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Original Article

Bile acids cause relaxation of the lower esophageal sphincter through G-protein-coupled bile acid receptors

Shih-Che Huang*

Department of Internal Medicine, E-Da Hospital and School of Chinese Medicine for Post-Baccalaureate, I-Shou University, Kaohsiung, Taiwan

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ABSTRACT

Objectives: Bile acids inhibit contraction of the gallbladder and intestine through the G-protein-coupled bile acid receptor (GPBAR). Perfusion of the esophagus with bile and acid (HCl) decreases lower esophageal sphincter (LES) pressure. The effects of bile acids on LES motility are not clear. The purpose of the present study was to investigate the effects of bile acids on LES motility *in vitro*.
Materials and Methods: We measured the relaxation of muscle strips isolated from guinea pig and rat LES caused by bile acids or the selective GPBAR agonist RG-239. Reverse transcription polymerase chain reaction (RT-PCR) was performed to determine *GPBAR* expression in rat LES.
Results: In carbachol-contracted guinea pig LES strips, lithocholic acid (LCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and cholic acid (CA) produced relaxation in a concentration-dependent manner. The relative potency was LCA $\geq$ DCA $>$ CDCA $>$ CA. RG-239 also induced concentration-dependent relaxation. This suggests that GPBAR mediates relaxation in guinea pig LES. DCA-induced LES relaxation was attenuated by the protein kinase A inhibitor KT 5720 but not by the protein kinase G inhibitor KT 5823 or the NO synthase inhibitor L-NNA. This suggests the involvement of cAMP. Separately, in endothelin-1-contracted rat LES strips, bile acids induced relaxation. The relative potency was LCA $=$ DCA $>$ CDCA $>$ CA. RT-PCR revealed GPBAR expression in rat LES.
Conclusion: These results demonstrate that bile acids cause relaxation of guinea pig and rat LES through GPBAR.

1. Introduction

Bile acids are steroid-like molecules produced by hepatic cholesterol metabolism. The principal primary bile acids are cholic acid (CA) and chenodeoxycholic acid (CDCA). Deoxycholic acid (DCA) and lithocholic acid (LCA) are secondary bile acids. Recent studies have shown that bile acids interact with two receptors, the cell-surface G-protein-coupled bile acid receptor (GPBAR), also known as TGR5 [1–3], and the nuclear farnesoid-X-receptor (FXR) [1,4,5]. GPBAR has been found in gastrointestinal tissues, including the stomach, small intestine, colon, gallbladder, and liver [1–3]. It regulates energy metabolism and signals through the cAMP pathway. The relative potency for bile acid interaction with GPBAR to increase cAMP is LCA $>$ DCA $>$ CDCA $>$ CA. FXR has been found in the liver and intestine. It inhibits transcription of the regulatory gene in bile acid synthesis in the liver. In the intestine, FXR also induces expression of fibroblast growth factor 15/19, which inhibits hepatic bile acid synthesis [1,4,5]. The relative potency for FXR-mediated responses is CDCA $>$ LCA $=$ DCA [1,4].

Previous *in vitro* studies have shown that bile acids inhibit gallbladder and intestinal motility [6–10]. Recent studies demonstrated that bile acids inhibit contraction in the guinea pig and mouse gallbladder and suppress contraction and induce peristalsis in the mouse intestine through GPBAR [7–10]. In addition, *in vivo* studies showed that injection of LCA promoted gallbladder filling in the mouse, gavage of bile acids delayed gastric emptying, and perfusion of the esophagus with bile and acid (HCl) decreased lower esophageal sphincter (LES) pressure [8,9,11]. Bile acids might be involved in the pathogenesis of gastroesophageal reflux disease (GERD), which includes an incompetent LES with abnormal relaxation and/or a hypotensive LES [12,13]. Bile reflux from the duodenum into the stomach and esophagus is common in GERD patients. Esophageal infusion of bile acids induces GERD symptoms in these patients. Esophageal bile acid concentrations are higher in

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* Corresponding author. Department of Internal Medicine, E-Da Hospital, 1, Yi-Da Road, Yan-Chau, Kaohsiung, Taiwan. Tel.: +886 7 6151100x5901; fax: +886 7 6150940.
E-mail addresses: shihchehuang@hotmail.com, huangshihche@gmail.com.

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patients with GERD than in healthy individuals. To the best of our knowledge, the effects of bile acids on LES motility are not clear. We hypothesized that bile acids influence LES motility through GPBAR. The aim of this study was to investigate the effects of bile acids mediated by GPBAR on guinea pig and rat LES contraction.

2. Materials and methods

Male Hartley guinea pigs and Sprague-Dawley rats were obtained from the National Laboratory Animal Center and BioLASCO Taiwan (Taipei, Taiwan), respectively. DCA, CDCA, and CA sodium salts, carbachol, papaverine, atropine, KT 5720, KT 5823, *N*(omega)-nitro-L-arginine (L-NNA), RG-239 hydrate, and buffer reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). REG-239 was dissolved in DMSO (1 mM stock solution) and diluted to 100 μM with 60% DMSO. LCA sodium salt was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Tetrodotoxin was purchased from Tocris Cookson (Bristol, UK). Reverse transcription polymerase chain reaction (RT-PCR) reagents were obtained from Invitrogen (Carlsbad, CA, USA) and primers for rat *GPBAR* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were purchased from Integrated DNA Technologies (Coralville, IA, USA). All procedures were performed in compliance with institutional guidelines. The protocol for this study was approved by the Institutional Animal Care and Use Committee of E-Da Hospital and Buddhist Tzu Chi General Hospital.

2.1. Measurement of relaxation of isolated guinea pig and rat LES strips

LES strips were isolated according to a procedure described previously [14–17]. Male guinea pigs (350–400 g) and rats (350–400 g) were euthanized with CO_2. The stomach and lower portion of the esophagus were removed and cut open in the longitudinal direction along the greater curvature. The mucosa was removed. A transverse strip (2 mm wide and 10 mm long) was cut from the LES, which was identified as a thickened muscle between the esophagus and the stomach [14–17].

Measurements of relaxation in guinea pig and rat LES strips were performed according to a procedure described previously [14–17]. In brief, guinea pig LES strips were placed in oxygenated standard incubation solution containing 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 14 mM glucose, 1.2 mM NaH_2PO_4, and 1.8 mM $CaCl_2$. Rat LES strips were placed in oxygenated Krebs–Henseleit solution containing 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 11.1 mM glucose, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 2.5 mM $CaCl_2$. The final pH at 37°C was 7.40 ± 0.05. Guinea pig and rat LES strips were attached to organ baths using surgical silk sutures and incubated at 37°C in the oxygenated standard incubation and Krebs–Henseleit solutions, respectively. The LES strips were attached to isometric transducers [FT.03 (Grass Technologies, West Warwick, RI, USA) for guinea pig LES and FORT 10 g (World Precision Instruments, Sarasota, FL, USA) for rat LES], which were connected to an integrated amplifier and computer recording system (BIOPAC Systems, Santa Barbara, CA, USA). The basal tension of the muscle strips was adjusted to 1.0 g [14–16]. Experiments were started after a 45-min equilibration period. To measure relaxation in contracted LES strips, bile acids and RG-239 were added to carbachol-contracted guinea pig LES or endothelin (ET)-1-contracted rat LES muscle strips 15 min after the stimulant addition. Relaxation responses were presented as a percentage of the relaxation induced by 100 μM papaverine. For studies using tetrodotoxin and atropine, LES muscle strips were exposed to the indicated concentration of these agents for 15 min and 6 min respectively, and then to 100 μM DCA [14–17]. For studies using signal transduction inhibitors, including the cGMP kinase inhibitor KT 5823 (3 μM), the cAMP kinase inhibitor KT 5720 (3 μM), and the NO synthase inhibitor L-NNA (1 mM), LES muscle strips were exposed to the indicated concentration of these inhibitors for 30 min and then to 100 μM DCA [18]. Only a single dose response, with or without tetrodotoxin, atropine, KT 5823, KT 5720, or L-NNA, was studied for each preparation.

2.2. RT-PCR for detection of GPBAR mRNA in rat LES

RT-PCR was performed to detect *GPBAR* and *GAPDH* mRNA in rat LES as previously described with minor modifications [19–21]. Total RNA was isolated from rat LES using TRIzol reagent and treated with RNAse-free DNAse I. The superscript II RNase H-reverse transcriptase system was used for reverse transcription. RT-PCR for rat *GPBAR* was performed with Taq polymerase at 94°C for 5 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 15 s, 72 °C for 30 s, and 72 °C for 5 min. PCR amplification of rat *GAPDH* was performed with Taq polymerase at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 48 °C for 30 s, 72 °C for 30 s, and 72 °C for 5 min. The PCR products were subjected to electrophoresis on a 1.5% agarose gel and analyzed. The following primers were used [19–21]: *GPBAR*, 5′-AAA GGT GGC TAC AAG TGC TTC-3′ (forward) and 5′-TTC AAG TCC AAG TCA GTG CTG-3′ (reverse); *GAPDH*, 5′-GAC CCC TTC ATT GAC CTC AAC T-3′ (forward) and 5′-CTC AGT GTA GCC CAG GAT GCC-3′ (reverse).

2.3. Data analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical analysis of data was performed by one-way analysis of variance (ANOVA) with the Dunnett *post hoc* procedure or a two-tailed unpaired Student *t* test when appropriate. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of bile acids on guinea pig LES relaxation

Addition of 100 μM DCA to resting guinea pig LES strips induced mild relaxation corresponding to 22 ± 8% of papaverine-induced relaxation ($n = 6$). We then studied the relaxation effects of bile acids of carbachol-contracted guinea pig LES strips. Carbachol (1 μM) increased the force of guinea pig LES strip contraction by 2.1 ± 0.3 g ($n = 20$) and this contraction reached a plateau within 15 min (Fig. 1). LCA addition to carbachol-contracted LES muscle strips at the plateau induced marked and sustained concentration-dependent relaxation (Figs. 1 and 2). LCA evoked detectable relaxation of carbachol-contracted LES strips at 10 μM. The highest LCA concentration tested (300 μM) induced 73 ± 4% relaxation of the carbachol-contracted LES (Fig. 2). DCA caused detectable relaxation of carbachol-contracted LES strips at 30 μM. The highest DCA concentration tested (300 μM) induced 72 ± 4% relaxation of

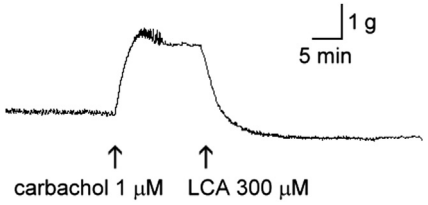

Fig. 1. Typical tracing showing relaxation of a guinea pig lower esophageal sphincter with 300 μM LCA.