled antibody, donkey anti-rabbit IgG ECL (Amersham Bioscience), goat anti-mouse IgG (Sigma-Aldrich), respectively. The target proteins were visualized by chemiluminescence (ECL Super- Signal West Pico Chemiluminescent, Pierce), analyzed using the ChemiDoc XRS System (Bio-Rad), and quantified by ImageJ (Schindelin et al., 2015).

2.5. Preparation of myotubes

Mouse C2C12 skeletal muscle cells were grown in Dulbecco's modified Eagle's medium (DMEM 25 mM glucose, Invitrogen) supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin (referred to as growth medium) before differentiation by incubating cells in DMEM supplemented with 2% horse serum (referred to as differentiation medium). C2C12 skeletal myotubes were used 7 days after induction of differentiation as described previously (Hirabara et al., 2010). C2C12 myotubes were incubated in the presence of 100 nM insulin for 1 h at 1 mM glucose and glutamine + BCH in the absence or presence of 20 μM EGCG before collection for immunoblotting performed according to the procedure described for islets.

Human skeletal muscle myoblasts were received from Dr. K. Bouzakri (biopsies obtained during abdominal surgery from Rectus abdominus muscle of donors who signed the informed consent at the Geneva University Hospital). The cells were grown in human skeletal muscle medium (Promo cell) before allowed to fuse and

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