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Induction of apolipoprotein A-I gene expression by glucagon-like peptide-1 and exendin-4 in hepatocytes but not intestinal cells

Joe M. Chehade[a], Rosalyn Alcalde[a], Emad Naem[a], Arshag D. Mooradian[a], Norman C.W. Wong[b], Michael J. Haas[a,*]

[a] Division of Endocrinology, Department of Medicine, University of Florida–Jacksonville College of Medicine, Jacksonville, FL 32209, USA
[b] University of Calgary, Calgary, Alberta, Canada

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ABSTRACT

Objective. Diabetic dyslipidemia is an important risk factor for the development of macrovascular complications. Recent clinical trials suggest that diabetics treated with glucagon-like peptide-1 (GLP-1) have normalized lipid levels, including an increase in plasma high-density lipoprotein cholesterol (HDLc) levels.

Methods. To determine if GLP-1 (7-36 amide) and the GLP-1-like insulinotropic peptide exendin-4 regulate expression of apolipoprotein A-I (apo A-I), the primary anti-atherogenic component of high-density lipoprotein (HDL), HepG2 hepatocytes and Caco-2 intestinal cells, representative of tissues that express the majority of apo A-I, were treated with increasing amounts of each peptide and apo A-I gene expression was measured in the conditioned medium.

Results. Apo A-I secretion increased in both GLP-1 and exendin-4-treated HepG2, but not Caco-2 cells, and this was accompanied by similar changes in apo A-I mRNA levels and apo A-I promoter activity. Induction of apo A-I promoter activity by GLP-1 and exendin-4 required an SP1-responsive element. Hepatic ATP binding cassette protein A1 (ABCA1) expression, but not scavenger receptor class B type1 receptor expression was also induced by GLP-1 and exendin-4.

Conclusions. These results suggest that GLP-1- and exendin-4-mediated changes in HDLc are likely due to changes in hepatic expression of apo A-I and ABCA1.

Abbreviations: ABCA1, ATP-binding cassette protein A1; Apo A-I, apolipoprotein A-I; AU, arbitrary units; CAT, chloramphenicol acetyltransferase; DMSO, dimethylsulfoxide; DPP-4, dipeptidyl peptidase IV; FBS, fetal bovine serum; For., forskolin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; HDLc, high-density lipoprotein cholesterol; IBMX, isobutylmethylxanthine; IRCE, insulin-responsive core element; NS, not significant; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PKA, protein kinase A; PPARα, peroxisome proliferator activated receptor alpha; RCT, reverse-cholesterol transport; SR-B1, Scavenger receptor class B member 1; SREBP-1C, sterol-responsive element binding protein-1C; Tris-Cl, tris(hydroxymethyl)aminomethane hydrochloride; TBST, tris-buffered saline-Tween 20.

* *Corresponding author*. Tel.: +1 904 244 8619; fax: +1 904 244 5650.
E-mail address: Michael.haas@jax.ufl.edu (M.J. Haas).

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1. Introduction

The incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released from the intestine in response to feeding, and in the presence of glucose, augments the release of insulin [1]. Furthermore, GLP-1 also suppresses secretion of glucagon from α-cells in the pancreas [1]. Both peptides have a short plasma half-life (1–5 min) and are degraded in the circulation by dipeptidyl peptidase IV (DPP-4) [2]. In contrast, exendin-4 possesses GLP-1-like activity but has an extended plasma half-life (2.4-h) [3]. In pancreatic β-cells and other cells, GLP-1 and exendin-4 bind the GLP-1 receptor stimulating adenylate cyclase activity resulting in elevated cAMP levels [4]. In pancreatic β-cells, GLP-1 is partially responsible for the amplifying effect on insulin release [5].

Recently GLP-1 agonists have been shown to modulate lipid metabolism. In *ob/ob* mice, exendin-4 was shown to reverse hepatic steatosis [6]. This was associated with an increase in hepatic cAMP production as well as a decrease in genes involved in fatty acid synthesis, including stearoyl-CoA desaturase 1 and sterol response element binding protein-1c (SREBP-1c) [6]. In contrast, expression of genes involved in fatty acid oxidation (peroxisome proliferator-activated receptor a (PPARα) and acetyl-CoA oxidase was increased in GLP-1-treated mice [6]. Others have reported that incretin mimetics may be used to treat non-alcoholic fatty liver disease in humans [7,8].

Most animal models and human studies suggest that incretin treatment is associated with changes in the lipid profile. In mice, treatment with GLP-1 alone resulted in higher high-density lipoprotein (HDL) levels [9]. Exendin-4-treatment likewise resulted in increased HDL levels in obese subjects with type 2 diabetes [10]. It is not clear how GLP-1 and exendin-4 elevate HDL levels. However, the apolipoprotein A-I (apo A-I) gene, which codes for the foremost anti-atherogenic factor in the HDL particle [11], is induced by both cAMP [12] and PPARα [13]. Therefore we hypothesized that GLP-1 and exendin-4 may enhance apo A-I gene expression by either or both mechanisms. To test this hypothesis, we examined the effects of both peptides on apo A-I gene expression in both HepG2 liver cells and Caco-2 intestinal cells.

2. Methods

2.1. Materials

Exendin-4 and GLP-1 [7–36] amide were purchased from Bachem (Torrance, CA). Acetyl-coenzyme A, and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO) and Lipofectamine was from Invitrogen (Gaithersburg, MD), and ^{14}C-chloramphenicol was purchased from Perkin Elmer-New England Nuclear (Boston, MA). Tissue culture media and fetal calf serum were purchased from BioWittaker (Walkersville, MD). All other reagents were from Sigma-Aldrich or Fisher Scientific (Pittsburgh, PA).

2.2. Cell culture

HepG2 cells were maintained in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum (FBS) and penicillin and streptomycin (100-U/ml and 100-μg/ml, respectively). Caco-2 (HTB-37) cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in Eagle's Minimal Essential Medium containing 10% FBS, non-essential amino acids, sodium pyruvate, and penicillin and streptomycin. Cells were maintained in a humidified environment at 37 °C and 5% CO_2. In each experiment, exendin-4 and GLP-1, dissolved in DMSO, were added to the serum-free medium at the indicated concentration. Cell viability was routinely measured by trypan blue exclusion [14], which was greater than 95% in all experiments.

2.3. Measurement of Apo A-I protein and mRNA

Apo A-I and albumin accumulation in the conditioned medium of HepG2 cells was measured by Western blot. Briefly, 5-μg of each protein sample [15] was fractionated by electrophoresis on a 10% sodium dodecylsulfate polyacrylamide gel, transferred to nitrocellulose [16], and incubated with antiserum to human apo A-I (diluted 1:1000) (Calbiochem, San Diego, CA) and albumin (1:5000) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). After incubation with a goat-anti-rabbit secondary antibody (1:10,000) and enhanced chemiluminescence with reagents from Pierce Chemical Co. (Rockford, IL), the signal was detected on film and quantified with a densitometer (Gel Logic Imaging System 100, Carestream Healthcare, New Haven, CT). Optical density was expressed in arbitrary units (A.U.)

Apo A-I, ABCA1, SR-B1, and GAPDH mRNA was measured by quantitative real-time PCR. Total RNA was isolated from control or treated cells as previously described [17].Total RNA was isolated from cells using TRIzol reagent and quantified by UV absorbance at 260 nm. Reverse transcription was performed with five-μg of total RNA and Avian Myeloblastosis Virus (AMV) reverse transcriptase (Promega, Madison, WI) in the presence of random primers. Two hundred and fifty-ng of cDNA was used in each PCR reaction, which contained primers for ABCA1, apo A-I, GAPDH, or SR-B1. Primer sequences were obtained from a database [18], validated in the laboratory, and are shown in Supplemental Table 1. Amplification was performed with SYBR Green methodology. For PCR, the apo A-I and GAPDH primer sets were amplified for 40 cycles at 95 °C, 52 °C, and 72 °C, each for one minute. For the ABCA1 and SR-B1 primers, amplification was for 40 cycles at 95 °C, 58 °C, and 72 °C. At the end of the amplification, a melt curve was performed to certify that only one product was amplified. Changes in gene expression were normalized to GAPDH gene expression using the $\Delta^{\Delta Ct}$ method.

2.4. Plasmids and transient transfection analysis

The plasmid pAI.474.CAT, containing the apo A-I promoter region from −474 and +7 bp was used to assess transcription-dependent changes in apo A-I gene expression. HepG2 cells were transfected as indicated in each figure using Lipofectamine. The plasmid pCMV.SPORT-β-gal (Invitrogen), expressing β-galactosidase under the control of the cytomegalovirus immediate-early promoter, was used to control for transfection efficiency. After 24-h, the culture medium