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Apolipoprotein A-IV enhances cholecystokinnin secretion

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ABSTRACT

Cholecystokinin (CCK) and apolipoprotein A-IV (ApoA-IV) are gastrointestinal peptides that play an important role in controlling energy homeostasis. Lymphatic ApoA-IV and plasma CCK secretion are mediated via a chylomicron formation-dependent pathway during a dietary lipid infusion. Given their similar roles as satiating proteins, the present study examines how the two peptides interact in their function. Specifically, this study sought to understand how ApoA-IV regulates CCK secretion. For this purpose, *Cck* gene expression in the small intestines of ApoA-IV knockout (ApoA-IV-KO) and wild-type (WT) mice were compared under an array of feeding conditions. When fed with a chow or high-fat diet (HFD), basal levels of *Cck* transcripts were significantly reduced in the duodenum of ApoA-IV-KO mice compared to WT mice. Furthermore, after an oral gavage of a lipid mixture, *Cck* gene expression in the duodenum was significantly reduced in ApoA-IV-KO mice relative to the change seen in WT mice. To determine the mechanism by which ApoA-IV modulates *Cck* gene expression, STC-1 cells were transfected with predesigned mouse lysophosphatidic acid receptor 5 (LPAR5) small interfering RNA (siRNA) to knockdown *Lpar5* gene expression. In this in-vitro study, mouse recombinant ApoA-IV protein increased *Cck* gene expression in enteroendocrine STC-1 cells and stimulated CCK release from the STC-1 cells. However, the levels of CCK protein and *Cck* expression were attenuated when *Lpar5* was knocked down in the STC-1 cells. Together these observations suggest that dietary lipid-induced ApoA-IV is associated with *Cck* synthesis in the duodenum and that ApoA-IV protein directly enhances CCK release through the activation of a LPAR5-dependent pathway.

1. Introduction

Obesity is a global epidemic that increases the risk of type II diabetes mellitus, cardiovascular disease, and many cancers [2,8,15,46]. Consumption of a high-fat diet (HFD) promotes positive energy balance, which is stored as fat and leads to subsequent development of obesity and insulin resistance [19,48]. Gastrointestinal peptides such as cholecystokinin (CCK) and apolipoprotein A-IV (ApoA-IV) are important satiating proteins for controlling energy homeostasis [21,47]. Both ApoA-IV and CCK inhibit gastric emptying and food intake [11,12,18,35,45]. There are two types of CCK receptors that exist in humans and rodents: CCK A receptors (CCK-AR), which are more abundant in peripheral tissues than in the brain, and CCK B receptors (CCK-BR), which are considered the gastric receptor and are abundantly present in both peripheral tissues and the brain [40]. Peripherally administered ApoA-IV and CCK do not cross the blood-brain barrier [31,39]. CCK acts by directly binding to CCK-AR on vagal afferent neurons in the intestinal enteric plexuses to relay satiation signals to the hypothalamus via vagal nerve endings in the nucleus of the solitary tract in the hindbrain [31,32]. ApoA-IV is involved in lipid-induced activation of the vagal afferent pathway and requires vagal nerve transmission to activate neurons in the hindbrain to suppress food intake [13,28,45]. Together, ApoA-IV and CCK work synergistically to reduce food intake, specifically, ApoA-IV requires a CCK-dependent pathway to induce neuronal activation in the NTS and suppress food intake [29,50]. Thus, the interaction of ApoA-IV and CCK are important in controlling energy homeostasis through the stimulation of vagal activity.

Dietary lipids and proteins stimulate CCK release in the small intestine [24,37]. In addition, different species have different gastrointestinal physiologies in response to nutrients. In humans, dietary lipids are the major physiological stimuli of CCK secretion, whereas in rats dietary proteins are the strongest stimulant of CCK secretion [9,25]. This response occurs in a dose-dependent manner, which CCK secretion by endocrine I cells in the small intestine is elevated with higher doses of lipids [24,37]. ApoA-IV, apolipoprotein A-I (ApoA-I) and

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apolipoprotein B (ApoB) are major protein constituents of lymphatic triglycerides (TG)-rich lipoproteins and are produced by enterocytes [14,17]. The ApoA-IV protein is the only apolipoprotein that responds to varying amounts of dietary lipids, whereas lymphatic secretion of ApoA-I and ApoB proteins remains consistent [1,16,17,38,43]. During a dietary lipid infusion, lymphatic ApoA-IV and plasma CCK secretion are mediated via a chylomicron formation-dependent pathway [17,34]. These findings imply that dietary lipids are important regulators of ApoA-IV and CCK production in the small intestine and induce secretion of CCK and ApoA-IV in a co-dependent manner. However, the effect of ApoA-IV on the regulation of intestinal CCK remains unknown. Protein and protein hydrolysates (peptones) directly interact with a lysophosphatidic acid receptor 5 (LPAR5 is also known as G protein-coupled receptor 93)-dependent pathway to produce a marked elevation in CCK release [5,10,25]. It was hypothesized that ApoA-IV enhances CCK levels through the activation of a LPAR5-dependent pathway as well. The present study was designed to determine whether intestinal *Cck* gene expression in ApoA-IV-KO and WT mice would be altered after diets with different fat contents, and whether ApoA-IV-induced CCK secretion by mouse enteroendocrine STC-1 cells is mediated via a LPAR5-dependent pathway.

2. Material and methods

2.1. Animals

ApoA-IV-KO mice were kindly provided by Dr. Jan Breslow (Rockefeller University, New York, NY) and were backcrossed for > 15 generations onto a C57BL/6J genetic background. All mice were genotyped by polymerase chain reaction (PCR) analysis of tail deoxyribonucleic acid (DNA) [6,20]. ApoA-IV-KO and wild-type (WT) mice (C57BL/6 J) were housed in an AAALAC-accredited facility under conditions of controlled illumination (12:12-h light-dark cycle, lights from 0600 to 1800 h). All animal protocols were approved by the Institutional Animal Care and Use Committee in Ohio University and the University of Cincinnati.

2.2. Animal feeding and small intestine collection

Starting at 10 weeks of age, male ApoA-IV-KO and WT mice received free access to water and chow diet (6% fat, LM-485 Mouse/Rat Sterilizable Diet, Envigo, Madison, WI). To confirm that the duodenum is a major site for CCK synthesis in ApoA-IV-KO and WT mice, the small intestines of 5 h-fasted, 18 weeks old WT and ApoA-IV-KO mice maintained on a chow diet were collected. The basal *Cck* gene expression levels of these samples were then determined for experiment 1. For the study of lipid-induced CCK synthesis in ApoA-IV-KO mice in experiment 2, chow-fed WT and ApoAIV-KO mice at 15 weeks of age received a lipid mixture containing 2.4 mg triolein, 0.2 mg cholesterol (CHOL) and 0.2 mg phospholipids (200 μl) by gavage after a 5-h fast. Two h later, the small intestine was collected on dry ice for the determination of duodenal CCK levels. To examine the influence of HFD on *Cck* gene expression in the duodenum in experiment 3, WT and ApoA-IV-KO mice at 12 weeks of age were fed with either a semi-purified high-fat diet (HFD, 20% butter fat by weight) or a matched low-fat diet (LFD, 5% butter fat content, Research Diets, Inc., New Brunswick, NJ) for 8 weeks [20]. After a 5-h fast, the duodenum was collected on dry ice for the determination of *Cck* gene expression using a real-time quantitative reverse transcription (qRT-PCR) analysis.

2.3. STC-1 cells

STC-1 cells, a mouse-intestinal cell-line, were provided by Dr. Douglas Hanahan (UCSF) [3,5]. Briefly, STC-1 cells were grown in 6-well plate with Dulbecco's modified Eagles medium, 10% fetal bovine serum, penicillin (100 IU/ml) and streptomycin (100 μg/ml). To determine basal level of CCK, the STC-1 cells (80% confluency) were incubated with Hanks' balanced salt solution (HBSS) as a vehicle for 0.5, 1 and 2 h. Then the media was collected for measuring the level of CCK. To determine whether CCK release by ApoA-IV increased in a dose-dependent manner, STC-1 cells were incubated with different doses of mouse recombinant ApoA-IV proteins (50, 100, 200 and 400 μg/ml) for 0.5, 1 or 2 h. After incubation the media was collected for CCK determination. To compare CCK secretion by STC-1 cells induced by bovine serum albumin (BSA) and ApoA-IV, STC-1 cells was incubated with BSA (Sigma-Aldrich, St. Louis, MO) or mouse recombinant ApoA-IV protein at 200 μg/ml.

To determine whether the stimulation of CCK by ApoA-IV is mediated via the LPAR5 pathway, *Lpar5*-expressing STC-1 cells were transfected with predesigned mouse *Lpar5* small interfering RNA (siRNA) to knockdown *Lpar5* gene expression. In a 35-mm tissue culture plate, 2×10^5 STC-1 cells per well were seeded in 2 ml-antibiotic-free normal growth medium supplemented with fetal bovine serum 24 h before transfection. Specific siRNAs targeting mouse *Lpar5* gene (catalog number sc-75195) and control siRNAs (catalog number sc-37007) were obtained from Santa Cruz Biotechnology (Santa Cruz Biotechnology, Inc., Dallas, TX) and utilized according to the manufacturer's instruction. Five groups for each treatment were used in this experiment, including five untransfected groups without either siRNA transfection medium or siRNA treatment as the negative controls, five groups incubated with a mixture of siRNA transfection medium plus *Lpar5* siRNA (60 pmols), and five groups incubated with mixture of siRNA transfection medium plus control siRNA (0.9 μmols). Briefly, 6 μl of control siRNAs (0.1 μmoles) were diluted in 200 μl siRNA transfection medium and 6 μl of siRNA transfection reagent (Santa Cruz) in each well of a 6-well culture plate. In parallel, 6 μl of the siRNA duplex (60 pmols siRNA) were diluted in 200 μl of the siRNA transfection Medium and 6 μl of the siRNA transfection reagent. The mixtures were incubated for 45 min at room temperature for complex formation and then added to the medium with STC-1 cells (80% confluency) without antibiotics at 37 °C. After a 7-h incubation, STC-1 cells were incubated with normal growth medium for an additional 18-h incubation. After 3 washes with ice-cold phosphate buffered saline (PBS, Gibco™, Fisher Scientific), STC-1cells were incubated with ApoA-IV (200 μg/ml) and vehicle (HBSS). After, the cells were washed with ice-cold PBS and harvested for qRT-PCR analysis, while the medium was assessed for CCK level using a CCK enzyme immunoassay kit assay.

2.4. qRT-PCR for Cck mRNA measurement

Total RNA was isolated from the duodenum, jejunum and ileum using Tri-Reagent (Ambion, Austin, TX). Total RNA was isolated, and first-strand cDNA was synthesized from 1 μg of total RNA [50]. qRT-PCR was performed using SYBR green RT-PCR master mixes at a 25-μl final reaction volume with the Bio-Rad iCycler iQ instrument (Hercules, CA) according to the manufacturer's instructions. Cyclophilin mRNA levels from each sample were used as internal controls to normalize mRNA levels. The sequences of the primers (Integrated DNA Technologies, Coralville, IA) were as follows: mouse *Cck*, 5′-CTA GCGCGATA CATCCAGCAGGTC-3′ (forward) and 5′-ACTTAATAAATAGATACTCAA ACC-3′ (reverse); mouse *Lpar5,* 5′-GCTCTGCCTGGGCGTGTGGGCTCTC ATCCTGC-3′ (forward) [5] and 5′-GCGTCGGGCCTCGCCAGTGTCCAG AAGAC-3′ (reverse); and mouse *cyclophilin*, 5′-TTCATGTGCCAGGGTG GTGACT-3′ (forward) and 5′-TCAGTCTTGGCAGTGCAGAT-3′ (reverse). Threshold cycle readings for each of the unknown samples were used, and the results were analyzed in Excel using the ΔΔCt method [27].

2.5. Measurement of the CCK release

CCK immunoreactivity was measured using an enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA) that cross-reacts at 100% with CCK-33 and CCK-8, and at 100% with