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Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1

Susumu Katsuma [a,b], Akira Hirasawa [b], Gozoh Tsujimoto [b,*]

[a] *Bioinformatics Center, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*
[b] *Department of Genomic Drug Discovery Science, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan*

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Abstract

Bile acids play essential roles in the absorption of dietary lipids and in the regulation of bile acid biosynthesis. Recently, a G protein-coupled receptor, TGR5, was identified as a cell-surface bile acid receptor. In this study, we show that bile acids promote glucagon-like peptide-1 (GLP-1) secretion through TGR5 in a murine enteroendocrine cell line STC-1. In STC-1 cells, bile acids promoted GLP-1 secretion in a dose-dependent manner. As STC-1 cells express TGR5 mRNA, we examined whether bile acids induce GLP-1 secretion through TGR5. RNA interference experiments showed that reduced expression of TGR5 resulted in reduced secretion of GLP-1. Furthermore, transient transfection of STC-1 cells with an expression plasmid containing TGR5 significantly enhanced GLP-1 secretion, indicating that bile acids promote GLP-1 secretion through TGR5 in STC-1 cells. Bile acids induced rapid and dose-dependent elevation of intracellular cAMP levels in STC-1 cells. An adenylate cyclase inhibitor, MDL12330A, significantly suppressed bile acid-promoted GLP-1 secretion, suggesting that bile acids induce GLP-1 secretion via intracellular cAMP production in STC-1 cells.

Keywords: TGR5; GLP-1; STC-1; Bile acid

Bile acids play an important role in the solubilization and absorption of dietary fat and lipid-soluble vitamins [1]. Bile acids are absorbed from the intestine by both active and passive mechanisms, and then return to the liver via the portal blood [2]. This circulation of bile acids is required for efficient micellarization and cholesterol homeostasis [3]. Bile acids also modulate the transcription of various genes for enzymes and transport proteins through binding to nuclear receptors, farnesoid X receptor, and pregnane X receptor [4–7].

Recently, two groups independently discovered a novel G protein-coupled receptor (GPCR), TGR5 (also known as BG37), which responds to bile acids [8,9]. Bile acids induced receptor internalization, activation of extracellular signal-regulated kinase, and intracellular cAMP production in TGR5-expressing HEK293 and CHO cells [8,9]. TGR5 mRNA is abundantly expressed in monocytes/macrophages and some representative intestinal cell lines, such as NCI-H716 and STC-1 [8,9]. Kawamata et al. [8] showed that bile acid treatment suppressed the cytokine productions in the rabbit alveolar macrophages and TGR5-expressing monocytic cell line THP-1, suggesting that TGR5 is implicated in the suppression of macrophage functions by bile acids. Also, bile acids dose-dependently induced the elevation of intracellular cAMP production in a human intestinal cell line NCI-H716, suggesting that an endogenous TGR5 functions as a bile acid receptor [9]. These findings indicate that TGR5 can transduce the bile acid-induced signaling independently of the nuclear receptor-mediated signaling.

* Corresponding author. Fax: +81 75 753 4523.
E-mail address: gtsuji@pharm.kyoto-u.ac.jp (G. Tsujimoto).

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In this study, we showed that bile acids promote glucagon-like peptide-1 (GLP-1) secretion in a murine enteroendocrine cell line STC-1. In addition, we revealed that bile acids promote GLP-1 secretion through a cell-surface bile acid receptor TGR5.

Materials and methods

Materials. Lithocholic acid and deoxycholic acid were from Nacalai Tesque. Linolenic acid, 8-bromo-cAMP, and MDL12330A were obtained from Sigma. Forskolin and Cholera toxin were purchased form Calbiochem.

Cell culture. Murine enteroendocrine cell line STC-1 [10] was maintained in Dulbecco's modified Eagle's medium (DMEM) containing 15% (v/v) horse serum (HS) and 5% (v/v) fetal bovine serum (FBS).

Real-time PCR analysis. Total RNA was isolated using Isogen (Nippon Gene) and subjected to polymerase chain reaction with reverse transcription (RT-PCR). Real-time PCR analysis was carried out using DNA Engine Opticon2 System (MJ Research) according to the manufacturer's instructions. PCR primers were as follows:

for mouse GPR120,
mGPR120-1—5′-GCATAGGAGAAATCTCATGG-3′ and
mGPR120-2—5′-GAGTTGGCAAACGTGAAGGC-3′;
for mouse GPR40,
mGPR40-2—5′-AGTCCTCGTCACACATATTG-3′ and
mGPR40-3—5′-AATGCCTCCAATGTGGATAG-3′;
for mouse TGR5,
mTGR5-F1—5′-ACTGGTCCTGCCTCCTTCTC-3′ and
mTGR5-R1—5′-GAAGACAGCTTGGGAGCTGC-3′.

Plasmids and transfection. Mouse TGR5 cDNA was isolated from STC-1 cells and cloned into pIRES-neo expression vector (Clontech). Plasmids were transfected into STC-1 cells using Lipofectamine PLUS or Lipofectamine 2000 reagents (Invitrogen) as described previously [11]. Two days after transfection, the concentration of GLP-1 was determined by enzyme immunoassay as described below.

Transfection of STC-1 cells with short hairpin RNA (shRNA) expression vector. DNA oligonucleotides targeting TGR5 were synthesized and inserted into the siRNA expression vector pSilencer4.0 (Ambion) as described before [11]. The sequences for two complementary oligonucleotides with single-stranded overhangs encoding restriction enzyme sites were as follows:

mTGR5 sense,
GATCCGCCTACTACTAGCCGGGCTTTCAAGAGAAGC
CCGGCTAGTAGTAGGCTTA;
mTGR5 antisense,
AGCTTAAGCCTACTACTAGCCGGGCTTCTCTTGAAA
GCCCGGCTAGTAGTAGGCG

Plasmids were transfected into STC-1 cells with Lipofectamine 2000 (Invitrogen). Two days after transfection, the concentration of GLP-1 was determined by enzyme immunoassay as described below. Transfected cells were also examined for GPR120 and TGR5 mRNA levels by real-time RT-PCR.

GLP-1 secretion. STC-1 cells were washed three times with Hanks' balanced salt solution (HBSS) and incubated for 60 min at 37 °C in HBSS containing various concentrations of fatty acids and bile acids. After incubation, the conditioned medium was collected and the concentration of GLP-1 was determined by enzyme immunoassay with a specific GLP-1 (7–36) amide Enzyme Immunoassay Kit (Wako).

Measurement of cAMP formation. Cyclic adenosine monophosphate (cAMP) levels in STC-1 cells were determined as reported previously [12]. Reactions were started by addition of test agents along with 500 μM of 3-isobutyl-1-methyl-xanthine (IBMX). After incubation for 10 min at 37 °C, intracellular cAMP was extracted. The level of cAMP was measured by enzyme immunoassay with an Amersham Biosciences cAMP assay system.

Statistical analysis. One-way analysis of variance (ANOVA) was used to evaluate treatment effects. If the ANOVA value was significant, comparisons between the control and treatment group were carried out using ANOVA followed by Dunnett's test to localize the significant difference. A P value of less than 0.05 was considered significant. All statistics were run with InStat 2.00 (GraphPad Software).

Results and discussion

Bile acids promote GLP-1 secretion in a murine enteroendocrine cell line STC-1 through TGR5

Recently, we showed that unsaturated free fatty acid, such as linolenic acid, promoted GLP-1 secretion in murine intestinal STC-1 cells [11]. To date, however, it has not been reported that bile acids promote GLP-1 release in intestinal cultured cells. In the present study, we examined whether bile acids promote GLP-1 secretion in STC-1 cells. As shown in Fig. 1, we first confirmed that linolenic acid-induced the release of GLP-1 in STC-1 cells. Also, we found that two bile acids, lithocholic acid (LCA) and deoxycholic acid (DCA), potently promoted GLP-1 secretion in a dose-dependent manner (Fig. 1). Maruyama et al. [9] recently reported that a cell-surface GPCR for bile acids, TGR5, is expressed in enteroendocrine STC-1, NCI-H716, and GLUTag cells, but not epithelial HT-29 and Caco-2 cells. Quantitative RT-PCR analysis showed that TGR5 mRNA was expressed at the same transcriptional level as two cell-surface free fatty acid receptors, GPR40 and GPR120, in STC-1 cells [11,13] (Fig. 2A). To examine whether bile acids promote GLP-1 secretion through TGR5 in STC-1

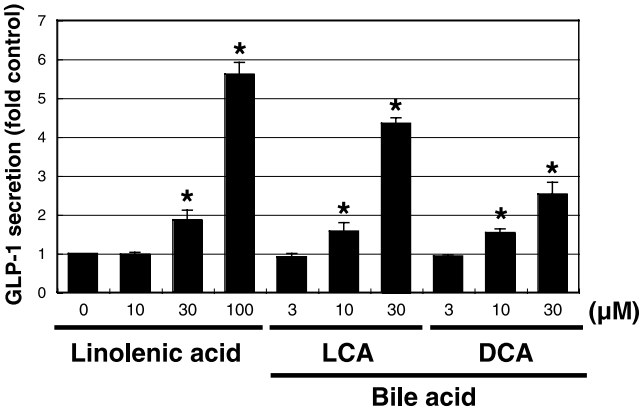

Fig. 1. Bile acids promote GLP-1 secretion in STC-1 cells. STC-1 cells were incubated for 60 min at 37 °C in HBSS containing various concentrations of linolenic acid and bile acids. After incubation, the conditioned medium was collected and the concentration of GLP-1 was determined by enzyme immunoassay. $*P < 0.05$.

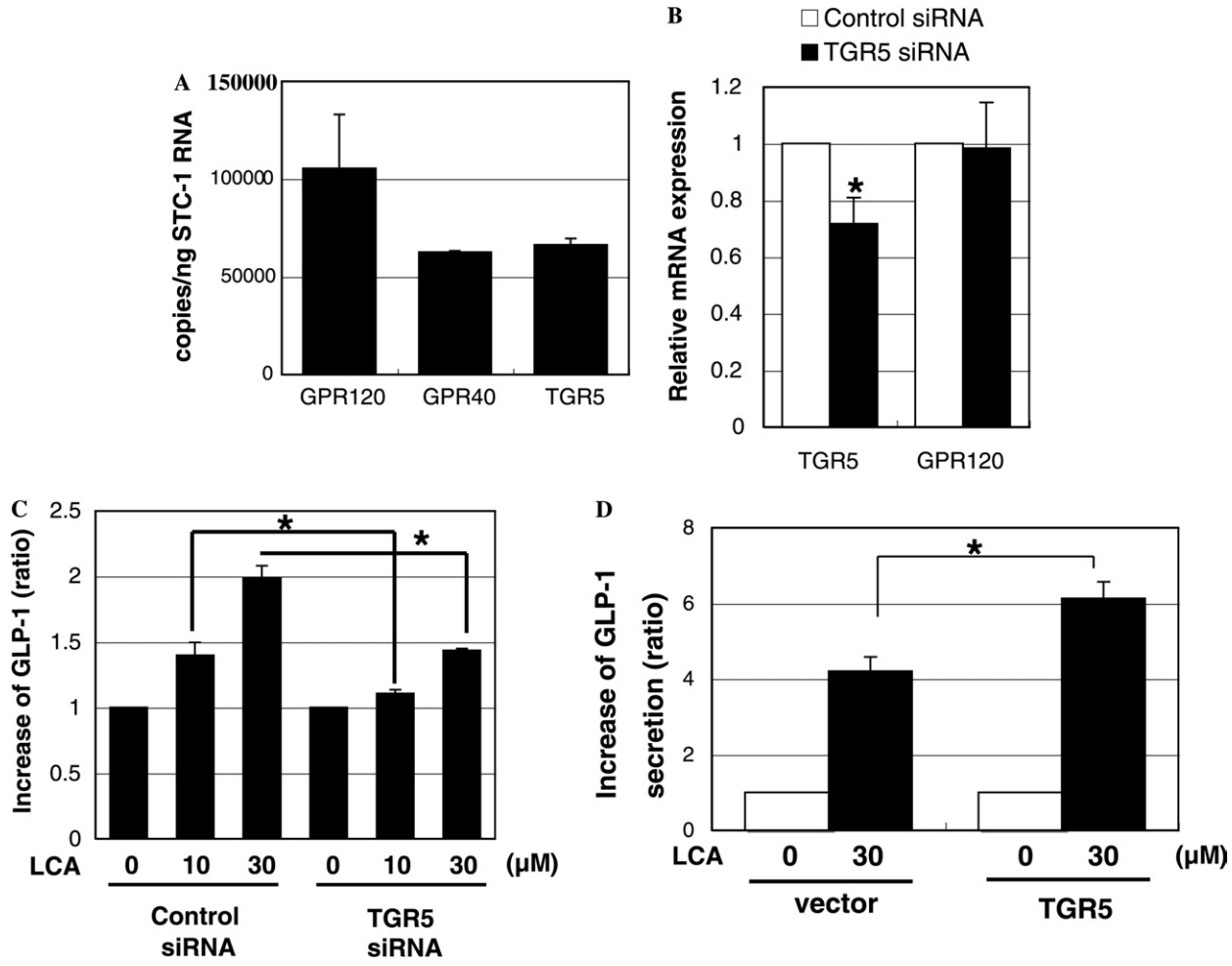

Fig. 2. Bile acids promote GLP-1 secretion in STC-1 cells through TGR5. (A) Expression of TGR5 mRNA. Total RNA was isolated from STC-1 cells and subjected to quantitative RT-PCR. (B) Effect of siRNA on expression of TGR5 mRNA in STC-1 cells. Transfected cells were examined for the levels of TGR5 and GPR120 mRNA by quantitative RT-PCR. mRNA levels were expressed as a percentage compared with those of control siRNA-transfected cells. $*P < 0.05$. (C) Effect of siRNA on LCA-promoted GLP-1 secretion in STC-1 cells. Transfected cells were incubated for 60 min at 37 °C in HBSS containing the indicated concentrations of LCA (0, 10, and 30 μM). After incubation, the conditioned medium was collected and the concentration of GLP-1 was determined by enzyme immunoassay. $*P < 0.05$. (D) Effect of transfection with TGR5 cDNA on LCA-promoted GLP-1 secretion in STC-1 cells. Transfected cells were incubated for 60 min at 37 °C in HBSS containing the indicated concentrations of LCA (0 and 30 μM). After incubation, the conditioned medium was collected and the concentration of GLP-1 was determined by enzyme immunoassay. $*P < 0.05$.

cells, we assessed the effects of inhibiting the expression and the function of TGR5 in STC-1 cells by using small interfering RNAs (siRNAs). Quantitative RT-PCR analysis showed that transfection with TGR5-specific siRNA reduced TGR5 mRNA expression by about 30% with little effect on GPR120 mRNA expression (Fig. 2B). Corresponding to the reduction of TGR5 mRNA expression, transfection with TGR5-specific siRNA significantly reduced LCA-induced GLP-1 secretion from STC-1 cells (Fig. 2C). In addition, we found that transfection with an expression plasmid containing murine TGR5 cDNA enhanced LCA-induced GLP-1 secretion in STC-1 cells (Fig. 2D). These results indicate that bile acid-induced GLP-1 secretion in STC-1 cells is mediated through TGR5.

Bile acids promote GLP-1 secretion via cAMP production

Previous reports showed that bile acids dose-dependently induced the production of cAMP in TGR5-expressing HEK293 and CHO cells or human enteroendocrine NCI-H716 cells [8,9]. We examined the effects of bile acids on intracellular cAMP production in STC-1 cells. As shown in Fig. 3A, bile acids induced cAMP production in a dose-dependent manner. To investigate the hypothesis that bile acids promote GLP-1 secretion by causing accumulation of intracellular cAMP, we examined the effect of an adenylate cyclase inhibitor, MDL12330A [14]. MDL12330A (10 μM) significantly reduced cAMP accumulation evoked by LCA, but did not affect the cAMP level in

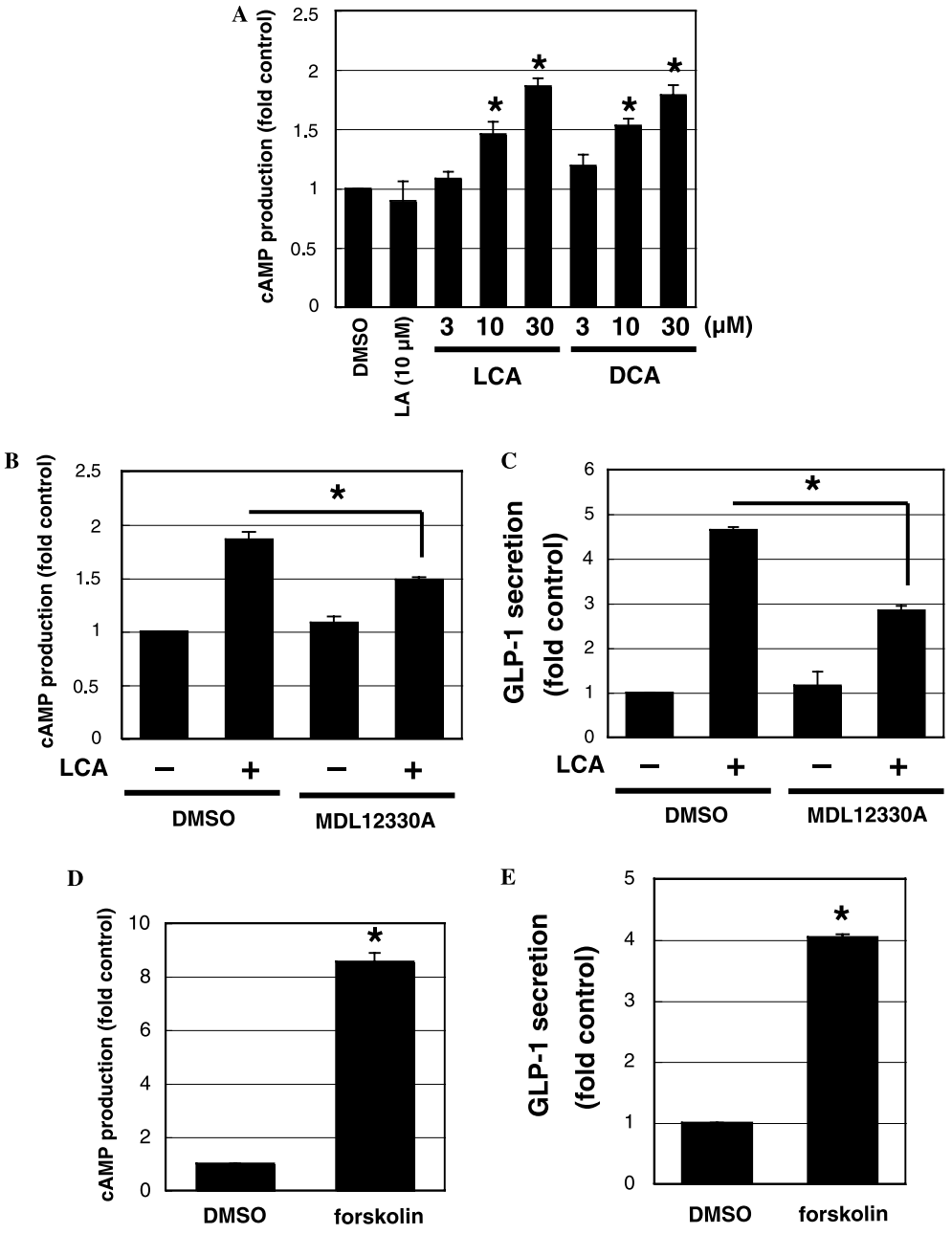

Fig. 3. Bile acids promote GLP-1 secretion through cAMP production. (A) The effect of bile acids on intracellular cAMP accumulation. STC-1 cells were stimulated with the indicated reagents and incubated for 10 min at 37 °C in the presence of IBMX (0.5 mM). LA, linolenic acid. $^*P < 0.05$. (B) The effect of MDL12230A on intracellular cAMP accumulation by LCA. MDL12230A (10 μM) was added 10 min before the stimulation of LCA (30 μM). $^*P < 0.05$. (C) The effect of MDL12230A on GLP-1 secretion by LCA. STC-1 cells were incubated for 60 min at 37 °C in HBSS with or without LCA (30 μM). After incubation, the conditioned medium was collected and the concentration of GLP-1 was determined by enzyme immunoassay. MDL12230A (10 μM) was added 10 min before the incubation period. $^*P < 0.05$. (D) The effect of forskolin on intracellular cAMP accumulation. STC-1 cells were stimulated with DMSO or forskolin (1 μM) and incubated for 10 min at 37 °C in the presence of IBMX (0.5 mM). $^*P < 0.05$. (E) The effect of forskolin on GLP-1 secretion. STC-1 cells were incubated for 60 min at 37 °C in HBSS with or without forskolin (1 μM). After incubation, the conditioned medium was collected and the concentration of GLP-1 was determined by enzyme immunoassay. $^*P < 0.05$.

the absence of LCA (Fig. 3B). Similarly, MDL12330A significantly inhibited LCA-promoted GLP-1 secretion, but did not affect GLP-1 secretion in the absence of LCA (Fig. 3C). In addition, we examined the effect of an adenylate cyclase activator, forskolin. Forskolin elicited an over 8-fold increase in cAMP production (Fig. 3D) and a 4-fold increase in GLP-1 secretion (Fig. 3E) in STC-1 cells. We also observed that other two cAMP elevators, 8-bromo-cAMP (a cell membrane-permeable cAMP analogue) and Cholera toxin (an activator of the alpha subunit of Gs protein), induced GLP-1 secretion in STC-1 cells (data not shown). These results sug-

gest that intracellular cAMP accumulation causes GLP-1 secretion in STC-1 cells. Taken together, these data show that bile acids induce GLP-1 secretion through TGR5 by causing intracellular cAMP accumulation in STC-1 cells.

In summary, we showed for the first time that bile acids promote GLP-1 secretion through TGR5 in murine enteroendocrine STC-1 cells. It was reported that GLP-1 release was promoted by bile acids in the isolated vascularly perfused rat colon [15]. Taken together with our present results, bile acids might evoke GLP-1 release in vivo through TGR5. Further studies are needed to understand the in vivo physiological roles of TGR5.

Acknowledgments

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