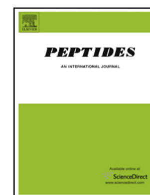
Peptides 48 (2013) 36–44

Contents lists available at ScienceDirect

Peptides

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



Functional activity of murine intestinal mucosal cells is regulated by the glucagon-like peptide-1 receptor

Mamdouh H. Kedees, Yelena Guz, Marine Grigoryan, Gladys Teitelman *

Department of Cell Biology, SUNY-Downstate Medical Center, Brooklyn, NY 11203, United States

ARTICLE INFO

Article history:
Received 2 May 2013
Received in revised form 19 July 2013
Accepted 22 July 2013
Available online 6 August 2013

Keywords:
Glucagon like peptide-1 receptor
Enterocytes
Paneth cells
Enteric neurons

ABSTRACT

To determine whether the glucagon-like peptide-1 receptor (GLP-1r) plays a role in the regulation of intestinal functional activity, we analyzed the distribution of the GLP-1r in mouse tissues and tested if tissues expressing the receptor respond to exendin-4 and exendin (9–39) amide, a GLP-1r agonist and antagonist respectively. In ileum, *Glp1r* mRNA level was two fold higher in extracts from epithelial cells than non-epithelial tissues. By immunohistochemistry, the receptor was localized to the mucosal cell layer of villi of ileum and colon, to the myenteric and submucosal plexus and to Paneth cells. Intravenous administration of exendin-4 to CD-1 mice induced expression of the immediate early gene *c-fos* in mucosal cells but not in cells of the enteric plexuses or in L cells of ileum. The induction of *c-fos* was inhibited by the voltage-gated sodium channel blocker tetrodotoxin. Exendin-4 also increased *c-fos* expression in ileal segments in vitro, suggesting that this action of the analog was independent of an extrinsic input. The induction of *c-fos* expression by exendin-4 was inhibited by exendin (9–39) amide, indicating that the action of exendin-4 was mediated by activation of the receptor. Our findings indicate that the GLP-1r is involved in ileal enterocyte and Paneth cell function, that the GLP-1 analog activates c-fos expression in the absence of an extrinsic input and that some of the actions of the receptor is/are mediated by voltage-gated Na channels.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is a 30 amino acid peptide produced by posttranslational processing of the proglucagon gene product in the intestinal L-cell, which are located mainly in ileum and colon. GLP-1 secreted after nutrient ingestion is rapidly degraded by the enzyme dipeptidyl-peptidase IV (DPP-IV) [17]). As a result, the biological half-life of GLP-1 is less than 5 min [15]. GLP-1 binds to a specific receptor (GLP-1r), a seven-transmembrane G protein-coupled receptor originally identified in islet β-cells [30] with a broad range of tissue-specific functions [9]. GLP-1r and GLP-1 containing nerve fibers are present in regions of the central nervous system (CNS) that regulate a diverse array of homeostatic functions including feeding behavior, gastric motility and glucoregulation [1]. The binding of GLP-1 to the GLP-1r on pancreatic beta cells increases transcription of the insulin gene and enhances both the stability of insulin mRNA and biosynthesis of insulin (reviewed in [19]).

It has recently been reported that GLP-1 regulates L cell number but that L cells do not express the receptor [12]. These observations suggest that GLP-1 may regulate L cell number through a paracrine and/or neuronal loop. To date, however, there is scant information of the tissue and cell-specific expression of the GLP-1r in the intestine. Previous studies documented the presence of GLP-1r in myenteric plexus of rodents [2,33] and humans [20] but its expression by other intestinal cells is not known. In the present study, we sought to determine whether non-neuronal cell types of the intestine also express the GLP-1r, if expression of the receptor was region- specific or, alternatively, was expressed throughout the intestinal tract. Second, we asked whether binding of a GLP-1 mimetic to the receptor activated downstream targets and, third, if the response of the receptor was mediated by a neuronal input.

2. Materials and methods

2.1. Animals

Three month old male CD-1 mice were purchased from Charles River. Animal protocols were approved by the Institutional Animal Care and Review Committee. Number of animals used is indicated in the individual experiments.

* Corresponding author at: Department of Cell Biology, SUNY-Downstate Medical Center, 450 Clarkson Ave, Brooklyn, NY, USA. Tel.: +1 718 270 2950; fax: +1 718 270 3732.
E-mail addresses: gteitelman@downstate.edu, gteitelman@comcast.net (G. Teitelman).

0196-9781/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.peptides.2013.07.022

2.2. Isolation of intestinal cells

Mice were anesthetized, and a 10 cm segment of ileum (proximal to the cecum), a 3 cm segment of the duodenum (proximal to the pylorus), and 4 cm of colon (next to the cecum) were used for isolation of epithelial cells following a previously described technique [34] with minor modifications [12]. Epithelial cell suspensions were collected in nuclease-free tubes filled with 1 ml phosphate buffer-saline (PBS) and centrifuged at 1000 rpm for 2 min at 4 °C. The pellet was collected for RNA isolation.

2.3. RNA isolation

Tissues (non-epithelial elements) or mucosal intestinal cells were collected and stored in RNA-later solution (Qiagen, Valencia, CA) or processed immediately for total RNA isolation using RNeasy kit (Qiagen) according to the manufacturer's protocol. RNA concentration and purity was checked by UV spectrometry at A260 and A280. Only samples giving OD ratio A260/A280 of 1.8–2.0 were used. RNA integrity was checked by gel electrophoresis, only samples demonstrating sharp bands of 28S and 18S ribosomal RNA were used. To eliminate genomic DNA contamination, DNase I digestion was performed at 37 °C for 30 min (TURBO DNA-free kit, Ambion, Austin, TX).

2.4. cDNA synthesis

Total RNA was transcribed using SuperScript III and accompanying reagents (Invitrogen, Grand Island, NY) to cDNA. The cDNA was diluted 1:10 in Nuclease-free water and used to study the relative gene expression.

2.5. RT-PCR

Expression of *Glp1r* in mouse ileum was initially demonstrated using classical semi-quantitative RT-PCR (Fig. 1). Primer sequences for *Glp1r* were as follows: 5-TGA ACC TGT TTG CAT CCTTCA-3 and 5-ACT TGG CAA GCC TGC ATT TGA-3, these primers were previously used by Suzuki et al. (2003). PCR Conditions were as follows: initial denaturation at 95 °C for 15 min followed by 30 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR products were separated on 2% agarose gel.

2.6. Quantitative real-time PCR

qRT-PCR was performed as previously described [18]. The level of the specific gene (X) transcript was normalized to the expression of TATA box binding protein (TBP) or 18S rRNA or equivalent as endogenous control. **Choice of house-keeping gene**: Initial experiments included testing a panel of 8 housekeeping genes Beta actin (*Actb*), Beta 2 microglobulin (*B2m*), Glyceraldhyde-3 phosphate dehydrogenase (*Gapd*), beta glucouronidase (*Gusb*), Hypoxanthine phosphoribosyltransferase 1 (*Hprt1*), Phosphoglycerate kinase (*Pgk*), Peptidylprolyl isomerase A (*Pp1a*), and Ribosomal protein L13A (*Rpl13a*), the mouse housekeeping gene set was purchased from Real Time Primers LLC, Elkins Park, PA) as well as *18SrRNA* (SuperArray) and TATA box binding protein (*Tpb*, SuperArray). *18SrRNA* and *Tpb* showed consistent expression and were used for quantitative Real-Time analysis.

For data analysis the $^{\Delta\Delta}$Ct method was used; for each gene, fold-changes were calculated as the difference in gene expression between samples. All reactions were run in triplicate and each gene expression assay was repeated at least three times using StepOne Plus (Applied Biosystems, Carlsbad, CA). mRNA values of cells and tissues were first normalized to the values of the house keeping gene in each specific experiment. Then, mRNA values of cells and tissues were compared. All primers used in qReal-Time PCR analysis in this report were purchased from SA biosciences/Qiagen. Primers sequences were not disclosed by SA biosciences. Melting curve analysis and Gel electrophoresis indicated that the primers amplify a single amplicon of the expected size (not shown). The validated primers from SuperArray have been extensively used by many researchers (http://www.sabiosciences.com/support_publication.php#qpcr).

2.7. Western Blot analysis

Tissues were homogenized for 5 min in ice-cold lysis buffer (50 mM Tris HCl, pH 7.4, 5 mM EDTA and 0.02% NaN_3) containing a cocktail of fresh protease inhibitors (Sigma, St Louis, MO). After homogenization, samples were centrifuged at 15,000 rpm for 10 min at 4 °C. Protein concentration in cleared lysates was measured using bicinchoninic acid colorimetric assay (BCA kit; Pierce, Rockford, IL). Samples were fractionated on a 12% Tris–HCl Mini-Protean 4–15% gradient TGX gels (Bio-Rad, Hercules, CA) that is able to detect bands from 6.5 to 200 kDa, electrophoretically transferred to Amersham Hybond ECL nitrocellulose membrane (Amersham, Piscataway, NJ). After 30 min blocking with a 5% milk solution at room temperature, membranes were incubated overnight at 4 °C with anti GLP-1r rabbit serum (a gift from J. Habener, Harvard University, diluted 1:1000 in 5% milk) or with an antibody from Novus Biologicals (Littleton, CO; NBP1-97308) at 1:1000 dilution. Next day, membranes were washed in TBS (0.5 M Tris Base, 9% NaCl, pH 8.4), incubated for 1 h at room temperature with Horseradish-Peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (Santa Cruz Laboratories, Santa Cruz, CA) diluted at 1:10,000 and bands were visualized using SuperSignal West Pico Chemiluminescent detection reagents (Thermo Scientific, Rockford, Il) and CL-XPosure autoradiography film (Thermo-Scientific). Bands were scanned with Epson Perfection 2400.

2.8. Preparation of tissue sections

Mice (not fasted) were perfused through the heart with 4% paraformaldehyde (PF). Samples of 3 cm duodenum (proximal to the pylorus), jejunum (6 cm from the pylorus), 8 cm ileum (proximal to the caecum) and 4 cm colon were collected and cleaned of mesenteric fat, gently flushed with phosphate buffer-saline (PBS), post-fixed for 1 h in 4% PF, cryopreserved in a 30% sucrose solution, embedded in Shandon M1 matrix (Thermo Scientific), and 20 μm frozen sections obtained using a cryostat microtome (Leica Jung 3050S).

2.9. Immunocytochemistry (ICC)

Sections were processed for immunostaining as previously described [32]. Briefly, sections were incubated sequentially in an empirically derived optimal dilution of control serum or primary antibody raised in species "X" overnight and with a 1:200 dilution of the secondary antibodies. For multiple label experiments using immunofluorescence, antibodies produced in different hosts were used. Alternatively, sections were incubated with primary antibodies and visualized with peroxidase–antiperoxidase (PAP) complex and diaminozenzidine (DAB) as a chromogen. To determine the localization of the GLP-1r protein in the gut, we used a specific antibody (a gift from J. Habener) previously described by Heller et al. [16] that was raised against a peptide that spans a 18 amino acid fragment (29–46) in the N-terminus of rat sequence; it shares 94% and 88% identity with the mouse and human sequence respectively as demonstrated by our multiple sequence alignment analysis using BLASTP and CLUSTALW (Biology workbench 3.2 free software (http://workbench.sdsc.edu/). The specificity of the

Download English Version:

https://daneshyari.com/en/article/2006195

Download Persian Version:

https://daneshyari.com/article/2006195

Daneshyari.com