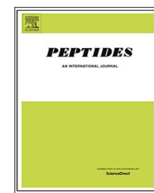
Peptides 96 (2017) 20–30

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides



Research article

Detailed characterisation of STC-1 cells and the pGIP/Neo sub-clone suggests the incretin hormones are translationally regulated

Anna L. Gillespie[a], Xiaobei Pan[a], Anna Marco-Ramell[b], Caroline Meharg[a], Brian D. Green[a,*]

[a] Institute for Global Food Security (IGFS), Queen's University Belfast, United Kingdom
[b] Biomarkers and Nutrimetabolomics Group, University of Barcelona, Spain

ARTICLE INFO

Keywords:
STC-1
GIP
GLP-1
Gut hormones
HCA
Gene translation
pGIP/Neo

ABSTRACT

STC-1 is a heterogeneous plurihormonal cell line producing several prominent gut peptide hormones. pGIP/Neo is a genetically selected sub-clone of STC-1 with augmented levels of glucose-dependent insulinotropic peptide (GIP). Morphometric parameters, hormone concentrations, mRNA transcripts, hormone immunocytochemistry and nutrient utilisation/production of these two cell lines were compared. Proglucagon-derived peptides (Glucagon-like peptide-1 (GLP-1) and – 2(GLP-2)) were lower in sub-clone cells than progenitor cells. High Content Analysis found altered intracellular GLP-1, GIP, cholecystokinin (CCK) and peptide YY (PYY) levels and differing hormone co-localisation. The proportion pGIP/Neo cells containing GIP immunoreactivity (82%) was greater than STC-1 (65%), as were the proportion with 'GIP only', 'GLP − 1 + GIP' or 'GIP + PYY' immunoreactivity. Most surprisingly mRNA transcripts of the proglucagon and GIP genes were inversely correlated to the levels of their translated peptides. This strongly suggests that proglucagon and GIP are encoded on 'translationally regulated genes' – a characteristic possessed by other endocrine hormones. Metabolomic profiling revealed differences in cellular nutrient utilisation/production and that under normal culture conditions both cell lines exhibit signs of overflow metabolism. These studies provide an insight into the metabolism and properties of these valuable cells, suggesting for the first time that incretin hormone genes are translationally regulated.

1. Introduction

Enteroendocrine cells are the most abundant endocrine cell in the human body [32]. They are specialised gastrointestinal cells secreting hormones into either, the circulation (for systemic actions), the extracellular space (for paracrine signalling), or to the enteric nervous system (for stimulation of nerve endings). There are the 'L' cells, originally classified on basis of enteroglucagon immunoreactivity. These are commonly distinguished from the 'K' cells which produce the incretin hormone, glucose-dependent insulinotropic peptide (GIP), and the 'I' cells which produce cholecystokinin (CCK). The term enteroglucagon encompasses the intestinal proglucagon-derived peptides: glucagon-like peptide (GLP)-1 (the other incretin hormone), the gut growth factor GLP-2, and the anorexigenic hormone oxyntomodulin [1]. The 'K' cells tend to predominate in the duodenum and decrease in number toward the distal end of the small intestine. 'L' cells predominate in the ileum and colon and decrease in number toward the proximal end of the small intestine [2]. However, this simplistic view is complicated by the fact that many enteroendocrine cells simultaneously express more than one hormone. For example, GLP-1 occurs in subsets of duodenal 'K' cells and also immunoreactive GIP occurs in subpopulations of proximal 'L' cells [2,3]. Ileal and colonic 'L' cells are well known to also express PYY or CCK [4]. The cellular colocalisation of hormones is supported by studies examining and manipulating hormone profiles *in vivo*. For example, the targeted ablation of proglucagon-expressing cells with diphtheria-toxin also leads to substantial losses in the number of PYY-, GIP- and CCK-positive cells [5]. Similar targeting of GIP-expressing cells reduces pro-CCK but not proglucagon mRNA transcripts in the duodenum, with a trend toward lower CCK levels in the remaining intestine [33]. Duodenal jejunum by-pass surgery in rats (which increases the levels of undigested nutrients arriving in the distal gut) increases the population of cells co-expressing GIP and GLP-1 in the jejunum [6]. Enteroendocrine cells therefore appear to be flexible, and it has been suggested that the 'L', 'K' and 'I' cells of the small intestine consist of a single cell type which exhibits a hormonal spectrum which is affected by its location within the gastrointestinal

Abbrievations: CCK, cholecystokinin; HCA, high content analysis; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide- 2; PYY, peptide YY; PC3, prohormone convertase 3; T2DM, type 2 diabetes mellitus; E2D2, ubiquitin-conjugating enzyme E2D2

* Corresponding author at: Institute for Global Food Security, Queen's University Belfast, Stranmillis Road, Belfast, Northern Ireland BT9 6AG, United Kingdom.
E-mail address: b.green@qub.ac.uk (B.D. Green).

http://dx.doi.org/10.1016/j.peptides.2017.08.010
Received 5 May 2017; Received in revised form 10 July 2017; Accepted 28 August 2017
Available online 10 September 2017
0196-9781/ © 2017 Elsevier Inc. All rights reserved.

tract but also its exposure to dietary nutrients [34].

Within type 2 diabetes mellitus (T2DM) research there is considerable interest in GLP-1, GIP, CCK, PYY and other enteroendocrine hormones. GLP-1 receptor agonists and inhibitors preventing GLP-1 degradation, for instance, are currently used in the clinic to treat patients with T2DM [7]. There is a demand for robust enteroendocrine cell models for the routine screening of nutrients and secretagogues [8]. Established cell lines used for this purpose include: GLUtag – derived from an intestinal endocrine tumour arising in the large bowel in proglucagon-simian virus 40 large T antigen transgenic mice [9]; NCI-H716 – a human cell line derived from a poorly differentiated adenocarcinoma of the caecum [10], and STC-1 which is a neuroendocrine cell line derived from tumours arising in transgenic mice [8].

Several years ago there was an interest in genetically engineering enteroendocrine cells to produce preproinsulin [11,12] as a novel therapy for type 1 diabetes. This attempted to exploit similarities between enteroendocrine ‘K’ cells and pancreatic β-cells *i.e.* mechanisms regulating hormone production/secretion, the timing of hormone release, and the presence of prohormone convertases (2 and 3) for post-translational processing [12]. The initial step before insertion of the insulin gene, involved initially selecting those cells with a ‘K’ cell phenotype *i.e.* those with an active GIP promoter. Ramshur et al. [12] sub-cloned the STC-1 cell line to produce the pGIP/Neo cell line. This involved transfection with a plasmid (pGIP/Neo) encoding neomycin phosphotransferase driven by the GIP promoter, with only those cells with an active GIP promoter surviving genetic selection [12]. pGIP/Neo cells therefore provide a unique opportunity to probe STC-1 which natively is a heterogeneous and plurihormonal population of cells [13]. Quantitative studies employing a specific GIP ELISA demonstrate that pGIP/Neo cells produce and secrete 3–6 times more GIP whilst retaining an ability to synthesize and secrete CCK and PYY [14]. However, further characterisation studies are needed to assess this potential model for GIP secretion. It has not yet been established if pGIP/Neo produces proglucagon-derived peptides or retains GLP-1 secretory activity. This study undertook the first detailed comparison of STC-1 and pGIP/Neo cells. Detailed characterisation studies involved not only assessing differences in the abundance/secretion of proglucagon peptides (GLP-1 and GLP-2), but also the application of high content analysis (HCA) to examine on a cell-by-cell basis how intracellular GLP-1, GIP, CCK and PYY (and their co-localisation) are affected. HCA was also employed to examine morphometric differences (nuclei size/shape/staining intensity) between progenitor STC-1 cells and pGIP/Neo cells. Quantitative measures of gene expression (transcript numbers) for each hormone, as well as, Prohormone convertase 3 (PC3) and Ubiquitin-conjugating enzyme E2D2 were undertaken. Finally, biochemical profiling of cell-cultured media assessed the similarities and differences in their nutrient utilisation/production. The findings bring forward new information relating to the metabolism of enteroendocrine cells and the regulation of incretin hormone biosynthesis.

2. Materials and methods

2.1. Materials

Antibodies anti-Gastric Inhibitory Polypeptide (GIP) (Alexa Fluor 647), anti-Cholecystokinin (CCK) (Alexa Fluor 555) and anti-Peptide YY antibody (Cy3) were purchased from antibodies-online GmbH (Aachen, Germany) and the Glucagon-like Peptide-1 (GLP-1) antibody (FITC) Biorbyt (Cambridge, UK). PBS tablets, Triton – X 100 ~ 10% solution and Formalin were purchased from Sigma (Dorset, UK).

2.2. Cell culture

STC-1 and pGIP/Neo cell lines were received as a gift from Dr. B. Wise (Washington University of St. Louis) with permission from Dr. D. Hanahan (Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland). STC-1 cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) containing high glucose, 4.5 g/L, with L-glutamine, and without sodium pyruvate (Gibco, Paisley, UK). The medium was supplemented with 17.5% foetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/L streptomycin. pGIP/Neo STC-1 cells were also cultured in DMEM containing high glucose, 4.5 g/L, with L-glutamine, and without sodium pyruvate, however the medium contained 10% FBS and geneticin (G418, 400 μg/mL, Sigma, Poole, Dorset, UK). Both cell medium were incubated in a 5% CO_2 humidified atmosphere at 37 °C. Cells underwent passage upon reaching 80–90% confluence and were used between passage numbers 15–50.

2.3. Media accumulation and intracellular content of GLP-1 and GLP-2

STC-1 and pGIP/Neo cell lines were seeded in 24-well plates at a density of 2×10^5/well and incubated in a 5% CO_2 humidified atmosphere at 37 °C overnight. Media was removed and 1 mL of fresh media added to each well and cells were incubated as above for 72 h upon which the media was collected, centrifuged to remove cellular debris and stored at −80 °C prior to ELISA analysis.

To assess the cellular hormone content acid/Ethanol (1.5% v/v HCl, 75% v/v ethanol, 23.5% v/v H_2O) was added and incubated overnight at 4 °C. The incubation solution was removed and centrifuged (900*g*, 5 min) to remove cellular debris. The supernatant was collected and the ethanol evaporated off using a Speedvac sample concentrator (Genevac, Ipswich, UK). Samples were reconstituted in PBST 0.1% BSA and stored at −80 °C prior to ELISA measurement.

2.4. Determination of GLP-1 and GLP-2 using ELISA

GLP-1 and GLP-2 (Total) concentrations were measured using ELISA kits purchased from Merck Millipore (Hertfordshire, UK). The assays were performed in accordance with the manufacturer’s instructions. The plates were read at 450 nm and 590 nm in a plate reader (Tecan, Safire[2]).

2.5. Immunofluorescence of GLP-1, GIP, CCK and PYY

Antibodies were prepared in 1% BSA in phosphate buffered saline with tween (PBST) immediately prior to adding to cells and protected from the light after preparation. Cells were seeded into 96-well plates (5×10^4 per well) and cultured overnight at 37 °C in a humidified atmosphere of 5% CO_2. Media was removed and cells fixed with 10% formalin for 10 min at room temperature. Cells were washed (x4) with PBS and permeabilisation solution added and incubated for 30 min at room temperature. Again, cells were washed (x4) with PBS before blocking solution (0.3 M glycine + 10% goat serum in 1% BSA in PBST (PBS + 0.01% Tween 20) was added for a further 1 h. The blocking solution was removed and the GLP-1, GIP, CCK or PYY antibody was added (1:200 dilution) and allowed to incubate overnight. Supernatant was removed and cells were washed with PBS (3 times × 5 min) in the dark, after which Hoechst staining solution (Thermo Scientific, Hampstead, UK) was added for 10 min. Cells were washed with PBS (x1) and PBS added to each well and the plate was sealed prior to reading using the ArrayScan® HCS Reader (Thermo, Loughborough, UK). The plate was evaluated at absorbance 350–461 nm and 485–520 nm for Hoechst dye and antibody fluorescence, respectively. Typical micrographs obtained from the ArrayScan® system can be found in Fig. 1.

2.6. HCA software parameters

We determined the percentage of cells with immunoreactivity to GLP-1, GIP, PYY or CCK by application of the HCS Studio Cell Analysis

Download English Version:

https://daneshyari.com/en/article/5514785

Download Persian Version:

https://daneshyari.com/article/5514785

Daneshyari.com