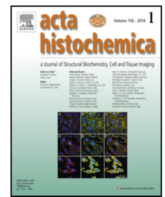
Acta Histochemica 117 (2015) 205–210

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis



Expression of cholecystokinin$_2$-receptor in rat and human L cells and the stimulation of glucagon-like peptide-1 secretion by gastrin treatment

Yang Cao, Xun Cao, Xiao-Min Liu*

Department of Endocrinology, First Affiliated Hospital of Harbin Medical University, Harbin 150001, Heilongjiang Province, China

ARTICLE INFO

Article history:
Received 18 October 2014
Received in revised form
30 November 2014
Accepted 18 December 2014

Keywords:
Gastrin
Glucagon-like peptide-1 (GLP-1)
NCI-H716 cell line
Cholecystokinin$_2$ receptor (CCK2R)
Rat colon

ABSTRACT

Gastrin is a gastrointestinal hormone secreted by G cells. Hypergastrinemia can improve blood glucose and glycosylated hemoglobin levels. These positive effects are primarily due to the trophic effects of gastrin on β-cells. In recent years, many receptors that regulate secretion of glucagon-like peptide 1 (GLP-1) have been identified in enteroendocrine L cell lines. This led us to hypothesize that, in addition to the trophic effects of gastrin on β-cells, L cells also express cholecystokinin$_2$-receptor (CCK2R), which may regulate GLP-1 secretion and have synergistic effects on glucose homeostasis. Our research provides a preliminary analysis of CCK2R expression and the stimulating effect of gastrin treatment on GLP-1 secretion in a human endocrine L cell line, using RT-PCR, Western blot, immunocytochemistry, and ELISA analyses. The expression of proglucagon and prohormone convertase 3, which regulate GLP-1 biosynthesis, were also analyzed by real-time PCR. Double immunofluorescence labeling was utilized to assess the intracellular localization of CCK2R and GLP-1 in L cells harvested from rat colon tissue. Our results showed that CCK2R was expressed in both the human L cell line and the rat L cells. We also showed that treatment with gastrin, a CCK2R agonist, stimulated the secretion of GLP-1, and that this effect was likely due to increased expression of proglucagon and PCSK1 (also known as prohormone convertase 3 (*PC3* gene)). These results not only provide a basis for the role gastrin may play in intestinal L cells, and may also provide the basis for the development of a method of gastrin-mediated glycemic regulation.

© 2014 Elsevier GmbH. All rights reserved.

Introduction

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from intestinal L cells in response to nutrient ingestion (Reimer, 2006; Tolhurst et al., 2009). GLP-1 influences glucose homeostasis in several ways, including stimulating glucose-dependent insulin secretion (Ranganath, 2008), promoting β-cell proliferation (Maida et al., 2009), suppressing glucagon release (Hare et al., 2009), reducing energy intake (Talsania et al., 2005; Tang-Christensen et al., 2001), and delaying gastric emptying (DeFronzo et al., 2008; Linnebjerg et al., 2008). In type 2 diabetes, the effects of incretins have been markedly reduced due to a defect in secretion of GLP-1 from intestinal L cells (Knop et al., 2007). This then leads to impairment of early-phase insulin secretion after food intake, and consequently results in postprandial hyperglycemia and hyperlipidemia (Deacon, 2007; Holst et al., 2009; Nauck and Meier, 2005; Rask et al., 2004). Therefore, identifying a method for restoring GLP-1 secretion by intestinal L cells may provide a viable therapeutic strategy for the management of metabolic syndrome. Many receptors involved in GLP-1 secretion have been identified in enteroendocrine L cells, including muscarinic receptors (Mortensen et al., 2009), leptin receptors (Anini and Brubaker, 2003), sulfonylurea receptor 1 (SUR1)/ATP-sensitive potassium (K_{ATP}) channel (Nielsen et al., 2007; Reimann and Gribble, 2002), Na^+/glucose co-transporter 1 (SGLT1) (Gribble et al., 2003), glucose transporter 2 (GLUT2) (Cani et al., 2007), sweet taste receptor (Jang et al., 2007), TGR5 (Katsuma et al., 2005), and G-protein coupled receptors (GPRs) (Hirasawa et al., 2005; Overton et al., 2008; Chu et al., 2008; Hara et al., 2009). Gastrin is a gastrointestinal hormone that is secreted by G cells. Previous studies found that gastrin secretion was indirectly elevated by proton pump inhibitors (PPI) and that this increase resulted in trophic effects on the regeneration and repair potential of the pancreas. Hypergastrinemia, therefore, indirectly caused an improvement in the levels of blood glucose and glycosylated hemoglobin (Wang et al., 1993; Bödvarsdóttir et al., 2010; Singh et al., 2012). The gastrin receptor

* Corresponding author at: Department of Endocrinology, First Affiliated Hospital of Harbin Medical University, No. 24 Youzheng Street, Harbin, Heilongjiang Province 150001, China.
E-mail address: liuxiaomin1957@126.com (X.-M. Liu).

http://dx.doi.org/10.1016/j.acthis.2014.12.007
0065-1281/© 2014 Elsevier GmbH. All rights reserved.

cholecystokinin$_2$-receptor (CCK2R/CCKBR), is normally expressed by gastric parietal cells, enterochromaffin-like (ECL) cells, smooth muscle cells, and the cells of the central nervous system. Previous reports have identified various receptors that regulate GLP-1 secretion in L cells. In certain cell types, stimulation of CCK2R induced secretion and controlled the expression of various genes. The aims of this study were to determine whether CCK2R is expressed in both a human L cell line and rat L cells, to examine whether gastrin could stimulate the secretion of GLP-1 in L cells, and to examine the possible mechanisms involved in gastrin-induced GLP-1 secretion.

Materials and methods

Cell culture

The NCI-H716 human intestinal cell line and the TT human medullary thyroid carcinoma cell line were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai Institute of Cell Biology, Shanghai, China). Cells were cultured by suspension in RPMI 1640 and Ham's F-12 media (Gibco, Grand Island, NY, USA), respectively, supplemented with 10% FBS (Gibco), 2 mM L-glutamine, 100 IU/mL penicillin, and 100 mg/mL streptomycin, and incubation at 37 °C, 5% CO_2.

Reverse transcription-polymerase chain reaction (RT-PCR)

RNA was isolated from NCI-H716 cells and TT cells (positive control) by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RT-PCR was performed using the OneStep RT-PCR kit (Qiagen, Hilden, Germany), according to manufacturer's protocols. The sequences of the primers used were the following: CCK$_2$/gastrin receptor (sense) 5′-CGTGTGCTGCAGTGCGTGCA-3′, (antisense) 5′-GGTGGTGTAGCTAAGCCT GG-3′; β-actin (sense) 5′-ATCTGGCACCACACCTTCTACA-3′, (antisense) 5′-GCT CATTGCCAATGGTGATGAC-3′. To detect CCK2R and β-actin, the following PCR conditions were used: 40 cycles of denaturing at 94 °C for 60 s, annealing at 60 °C for 60 s, and extension at 72 °C for 120 s. PCR-products were analyzed on a 1.2% agarose gel.

Western blot

A total of 3×10^6 NCI-H716 and TT cells were harvested by scraping (TT cells) and/or by resuspension. Cells were centrifuged at 12,000 × g for 10 min at 4 °C and then lysed using RIPA buffer (Beyotime Institute of Biotechnology, China). Concentrations of the resulting supernatants were determined using bicinchoninic acid (BCA) protein assay reagent (Beyotime Institute of Biotechnology, Jiangsu, China). Forty micrograms of each sample was then separated by SDS–PAGE. Proteins were then transferred and incubated in blocking buffer. Membranes were incubated overnight with a 1:2000 dilution of CCK2R-specific primary antibody (ab77077; Abcam, Cambridge, UK) in blocking buffer. Membranes were then incubated with a goat-specific, horseradish peroxidase-conjugated secondary antibody (ZSGB-BIO, Beijing, China) for 1 h, and protein complexes were detected using enhanced chemiluminescence solution (Beyotime Institute of Biotechnology).

Immunocytochemical staining

NCI-H716 cells were grown on coverslips, fixed in 4% paraformaldehyde in PBS for 10 min, washed with PBS, and cooled in 100% methanol at −20 °C for 20 min. Cells were then washed with PBS. After blocking with protein blocking solution, coverslips were incubated overnight with a 1:100 dilution of anti-CCK2R primary antibody (Abcam) at 4 °C. Coverslips were then washed, incubated with a 1:200 dilution of a FITC-conjugated (green) rabbit anti-goat secondary antibody (Boster, Wuhan, China) for 30 min at room temperature in the dark, and again washed with phosphate buffered saline (PBS). Cell nuclei were then counterstained by incubation with DAPI stain for 5 min. After a final wash with PBS, coverslips were mounted on slides by using the SlowFade Antifade Kit (Life Technologies, Carlsbad, CA, USA). For negative controls, the primary antibody was omitted.

GLP-1 secretion study

Two days before each experiment, NCI-H716 cells (10^6 cells/well) were seeded in 12-well plates pre-coated with Poly-L-lysine. On the day of the experiment, the culture medium was replaced with fresh medium or with medium containing gastrin (10^{-6}, 10^{-7}, or 10^{-8} M final concentration) (Bachem, Bubendorf, Switzerland). After incubation at 37 °C for 2 h, supernatants were collected and 50 μg/mL phenylmethylsulfonyl fluoride (PMSF) protease inhibitor was added. The concentrations of GLP-1 in the culture supernatants were measured using a GLP-1 active ELISA kit (USCN, Wuhan, China), according to the manufacturer's protocol.

Quantitative RT-PCR

Quantitative RT-PCR (qRT-PCR) was used to measure proglucagon (*gcg* gene) and PCSK1 mRNA (prohormone convertase 3 (*PC3* gene)) levels in human NCI-H716 cells (Liu et al., 2013). After washing with PBS three times, total RNA from NCI-H716 cells was isolated using TRIzol reagent (Invitrogen), according to the manufacturer's instructions. The purity and concentration of RNA was determined by measuring the absorbance at 260 and 280 nm. *A*260/*A*280 ratio of at least 1.7 was considered indicative of sufficient purity (Reimer et al., 2001). RNA (1 μg) was reverse-transcribed into cDNA by using oligo(dT)^{18} primers (Takara Biotechnology, Otsu, Japan) with the following cycling conditions: 37 °C for 15 min, 85 °C for 5 s, and 4 °C. qRT-PCR reactions were performed using the ABI7500Fast detection system. The sequences of the primers used in this study were as follows: proglucagon (sense) 5′-TGTCAGCGTAATATCTGTGAGGC-3′, (antisense) 5′-AGCAGGTGAAGAGA GAGCAA GC-3′; *PC3* (sense) 5′-CAGAAG GCTTTTGAATATGGTGT-3′, (antisense) 5′-GGAGGCACTGCTGATGGAGAT-3′; β-actin (sense) 5′-CAGTCGG TTGGAGCGAGCAT-3′, (antisense) 5′-GGACTTCC TGTAACAACGCAT CT-3′.

After denaturing at 95 °C for 2 min, amplification was achieved by performing 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Melting curves were performed to evaluate the specificity of the PCR reaction. Relative expression of each gene was calculated according to a comparative Ct method, using the formula: $RQ = 2^{-\Delta\Delta C_t}$.

Localization of CCK2R and GLP-1 in the rat colon

Rat colon tissue was harvested, fixed by treatment with 4% paraformaldehyde overnight, and then incubated in 10% sucrose at 4 °C until it was completely submerged in the solution. The excised rat colons were embedded in OCT medium (Sakura Finetek, Torrance, CA, USA) and snap frozen in liquid nitrogen. The samples were cut into 5-μm-thick sections and rehydrated by incubating in 0.01 M PBS (pH 7.4) three times for 10 min each. Rehydrated sections were blocked in 5% BSA for 30 min at room temperature and then incubated overnight with a 1:100 dilution of rabbit anti-rat GLP-1 antibody (Abcam) at 4 °C. The following day, sections were rinsed with PBS and incubated in a 1:100 dilution of Alexa Fluor® 594-conjugated Affinipure goat anti-rabbit IgG (ZSGB-BIO, Beijing, China) for 1 h at 37 °C in the dark. To detect CCK2R, we repeated this process using a goat anti-rat CCK2R antibody and a FITC-labeled,

Download English Version:

https://daneshyari.com/en/article/1923575

Download Persian Version:

https://daneshyari.com/article/1923575

Daneshyari.com