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Oleic acid stimulates glucagon-like peptide-1 release from enteroendocrine cells by modulating cell respiration and glycolysis

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ABSTRACT

Objective. Glucagon-like peptide-1 (GLP-1) is a potent satiating and incretin hormone released by enteroendocrine L-cells in response to eating. Dietary fat, in particular monounsaturated fatty acids, such as oleic acid (OA), potently stimulates GLP-1 secretion from L-cells. It is, however, unclear whether the intracellular metabolic handling of OA is involved in this effect.

Methods. First we determined the optimal medium for the bioenergetics measurements. Then we examined the effect of OA on the metabolism of the immortalized enteroendocrine GLUTag cell model and assessed GLP-1 release in parallel. We measured oxygen consumption rate and extracellular acidification rate in response to OA and to different metabolic inhibitors with the Seahorse extracellular flux analyzer.

Results. OA increased cellular respiration and potently stimulated GLP-1 release. The fatty acid oxidation inhibitor etomoxir did neither reduce OA-induced respiration nor affect the OA-induced GLP-1 release. In contrast, inhibition of the respiratory chain or of downstream steps of aerobic glycolysis reduced the OA-induced GLP-1 release, and an inhibition of the first step of glycolysis by addition of 2-deoxy-D-glucose even abolished it.

Conclusion. These findings indicate that an indirect stimulation of glycolysis is crucial for the OA-induced release of GLP-1.

1. Introduction

GLP-1 is a potent satiating and incretin hormone synthesized and released by EC in response to eating [1]. Different mechanisms have been proposed for the stimulatory effect of macronutrients and their digestion products on GLP-1 release [2–5]. Most of the studies investigating the mechanisms of GLP-1 secretion from EC focused on the role of

Abbreviations: GLP-1, glucagon like peptide-1; OA, sodium salt of oleic acid; EC, enteroendocrine cells; GPR, G protein-coupled receptors; FAT, fatty acid transporters; CD36, class B scavenger receptor cluster-of-differentiation 36/fatty acid translocase; XF24, Seahorse extracellular flux analyzer; OCR, oxygen consumption rate; ECAR, extracellular acidification rate; CGM, complete growth medium; FBS, fetal bovine serum; Oligo, oligomycin; DMSO, dimethyl sulfoxide; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; Rot, rotenone; Anti, antimycin; BA, butyric acid; L-Glut, L-glutamine; 2DG, 2-deoxy-D-glucose; Eto, etomoxir; UK, UK-5099; DPPIVi, dipeptidyl peptidase-4 inhibitor; SEM, standard error of the mean; mFAO, mitochondrial fatty acid oxidation; CPT-1, carnitine palmitoyltransferase-1; MPC, mitochondrial pyruvate carrier; TCA, tricarboxylic acid cycle; UCP2, uncoupling protein 2.

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different receptors or transporters located on the luminal or basolateral membrane of EC [6–8]. Only few studies addressed a possible effect of intracellular metabolism in this context [9–11], and there is no consensus about the metabolic pathways possibly involved.

Fatty acids, especially monounsaturated fatty acids, potently stimulate GLP-1 release *in vivo* [12,13]. GPR, such as GPR 40 [14], GPR 43 [15] and GPR 120 [1,16,17] have been implicated in fatty acid-induced GLP-1 release. *In vitro*, the monounsaturated fatty acid, OA, potently stimulates the release of GLP-1 without impairing cell viability [2–5,18,19]. Besides stimulating the release of GLP-1 via GPR 120, free OA is also actively taken up by GLUTag cells via the FAT 1, 2 and 4 as well as via CD36 [6–8,20]. A specific inhibition of CD36 with sulfo-*N*-succinimidyl oleate antagonized the uptake of OA and decreased the OA-induced release of GLP-1 *in vitro* [9–11,20]. Also, mice lacking FAT 4 display a decreased release of GLP-1 in response to OA suggesting a possible role of intracellular OA handling in the release of GLP-1. OA could influence exocytosis of GLP-1 vesicles by direct activation of the isoenzyme PKCζ [11–13,19] or by modulating mitochondrial energy metabolism [14,21], but the mechanisms that link intracellular OA handling and GLP-1 release are still unclear.

We here investigated the relationship between cellular bioenergetics and GLP-1 release in the EC line model GLUTag. In a first experiment we characterized the optimal medium for the bioenergetics measures. It is crucial for such experiments that the cells are metabolically active and respond to metabolic inhibitors and stimulators. We used the XF24 to simultaneously assess OCR, an indicator of respiratory chain activity, and ECAR, an indicator for the glycolytic flux [15,22]. We monitored the metabolic response of the EC to OA, and to different inhibitors of the potential intracellular pathway of OA metabolism, and measured GLP-1 release into the medium.

2. Experimental Procedures

2.1. Cell Culture

Differentiated GLUTag cells (kindly provided by Dr. D. Drucker, University of Toronto, Toronto, Canada) were cultured in CGM (DMEM 1 g/L glucose (Life Technologies 31885)) supplemented with 10% FBS (Life Technologies 16000) and 1% PenStrep (Life Technologies 10378) at 37 °C, 95% rHu and 5% CO_2. For all experiments GLUTag cells were used between passage numbers 15 and 25.

2.2. Compounds and Concentrations

Oligo 1 μg/mL (Sigma O4876 from 10 mg/mL stock, dissolved in DMSO (Sigma D2438)), FCCP 0–1.25 μmol/L (Sigma C2920 from 2.5 mmol/L DMSO stock), Rot 1.5 μmol/L (Sigma R8875 from 2.5 mmol/L DMSO stock), Anti 2 μg/mL (Sigma A8674 from 2 mg/mL DMSO stock) glucose 1–40 mmol/L (Fisher Scientific 50-99-7 from 3 M NaCl stock), OA 62.5–1000 μmol/L (Sigma O7501 from 5 mmol/L NaCl stock), 2DG 10–200 mmol/L (Sigma D6134 from 2 M NaCl stock) and Eto 0.5–4 mmol/L (Labor Dr. Heusler HHAC-Bez. 31770/4, from 4 M NaCl stock), and UK 5–135 μmol/L (Sigma PZ0160 from 69.37 mmol/L DMSO stock) were used. The medium and all compounds were adjusted to pH 7.4 and 37 °C before every experiment.

2.3. XF24 Experiments

The XF24 (Seahorse Bioscience) was used to continuously monitor oxygen consumption and media acidification. One day prior to the experiments, 60 K cells were seeded in CGM in a customized XF24 cell plate and cultured overnight at 37 °C, 95% rHu and 5% CO_2. One hour prior to the experiment, cells were washed with XF assay medium (XFDMEM, Seahorse Bioscience, 102353) supplemented with 1 g/L glucose, 1 mmol/L sodium pyruvate (Life Technologies 11360-088) and 1% PenStrep and incubated with 500 μL XF assay medium for one hour at 37 °C, 95% rHu and 0% CO_2. After 15 min equilibration time, OCR and ECAR were assessed every 9 min (5 min mix, 2 min wait, 2 min measure). The different compounds were added to the injection ports of the XF cartridge in 10× concentration and were all prepared in NaCl (0.9% B. Braun).

2.4. GLP-1 Secretion Experiments

To measure GLP-1 release, 150 K GLUTag cells were seeded in 24-well plates and cultured for 1.5 days to reach 80% confluence. Then the cells were washed twice with PBS and incubated in assay medium (DMEM 1 g/L glucose supplemented with 1 mmol/L pyruvate, 1% PenStrep, 0.1 mmol/L, DPPIVi (ANAWA Trading SA)) containing the test compound dissolved in NaCl against medium with NaCl as a control. After the desired incubation period, the medium was collected for GLP-1 measurements using Active GLP-1 (ver. 2) Assay Kit (Mesoscale Discovery, K150JWC-1) according to the manufacturer's instructions. All results were normalized to GLP-1 secretion in experimental medium containing saline and are expressed as % changes of the control.

2.5. Data Analysis and Statistics

All results are presented as means ± SEM of absolute values, % changes or fold changes, as specified in the figure legends. Statistical significance was tested using Student's t test or One-Way or Two-Way ANOVA with Tukey's multiple comparison test as indicated in the figure legends. In all experiments $p < .05$ was used as threshold for significance.

3. Results

3.1. Glucose and Pyruvate were Necessary to Keep GLUTag Cells Metabolically Active

Bioenergetics measurements of cellular metabolism with the XF require metabolically active cells. Therefore, to determine the optimal medium we incubated cells in modified and non-buffered XFDMEM basal medium supplemented with 1% PenStrep with or without 5.5 mmol/L glucose and/or 1 mmol/L pyruvate and measured OCR and ECAR (Fig. 1A and B). First we measured basal cellular metabolism and