The Intestinal Glucose–Apelin Cycle Controls Carbohydrate Absorption in Mice

CÉDRIC DRAY,[1,2,*] YASSINE SAKAR,[3,*] CLAIRE VINEL,[1,2] DANIELE DAVIAUD,[1,2] BERNARD MASRI,[2,4] LUC GARRIGUES,[2,5] ESTELLE WANECQ,[1,2] SYLVAIN GALVANI,[1,2] ANNE NEGRE–SALVAYRE,[1,2] LARRY S. BARAK,[6] BERNARD MONSARRAT,[2,5] ODILE BURLET–SCHILTZ,[2,5] PHILIPPE VALET,[1,2] ISABELLE CASTAN–LAURELL,[1,2] and ROBERT DUCROC[3]

[1]INSERM Unité 1048, Institut des Maladies Métaboliques et Cardiovasculaires, Toulouse, France; [2]Université de Toulouse, Université Paul Sabatier, Toulouse, France; [3]INSERM Unité 773, Centre de Recherche Biomédicale Bichat Beaujon, CRB3; UFR de Médecine site Bichat Paris 7 - Denis Diderot; IFR02 Claude Bernard, Paris, France; [4]INSERM UMR1037, Cancer Research Center of Toulouse, Toulouse, France; [5]CNRS, Institut de Pharmacologie et de Biologie Structurale, Toulouse, France; and [6]Department of Cell Biology, Duke University Medical Center, Durham, North Carolina

BACKGROUND & AIMS: Glucose is absorbed into intestine cells via the sodium glucose transporter 1 (SGLT-1) and glucose transporter 2 (GLUT2); various peptides and hormones control this process. Apelin is a peptide that regulates glucose homeostasis and is produced by proximal digestive cells; we studied whether glucose modulates apelin secretion by enterocytes and the effects of apelin on intestinal glucose absorption. **METHODS:** We characterized glucose-related luminal apelin secretion in vivo and ex vivo by mass spectroscopy and immunologic techniques. The effects of apelin on ^{14}C-labeled glucose transport were determined in jejunal loops and in mice following apelin gavage. We determined levels of GLUT2 and SGLT-1 proteins and phosphorylation of AMPKα2 by immunoblotting. The net effect of apelin on intestinal glucose transepithelial transport was determined in mice. **RESULTS:** Glucose stimulated luminal secretion of the pyroglutaminated apelin-13 isoform ([Pyr-1]-apelin-13) in the small intestine of mice. Apelin increased specific glucose flux through the gastric epithelial barrier in jejunal loops and in vivo following oral glucose administration. Conversely, pharmacologic apelin blockade in the intestine reduced the increased glycemia that occurs following oral glucose administration. Apelin activity was associated with phosphorylation of AMPKα2 and a rapid increase of the GLUT2/SGLT-1 protein ratio in the brush border membrane. **CONCLUSIONS: Glucose amplifies its own transport from the intestinal lumen to the bloodstream by increasing luminal apelin secretion. In the lumen, active apelin regulates carbohydrate flux through enterocytes by promoting AMPKα2 phosphorylation and modifying the ratio of SGLT-1:GLUT2. The glucose-apelin cycle might be pharmacologically handled to regulate glucose absorption and assess better control of glucose homeostasis.**

Keywords: Calorie Intake; Mouse Model; Diabetes; Adipokine.

Apelin is the endogenous ligand for the G protein–coupled receptor APJ,[1,2] acting under several molecular forms ([Pyr-1]-apelin-13, apelin-13, -17, and -36) processed from a 77–amino acid precursor.[3] The active forms of apelin are present in peripheral tissues, including lungs, heart, adipose, and pancreas (for reviews, see Carpene et al[4] and Castan-Laurell et al[5]). In the gastrointestinal tract, messenger RNA (mRNA) apelin-expressing cells were found in rat and mouse stomach, mouse duodenum, and human and mouse colon.[1,6] APJ immunostaining has also been described in the epithelium, goblet cells, and crypt cells of the small intestine and in the smooth muscle layer of the gastrointestinal tract in the rat.[7] APJ is also located in the enteric blood vessels.[7] Thus, the apelin/APJ system may have a potential role in the digestive tract.

Recent studies have established that apelin is involved in glucose homeostasis.[8] We showed that intravenous injection of physiological doses of apelin decreased glycemia and stimulated glucose uptake in skeletal muscles of lean and obese insulin-resistant mice.[9] Moreover, apelin-stimulated glucose transport in muscle was dependent on AMP-activated protein kinase (AMPK) activation. Similar results were described in cultured C2C12 myotubes by Yue et al, who also showed that apelin-deficient mice exhibit decreased insulin sensitivity.[10] Taken together, such studies support the assumption that apelin plays a physiological role in glucose metabolism and maintenance of insulin sensitivity.[8]

We showed that leptin and resistin β, two adipokines secreted in the gastrointestinal lumen by gastric and intestinal endocrine cells, regulate the activity of the sugar transporters in enterocytes by an AMPK-dependent mechanism.[11–13] The net effect of this regulation of hexose transporters was an increase of sugar uptake with significant consequences on splanchnic metabolism. Interestingly, the adipokine apelin shares several features with these peptides, such as (1) the ability to be produced in

*Authors share co-first authorship.

Abbreviations used in this paper: AMPK, AMP-activated protein kinase; BBM, brush border membrane; GLUT2, glucose transporter 2; Isc, short-circuit current; KRB, Krebs–Ringer solution; mRNA, messenger RNA; MS, mass spectrometry; PBS, phosphate-buffered saline; SGLT-1, sodium-glucose transporter 1.

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2013.01.004

the gastrointestinal tract, (2) an implication in glucose metabolism, and (3) the control of insulin sensitivity via AMPK.[10,11] Recent studies brought evidence of a putative regulation of apelin by glucose in different tissues. Indeed, increased amounts of apelin in response to different glucose levels[14,15] were shown in human endothelial as well as in pancreatic beta cells.

This study was designed to characterize the relationship between apelin and glucose in the intestine. We show that D-glucose specifically promotes [Pyr-1]-apelin-13 secretion in the intestine, and we further show in vitro and in vivo the capacity of apelin to increase glucose flux from the lumen toward the bloodstream by interacting with the APJ receptor present in enterocytes. This effect appears to involve AMPK-dependent control of sodium glucose transporter 1 (SGLT-1) and glucose transporter 2 (GLUT2) expression in the membrane of apical enterocytes by apelin. Moreover, pharmacologic inhibition of endogenous apelin action by a selective APJ antagonist resulted in a decrease of glycemia, supporting the existence of a glucose/apelin cycle that regulates intestinal absorption of carbohydrates.

Materials and Methods

Animals

Male C57BL/6J mice (Centre Elevage Janvier, Le Genest-St-Isle, France) had free access to water and standard food. They were treated in accordance with European Community guidelines concerning the care and use of laboratory animals.

Nanoflow Liquid Chromatography–Tandem Mass Spectrometry Analysis

The gastric contents were filtrated with a 10-kilodalton membrane and injected on a NanoRS 3500 chromatographic system (Dionex, Amsterdam, The Netherlands) coupled to an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Five microliters of each sample was separated on a 75 μm ID $\times$ 15 cm C18 column (Proxeon Biosystems, Odense, Denmark). Peptides were eluted using a 5% to 50% linear gradient of solvent B in 105 minutes (solvent A was 0.2% formic acid and solvent B was 0.2% formic acid in 80% ACN). Full mass spectrometry (MS) scans were acquired in the Orbitrap on the 300–2000 m/z range with the resolution set to 60,000. An inclusion list corresponding to several charge states (2+, 3+, 4+) of [Pyr-1]-apelin-13 was used to select these ions for CID fragmentation, and the resulting fragment ions were analyzed in the linear ion trap (LTQ, Thermo Fisher Scientific). Dynamic exclusion was used within 60 seconds to prevent repetitive selection of the same peptide.

Fluorescence Immunohistochemical Studies and Confocal Microscopy

Immunohistochemical staining was performed as previously described[13] using anti-APJ polyclonal antibody (1/100; Novus Biologicals, Litlletown, CO), anti-apelin polyclonal antiserum (1/200; Covalab, Villeurbanne, France), and anti-GLUT2 antibody (1/100; Abcam 54460, Paris, France). Nuclei were stained with TOPRO-III (1/1000; Invitrogen, Saint Aubin, France). Fluorescence analysis was performed using an LSM510 confocal laser scanning microscope (Zeiss, Le Pecq, France). Samples were visualized with a 25$\times$ objective lens (Plan-Apochromat, N.A. 1.4, oil) and excited using 3 laser lines (488, 543, and 633 nm). For APJ and GLUT2 detection, control was achieved using an immunoglobulin G mouse serum at the same concentration as the antibody. The specificity of apelin immunostaining was tested using primary antisera preabsorbed with an excess amount of homologous antigen ([Pyr1]-Apelin-13, 10^{-6} mol/L). Densitometric quantifications of fluorescence intensity were assessed by ImageJ software. The results represent the apelin-integrated density – (total area $\times$ mean fluorescence of background) of 3 different pictures per mouse and 4 mice per group.

Tissue Preparation and Short-Circuit Measurement

Mice were fasted for 16 hours and killed. The proximal jejunum was dissected, and adjacent samples were mounted in Ussing chambers. The tissues were bathed with Krebs–Ringer solution (KRB) with 10 mmol/L glucose at 37°C (pH 7.4) and gassed with 95% O_2/5% CO_2. Electrogenic ion transport was monitored as previously described.[11] KRB alone (vehicle) or containing apelin (10^{-10} to 10^{-6} mol/L) was added in the mucosal bath 2 minutes before challenge with 10 mmol/L glucose. Similar tests were performed with 100 nmol/L apelin incubated overnight at 4°C with 1/100 rabbit polyclonal antibody raised against apelin (Covalab).

Transport of Transmural Hexoses

The experiments were performed using jejunal sacs from fasted mice. The proximal jejunum was dissected and rinsed in cold saline solution. Jejunal sacs (4 cm long) were prepared for D-[1-^{14}C] glucose (49.5 mCi/mmol) transport as previously described.[12] The corresponding jejunal sacs were filled with 1 mL of KRB without (control) or with 1 nmol/L apelin and containing 0.02 μCi/mL of the isotopic tracer D-[1-^{14}C] glucose (49.5 mCi/mmol) and glucose to obtain a final concentration of 30 mmol/L. Similarly, we studied paracellular transport with 30 mmol/L mannitol and the isotopic tracer D-[1-^{14}C] mannitol (59 mCi/mmol) at 0.2 μCi/mL.

SGLT-1, GLUT2, AMPK, and APJ Western Blot

Fasted animals were anesthetized and underwent laparotomy for in situ experiments. Three jejunal segments (5 cm long) were tied and filled with 3 mL of KRB without (control) or with 1 nmol/L apelin. After 3 minutes of in situ incubation, 3 mL of 60 mmol/L glucose solution was injected into the lumen to obtain a final concentration of 30 mmol/L. After a further 5 minutes, these sacs were removed and opened along the mesenteric border and the mucosa was scraped off on ice with a glass blade.

For APJ determination, mice were gavaged with water (control) or [Pyr-1]-apelin-13 (200 pmol/kg in 100 μL). After 10 minutes, mice were killed and whole intestine was dissected on ice. The total cell protein extracts and the brush border membranes (BBMs) were prepared from the scrapings as previously described.[11] Solubilized proteins were resolved by electrophoresis on 10% sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and immunoblotting was performed. The following antibodies were used at a 1:1000 dilution: SGLT-1 (AB 1352; Chemicon International, Temecula, CA) and GLUT2, phospho-AMPK-α1/2 (Thr172), and AMPKα 1/2 (sc-9117, sc-33524, and sc-25792, respectively; Santa Cruz Biotechnology, Santa Cruz, CA); a 1:500 dilution was used for APJ (NLS 64; Novus Biolog-