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

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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 > 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.

Conclusion: These results demonstrate that bile acids cause relaxation of guinea pig and rat LES through GPBAR.

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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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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 5723, N(omega)-nitro-L-arginine (L-NNA), and buffer reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The 12-electrode computer recording system was used for reverse transcription. RTPCR 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 GCC TAC AAG TGC TTC-3' (forward) and 5'-TCA 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 CAG CCC-3' (reverse).

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 GCC TAC AAG TGC TTC-3' (forward) and 5'-TCA 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 CAG CCC-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.
carbachol-contracted LES (Fig. 2). CDCA and CA caused mild relaxation. The highest CDCA and CA concentrations tested (300 μM) induced 42 ± 6% and 25 ± 1% relaxation, respectively (Fig. 2). The GPBAR selective agonist RG-239 [22] caused detectable relaxation of carbachol-contracted LES strips at 3 μM. The highest RG-239 concentration tested (10 μM) stimulated strong relaxation and abolished carbachol-induced contraction of the LES (Fig. 3). The relaxation induced by 100 μM DCA (59 ± 3%) was not affected by 1 μM tetrodotoxin (58 ± 5%; p = 0.88).

3.2. Effects of signal transduction inhibitors on DCA-induced guinea pig LES relaxation

In carbachol-contracted guinea pig LES strips, the relaxation response induced by DCA was inhibited by the cAMP kinase inhibitor KT 5720 but not the cGMP kinase inhibitor KT 5823 or the NO synthase inhibitor L-NNA (Fig. 4). Specifically, with 3 μM KT 5720, 100 μM DCA induced 33 ± 5% (p = 0.003 vs. DCA alone, ANOVA; Fig. 4). In contrast, in the presence of 3 μM KT 5723 and 1 mM L-NNA, 100 μM DCA induced 62 ± 7% and 63 ± 3% relaxation, respectively (p = 0.89 and 0.92 vs. DCA alone, ANOVA; Fig. 4).

3.3. Effects of bile acids on rat LES relaxation

We tested the relaxation effects of bile acids on ET-1-contracted rat LES strips because ET-1 alone does not contract guinea pig LES strips. Addition of LCA, DCA, CDCA, and CA to ET-1-contracted rat LES muscle strips at the plateau induced concentration-dependent relaxation (Fig. 5). LCA and DCA were the most potent. LCA caused detectable relaxation at 1 μM. The highest LCA concentration tested (100 μM) induced 68 ± 4% relaxation of ET-1-contracted LES (Fig. 5). DCA caused detectable relaxation of ET-1-contracted LES strips at 1 μM. The highest DCA concentration tested (100 μM) induced 82 ± 7% relaxation (Fig. 2). CDCA and CA caused mild relaxation. The highest CDCA and CA concentrations tested (100 μM) induced 48 ± 8% and 27 ± 2% relaxation, respectively (Fig. 5). The relaxation induced by 100 μM DCA was not affected by 1 μM atropine (70 ± 10%; p = 0.37 compared with DCA alone).

3.4. GPBAR expression in rat LES

RT-PCR experiments were performed to determine GPBAR expression in rat LES. Fig. 6 shows RT-PCR results for GPBAR and GAPDH mRNA. Amplification revealed 103- and 732-bp products for GPBAR and GAPDH, respectively, as predicted [19,21].

4. Discussion

Previous studies have shown that bile acids cause relaxation of the smooth muscle of the gallbladder and intestine through GPBAR [6–10]. Little information is available about the effects mediated by bile acids on the smooth muscle of the lower esophagus. The present study demonstrates that bile acids induce relaxation of both guinea pig and rat LESs.

Fig. 2. Relaxation effect of bile acids LCA, DCA, CDCA and CA on guinea pig lower esophageal sphincter strips contracted with 1 μM carbachol. Values are expressed as a percentage of the relaxation induced by 100 μM papaverine. Results are from at least three experiments. Vertical bars represent ± SEM.

Fig. 3. Relaxation effect of the GPBAR selective agonist RG-239 and DMSO (vehicle) on guinea pig lower esophageal sphincter strips contracted with 1 μM carbachol. Values are expressed as a percentage of the relaxation induced by 100 μM papaverine. Results are from at least three experiments. Vertical bars represent ± SEM.

Fig. 4. Relaxation effect of DCA (100 μM) in the absence or presence of KT5720 (3 μM), KT5823 (3 μM), and L-NNA (1 mM) on guinea pig lower esophageal sphincter strips contracted with 1 μM carbachol. Values are expressed as a percentage of the relaxation induced by 100 μM papaverine. Results are from at least three experiments. Vertical bars represent ± SEM. * p < 0.05 compared with DCA alone.

Fig. 5. Relaxation effect of bile acids LCA, DCA, CDCA and CA on rat lower esophageal sphincter strips contracted with 100 nM ET-1. Values are expressed as a percentage of the relaxation induced by 100 μM papaverine. Results are from at least three experiments. Vertical bars represent ± SEM.
GPBAR in the upper gastrointestinal tract. The present study demonstrates that bile acids cause relaxation of guinea pig and rat LES. It also provides evidence that GPBAR mediates LES relaxation. Thus, GPBAR modulates not only the gallbladder and intestine but also LES motility.

LCA, DCA, CDCA, and CA induced concentration-dependent relaxation in carbachol-contracted guinea pig LES strips. The relative relaxation potency of the bile acids was LCA > DCA > CDCA > CA. In addition, the GPBAR specific agonist RG-239 [22] induced concentration-dependent relaxation of carbachol-contracted guinea pig LES strips. Similarly, bile acids induced concentration-dependent relaxation of ET-1-contracted rat LES strips. The relative relaxation potency of the bile acids was LCA > DCA > CDCA > CA. RT-PCR revealed GPBAR expression in rat LES. This indicates that GPBAR mediates LES relaxation. GPBAR-mediated LES relaxation might involve CAMP, as the DCA-induced relaxation responses were attenuated by the cAMP kinase inhibitor KT-5720 but not the cGMP kinase inhibitor KT-5823 or the NO synthase inhibitor L-NNA. This is in agreement with previous studies showing that CAMP is involved in GPBAR signaling and is one of the major relaxation pathways in the LES [3,8,23].

Bile reflux from the duodenum into the stomach and esophagus occurs in GERD. The reflux gastric juices of GERD patients contain bile acids up to millimolar concentrations [12], which exceed those tested in the present study. Further studies of the effects of bile acids on human LES are warranted to evaluate the involvement of GPBAR in the pathogenesis of GERD.

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