METABOLISM CLINICAL AND EXPERIMENTAL 65 (2016) XXX–XXX

Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

The bile acid TUDCA increases glucose-induced insulin secretion via the cAMP/PKA pathway in pancreatic beta cells

Jean Franciesco Vettorazzi[a,d], *Rosane Aparecida Ribeiro*[b], *Patricia Cristine Borck*[a], *Renato Chaves Souto Branco*[a], *Sergi Soriano*[c], *Beatriz Merino*[d], *Antônio Carlos Boschero*[a], *Angel Nadal*[d], *Ivan Quesada*[d,1], *Everardo Magalhães Carneiro*[a,*,1]

[a] *Department of Structural and Functional Biology, Institute of Biology, University of Campinas (UNICAMP), 13083-970 Campinas, SP, Brazil*
[b] *Integrated Laboratory of Morphology, Centre for Ecology and Socio-Environmental — NUPEM, Federal University of Rio de Janeiro (UFRJ), Macaé, Rio de Janeiro, Brazil*
[c] *Department of Physiology, Genetics and Microbiology, University of Alicante, 03080 Alicante, Spain*
[d] *Institute of Bioengineering and the Biomedical Research Center in Diabetes and Associated Metabolic Disorders (CIBERDEM), Miguel Hernández University, 03202, Elche, Spain*

ARTICLE INFO

Article history:
Received 11 March 2015
Accepted 12 October 2015

Keywords:
β-cell
Bile acids
Insulin secretion
TUDCA

ABSTRACT

Objective. While bile acids are important for the digestion process, they also act as signaling molecules in many tissues, including the endocrine pancreas, which expresses specific bile acid receptors that regulate several cell functions. In this study, we investigated the effects of the conjugated bile acid TUDCA on glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells.

Methods. Pancreatic islets were isolated from 90-day-old male mice. Insulin secretion was measured by radioimmunoassay, protein phosphorylation by western blot, Ca^{2+} signals by fluorescence microscopy and ATP-dependent K^+ (K_{ATP}) channels by electrophysiology.

Results. TUDCA dose-dependently increased GSIS in fresh islets at stimulatory glucose concentrations but remained without effect at low glucose levels. This effect was not associated with changes in glucose metabolism, Ca^{2+} signals or K_{ATP} channel activity; however, it was lost in the presence of a cAMP competitor or a PKA inhibitor. Additionally, PKA and CREB phosphorylation were observed after 1-hour incubation with TUDCA. The potentiation of GSIS was blunted by the Gα stimulatory, G protein subunit-specific inhibitor

Abbreviations: 6E-CDCA, 6-Ethyl-chedeoxycholic acid; ADP, Adenosine diphosphate; AKT or PTB, Protein kinase B; ATP, Adenosine triphosphate; AUC, Area under curve; BSA, Bovine serum albumin; cAMP, Cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; DZX, Diazoxide; FXR, Farnesoid X receptor; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; GLP-1, Glucagon-like peptide 1; GLUT-2, Glucose transporter 2; GSIS, Glucose-stimulated insulin secretion; H89, Protein kinase A inhibitor; INT-777, 6-Alpha-ethyl-23(S)-methyl-cholic acid; K_{ATP}, ATP-sensitive K^+ channel; KLF11, Kruppel-like factor 11; NAD(P)H, Nicotinamide adenine dinucleotide phosphate; NF449, Gαs subunit G protein antagonist; OA, Oleanolic acid; OCA, Obeticholic acid; PKA, Protein kinase A; Rp-cAMPS, Competitive inhibitor of the activation of cAMP-dependent protein kinases by cAMP; TCDC, Taurochenodeoxycholic acid; TGR5, G protein-coupled bile acid receptor 1; TβMCA, Tauro β-muricholic acid; TUDCA, Tauroursodeoxycholic acid; UDCA, Ursodeoxycholic acid.

* *Corresponding author at:* Department of Structural and Functional Biology, Institute of Biology, University of Campinas (UNICAMP), Monteiro Lobato Street, 13083-970, Campinas, SP, Brazil. Tel.: +55 1935216203.
E-mail address: emc@unicamp.br (E.M. Carneiro).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.metabol.2015.10.021

NF449 and mimicked by the specific TGR5 agonist INT-777, pointing to the involvement of the bile acid G protein-coupled receptor TGR5.

Conclusion. Our data indicate that TUDCA potentiates GSIS through the cAMP/PKA pathway.

1. Introduction

Bile acids are molecules derived from cholesterol and synthesized in hepatocytes. They facilitate the digestion and absorption of dietary lipids and fat-soluble vitamins and regulate cholesterol excretion and sterol homeostasis. Before secretion into the gallbladder and duodenum, bile acids undergo a conjugation process with glycine or taurine, which increases their solubility and decreases the toxicity of these compounds [1–3]. In addition to the digestive function of bile acids, the discovery of bile acid receptors in the last couple of years has emphasized their role as extracellular messengers, which produce both genomic and non-genomic effects through multiple signaling pathways [1,2,4,5]. Many tissues, including the endocrine pancreas, express bile acid receptors [6,7]. The most important of these receptors are the nuclear receptor farnesoid X receptor (FXR) and the G protein-coupled bile acid receptor TGR5 [1,2,8].

The activation of FXR can regulate several processes in pancreatic β-cells. In the insulin-producing cell line βTC6, the FXR agonist 6-ethyl-chenodeoxycholic acid (6E-CDCA) increased the expression of insulin and the glucose-regulated transcription factor KLF11. It also induced AKT phosphorylation and GLUT-2 translocation to the plasma membrane, promoting glucose uptake [9]. The activation of FXR by the taurine-conjugated bile acid taurochenodeoxycholic acid (TCDC) increased glucose-stimulated insulin secretion (GSIS) in isolated mouse islets. This effect was associated with the inhibition of ATP-dependent K^+ (K_{ATP}) channels, changes in β-cell electrical activity, and increased Ca^{2+} influx [7]. The use of FXR ligands has also been explored in the treatment of glucose homeostasis disorders. The FXR ligand 6-ethyl-chenodeoxycholic acid (6E-CDCA) decreased glucose, triglyceride and cholesterol levels in db/db mice and Zucker fa/fa rats, improving glucose homeostasis in these diabetic models [8]. The FXR agonist obeticholic acid (OCA) ameliorated insulin sensitivity and the metabolic profile in patients with type-2 diabetes [9]. Activation of the G protein-coupled bile acid receptor TGR5 can also regulate pancreatic β-cell function. The TGR5 ligands oleanolic acid (OA) and INT-777 stimulated GSIS in the insulin-producing cells MIN-6 and human islets [6]. This effect depended on the activation of the Gα stimulatory TGR5 subunit, increasing adenylyl cyclase activity, cAMP levels, and cytosolic Ca^{2+} concentrations [6]. In rodents, synthetic TGR5 agonists diminished plasma glucose and insulin levels and protected against high-fat diet-induced obesity [10]. TGR5 was also shown to be involved in glucose homeostasis through stimulation of the incretin glucagon-like peptide 1 (GLP-1) secretion [11,12].

Although bile acids have recently been shown to be signaling messengers that are able to regulate some cellular processes in the endocrine pancreas, there is little information regarding their receptors, their molecular mechanisms and the actions involved. In this study, we analyzed the effects of the taurine-conjugated bile acid tauroursodeoxycholic acid (TUDCA) on the insulin secretory function of pancreatic β-cells. TUDCA and ursodeoxycholic acid (UDCA) are used for the treatment of different liver diseases, such as primary biliary cirrhosis and cholesterol gallstones, but they also seem to have therapeutic potential in non-liver diseases, such as neurological, retinal, metabolic and myocardial disorders [13,14]. These effects seem to be associated with their anti-apoptotic properties. Additionally, studies in experimental models of obesity have reported that TUDCA can act as a chemical chaperone that ameliorates insulin resistance by reducing endoplasmic reticulum stress and the unfolded protein response [15]. Here, we show that TUDCA potentiates GSIS in pancreatic β-cells, likely through the bile acid receptor TGR5 and activation of the cAMP/PKA pathway.

2. Materials and Methods

2.1. Reagents

TUDCA was purchased from Calbiochem (São Paulo, SP, Brazil, cat. 580549), and ^{125}I was purchased from Genesis (São Paulo, SP, Brazil). Western blot reagents were purchased from Bio-Rad (Madrid, Spain), and antibodies were purchased from Cell Signaling (Barcelona, Spain). The remaining reagents were purchased from Sigma Chemical (St. Louis, MO).

2.2. Animals

All experiments involving animals were approved by the Animal Care Committee at UNICAMP (License Number: 2234-1) and Miguel Hernández University (Ref. UMH.IB.IQM.01.13). Male 90-day-old C57Bl/6 mice were obtained from the breeding colony at UNICAMP and UMH and were maintained at 22 ± 1 °C on a 12-h light–dark cycle with free access to food and water. Mice were euthanized in a CO_2 chamber and decapitated for pancreatic islet isolation by collagenase digestion of the pancreas, as previous described [16].

2.3. Insulin Secretion

For static insulin secretion, pancreatic islets (4 islets per well) were incubated for 30 min with Krebs-bicarbonate buffer (KBB; (in mmol/L) 115 NaCl, 5 KCl, 2.56 $CaCl_2$, 1 $MgCl_2$, 10 $NaHCO_3$, 15 HEPES), supplemented with 5.6 mmol/L glucose and 0.3% BSA and equilibrated with a mixture of 95% O_2/5% CO_2 to regulate the pH at 7.4. After 30 min of preincubation time, the medium was removed and immediately replaced with fresh KBB medium containing different glucose and TUDCA concentrations, as well as the different reagents indicated in the experiments. After 1 h of incubation time, the medium was removed and stored at −20 °C. For islet insulin content, groups of four islets were collected and transferred to tubes containing

Please cite this article as: Vettorazzi JF, et al, The bile acid TUDCA increases glucose-induced insulin secretion via the cAMP/PKA pathway in pancreatic beta cells, Metabolism (2015), http://dx.doi.org/10.1016/j.metabol.2015.10.021