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Bile Acid Modulation of Gastroinstinal Smooth Muscle Contraction and Ionic Currents

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We have examined whether bile acids can affect the electrical and mechanical activities of circular smooth muscle of canine colon and ileum, using isometric tension measurement or patch clamp technique. It was found that a dilution of canine bile (0.03~2% by volume) enhanced or inhibited the amplitude of spontaneous contractions. An individual component of bile, deoxycholic acid (DCA) enhanced the frequency and amplitude of the spontaneous contractile activity at 10^{-6} M, while DCA at 10^{-4} M inhibited the contraction. Similarly, the response to cholic acid was excitatory at 10^{-5} M and inhibitory at 3×10^{-4} M. Taurocholic acid at 10^{-4} M enhanced the amplitude of muscle contraction. Electrically, canine bile at 1% reversibly depolarized the colonic myocytes under current clamp mode. Bile acids also elicited non-selective cation currents under voltage clamp studies, where K^+ currents were blocked and the Cl^- gradient was adjusted so that E_{Cl}^- was equal to -70 mV, a holding potential. The non-selective cation current might explain the depolarization caused by bile acids in intact muscles. Furthermore, the bile acid regulation of electrical and mechanical activities of intestinal smooth muscle may explain some of the pathophysiological conditions accompanying defects in bile reabsorption.

Key Words: Bile acids, Gastrointestinal motility, Non-selective cation current

INTRODUCTION

Bile acids are synthesized from cholesterol in the liver and stored in the gall bladder. They are secreted into the small intestine in response to cholecystokinin that is released in the presence of fat digestion products in the duodenum. After lipid emulsification and mixed micelles formation, bile acids are actively reabsorbed by the distal ileum. Reabsorbed bile acids are taken up by the hepatocytes with portal blood, where conjugation with glycin or taurin occurs. This enterohepatic circulation occurs several times daily and recirculates bile acids (Hofmann, 1976).

Bile acids malabsorption has been associated with chronic diarrhea in Crohn's disease (Nyhlin et al, 1994) and AIDS (Sciaretta et al, 1994) or that of unknown origin (Eusufzai, 1993). Under normal conditions, due to the efficient enterohepatic circulation, only less than 5% of bile acids reaches the colon (Samuel et al, 1968). Under some pathological conditions such as with ileal mucosa inflammation or after extensive ileocecal resection which are common in inflammatory bowel disease, the reabsorption of bile acids is compromised. During these conditions, increased amounts of bile acids pass into the colon. The presence of a high concentration of bile acids may alter the colonic motility, resulting in clinical symptoms such as diarrhea. In the present study, this hypothesis was tested by examining the effects of bile acids on electrical and mechanical activities of canine colonic and ileal smooth muscles.

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METHODS

Preparation of muscle strips and cells

Proximal (10~15 cm downward from ileocolonic sphincter) colon and ileum (10~15 cm upward from

ileocolonic sphincter) were removed from dogs of either sex. Isolated colonic or ileal segment was opened along the mesenteric border, and fecal material was removed by washing with Krebs-Ringer bicarbonate solution. The resulting sheets of colon or ileum were pinned-out in a dissecting dish. Muscle strips (1 mm × 10 mm) were cut parallel to the longitudinal fibers, and the strips were used for contractile studies.

For patch clamp experiments, a thin strip of tissue along the submucosal surface of the circular muscle layer was carefully dissected from each strip of colonic muscle and cut into small pieces (1 ~ 2 mm^3). The muscles were incubated in oxygenated, Ca^{2+}-free Hanks' solution of the following composition (in mM): Na^+, 141; K^+, 5.8; Cl^-, 130.4; HCO_3^-, 15.5; HPO_4^{2-}, 0.34; $H_2PO_4^-$, 0.44; dextrose, 10; sucrose, 2.9. After equilibration with 95% O_2/5% CO_2, this solution had a pH of 7.4 at room temperature. The tissue pieces were transferred to the dispersion solution containing (per ml of Hanks' Ca^{2+}-free solution) collagenase CLS II (196 U/ml, Worthington Biochemical, Lakewood, NJ), 1.5 mg bovine serum albumin (fatty acid free; Sigma, St. Louis, MO), 2 mg trypsin inhibitor (Sigma, St. Louis, MO), 0.11 mg Na_2ATP (Sigma, St. Louis, MO), and incubated at 37°C for 20 min. The supernatant was removed, and the tissue pieces were resuspended in Ca^{2+}-free Hanks' solution. Gentle trituration detached single smooth muscle cells. The cells were plated into plastic culture dishes and incubated at 37°C (90% humidity and 95% O_2/5% CO_2) for 30 min. Cells were stored at 4°C and used within 10 h.

Recordings of isometric tension from intact muscles

The muscle strip was mounted horizontally in the chamber, securing one end to a fixed point and the other to the lever arm of an isometric force transducer (Grass FT.03, Grass Instruments, Inc., Quincy, MA). The force transducer was mounted on a micro-manipulator, and a resting load of 1 g was imposed on the preparation. Each muscle strip was equilibrated at the resting load for at least 60 min in advance of experiment. Increasing concentrations of bile acids were applied to each muscle strip in the perfusion solution and the isometric tension developed was recorded using the chart recorder.

Patch clamp experiments

To measure the nonselective cation current, the perfusion buffer contained (in mM): Na^+, 130; K^+, 5.8; Ca^{2+}, 1.8; Mg^{2+}, 0.9; Cl^-, 135; HCO_3^-, 4.17; HPO_4^{2-}, 0.34; $H_2PO_4^-$, 0.44; SO_4^-, 0.4; dextrose, 10; sucrose, 2.9; HEPES, 10. Solution pH was adjusted to 7.4 with NaOH. In these experiments the pipette solution contained (in mM): Cs^+, 130; Na^+, 14; Mg^{2+}, 2; Aspartate, 110; Cl^-, 10; ATP, 5.0; GTP, 1.0; Phosphocreatine, 2.0; HEPES, 5; EGTA, 0.1. The pH was adjusted to 7.2 with CsOH, and the solution was filtered and stored frozen.

Pipette resistance was 1 ~ 3 MΩ. After obtaining gigaseals and access to the cell interior was gained, 3 ~ 5 min were allowed for cell dialysis. Dialyzed cells were used in all experiments. Temperature was continuously monitored and regulated at 33 ± 1°C with a bipolar temperature controller (Medical Systems Co., Greenvale, NY). The cells had an average capacitance of 100 pF as reported by Langton et al. (1989). Voltage clamp test pulse protocols were delivered, and membrane current responses were recorded by means of a patch clamp amplifier (Axopatch 1A, Axon Instruments, Foster City, CA) interfaced to a 12-bit analog-to-digital converter (TL-1, Axon Instruments, Foster City, CA) and a computer running pClamp software (Axon Instruments, Foster City, CA). Currents were lowpass filtered at 2 kHz. Currents displayed in figures were not corrected for leakage or capacitance, but reported potentials were corrected for junction potentials (i.e. 7 and 12 mV for HEPES-buffered and NMDG-substituted buffer, respectively). Data were analyzed with pClamp software and plotted using Sigmaplot software (Jandel Scientific, Chicago, IL).

Canine bile preparation

Canine bile was collected from the gallbladder of each dog using a 5 ml syringe and stored at 4°C until use. Immediately before each experiment, the bile was diluted by volume from 0.03% to 2% with perfusion buffer solution. The diluted canine bile was applied on the muscle strips and/or myocytes prepared only from the same dog. In general, the bile was used within 6h after being collected.

Drugs

Chlolic Acid, deoxycholic acid and taurocholic acid

were obtained from Sigma (St. Louis, MO) and dissolved in distilled water at 10^{-1} M which was diluted with the perfusion buffer immediately before each experiment.

RESULTS

Bile acid effects on muscle contractility

Bile acids produced both contraction and relaxation of the GI smooth muscles. Diluted canine bile (0.03 or 1% by volume) either transiently enhanced or inhibited the phasic contractile activity of canine ileal smooth muscle (Fig. 1). In canine colonic muscle, individual components of bile such as deoxycholic acid (DCA, pH 7.3 at 10^{-4} M, n=4, Fig. 2A), cholic acid (CA, n=3, Fig. 2B) exerted a concentration-dependent excitation and inhibition of the contractile activity. In general, the amplitude and/or frequency of the phasic activity was enhanced by bile acids. Taurocholic acid (TCA) increased the amplitude of spontaneous phasic contraction at 10^{-4} M (n=3), while canine bile (2% by volume, pH 7.2~7.3, 320 mOsm) almost abolished the phasic contractile activity (n=3, Fig. 2C).

Bile acid effects on membrane potential

Patch clamp studies were performed on freshly dispersed smooth muscle cells from the circular muscle layer of the colon (Fig. 3). The effects of bile acids were examined under current clamp mode at 33 $\pm$1°C. Perfusion of canine bile (1% by volume) depolarized a single myocyte by about 40 mV in this particular cell and averaged as 19$\pm$14 mV (mean$\pm$S.D., n=4) which was almost completely and immediately reversed upon wash out of the bile.

Bile acid-induced current

To determine the ionic mechanisms responsible for the effects of bile acids, voltage clamp studies were performed on freshly dispersed colonic smooth muscle cells and the effects of bile acids were examined at 33$\pm$1°C (Fig. 4). Potassium currents

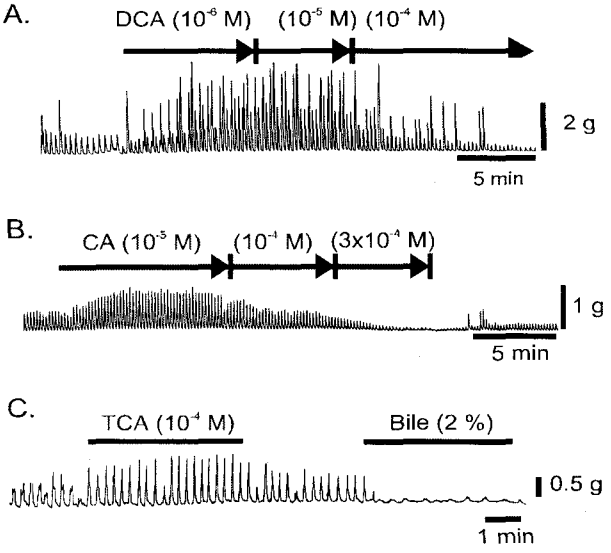

Fig. 2. Individual components of the bile, deoxycholic acid (DCA, pH 7.3 at 10^{-4} M), cholic acid (CA) and taurocholic acid (TCA) exerted concentration-dependent excitatory and inhibitory actions on contractile activity of canine colonic circular muscle. (C) Addition of diluted fresh bile (2%, pH 7.2~7.3, 320 mOsm) almost abolished spontaneous phasic contractile activicty.

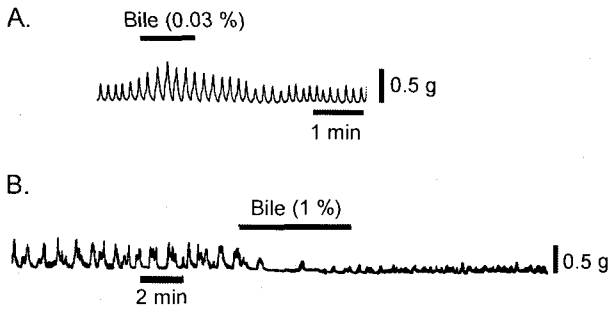

Fig. 1. Dilutions of canine bile enhanced or suppressed the contractile activity of canine ileal circular muscle. The pH of the bile at 2% (by volume) was 7.2~7.3 and osmolarity was 320 mOsm.

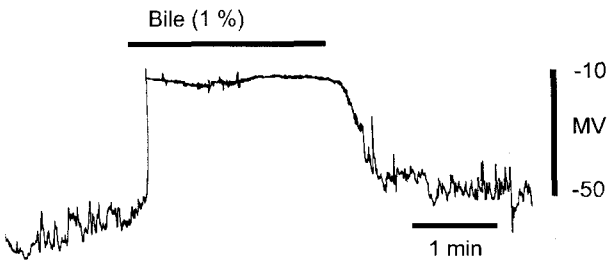

Fig. 3. Electrical effects of canine bile on canine colonic circular muscle. Perfusion of the bile depolarized the membrane of a single myocyte by about 40 mV in this particular case under current clamp mode.

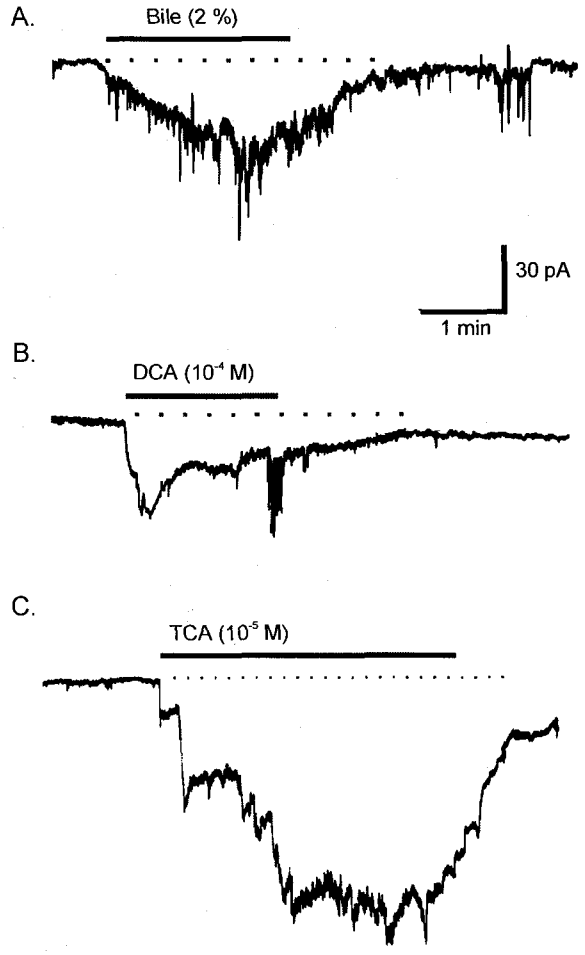

Fig. 4. Diluted canine bile and individual components of the bile, deoxycholic acid (DCA, pH 7.3 at 10^{-4} M) and taurocholic acid (TCA) activated inward currents in single colonic myocytes at a holding potential of −70 mV (set for Cl^- equilibrium potential).

were blocked by internal dialysis with Cs^+, and the Cl^- gradient was adjusted so that E_{Cl}^- was equal to −70 mV (see Methods). Canine bile (1% by volume) induced an inward current at a holding potential of −70 mV (Fig. 4A). Previously, we have shown that a similar current, identified as a nonselective cation current, can be activated by muscarinic stimulation (I_{ACh}, Lee et al, 1993). DCA (10^{-4} M, n=3) and TCA (10^{-5} M, n=3) applied by bath perfusion also activated similar inward currents at −70 mV under the identical condition (Fig. 4B & C). To examine whether this current is also carried by Na^+ as was seen in I_{ACh} (Lee et al, 1993), extracellular Na^+ was reduced from 130 mM to 35 mM by replacing Na^+ with impermeant N-Methyl-D-Glucamine (NMDG) during the exposure to DCA (10^{-4} M, pH 7.3) or

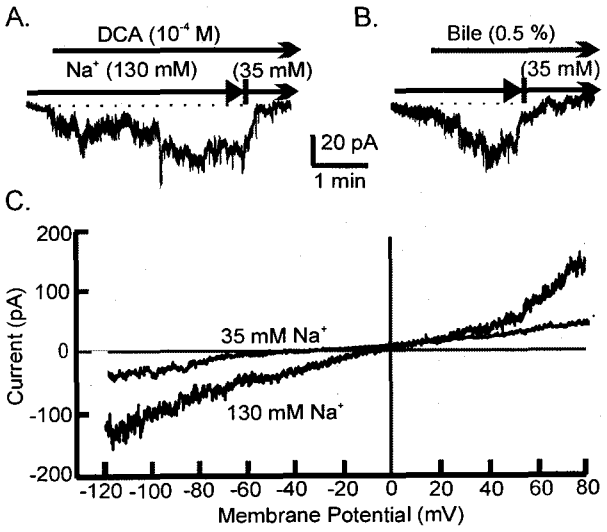

Fig. 5. The bile acid-induced currents are Na^+-dependent. (A & B) Reduction in external Na^+ concentration ($[Na^+]_o$) to 35 mM substantially decreased the currents activated by DCA and fresh bile. (C) Reduction in $[Na^+]_o$ also shifted the reversal potential of the current activated by fresh bile (0.5%) to more negative potentials.

canine bile (0.5%) (Fig. 5A & B). This maneuver almost abolished inward currents developed. In another experiment, voltage ramps were used to depolarize cells from 120 to +80 mV (ramp speed 0.1 V/s), and current responses were recorded in the presence of canine bile (0.5%) with normal Na^+ (130 mM) and low Na^+ (35 mM Na^+ replaced with NMDG) (n=3, Fig. 5C). In the presence of normal Na^+, the current activated by canine bile was linear with slight inward rectification at negative potentials with reversal potential close to 0 mV, suggesting the current perhaps is non-selective cation current. In the presence of low Na^+, the reversal potential of the current was shifted to more negative potentials between −35 and −40 mV. These findings suggest that the inward current elicited by bile acids at physiological condition is carried predominantly by Na^+.

DISCUSSION

The present study demonstrates the evidences that bile acids modulate contractile activity of gastrointestinal smooth muscle. The effects of bile acids in this study are not due to a change in osmolarity or pH, since they were within normal range at the concentrations of bile acids used. Furthermore, the

effects were less likely from detergent action of bile acids (Chadwick et al, 1979; Fasano et al, 1990) or bile acids being uptaken by the cell (Schwarz et al, 1975; Blitzer & Donovan, 1984). Rather, bile acids must have directly modulated membrane protein, supported by the findings that the responses were reversed upon wash out of the bile (Fig. 1, 3, 4) and the response to each individual component of bile acids was not uniform in the patterns (Fig. 2).

Previously in rat hepatocytes, bile acid (TCA at 5~100 μM) depolarized membrane by increasing Na^+ conductance (Wehner, 1993). Similarly in canine colonic smooth muscle, Na^+ conductance (I_{NSC}) was responsible for membrane depolarization responses to excitatory neurotransmitters such as acetylcholine (Lee et al, 1993) or tachykinins (Lee et al, 1995). Therefore, activation of I_{NSC} also may explain, at least in part, the membrane depolarization caused by bile acids. Subsequent increase in open probability of voltage-activated Ca^{2+} channel causing Ca^{2+} influx will eventually lead to muscle excitation by increasing intracellular Ca^{2+} level ($[Ca^{2+}]_i$). This hypothesis is partly supported by our preliminary finding that canine bile (1%) increased $[Ca^{2+}]_i$ in isolated colonic myocytes, which was measured using fluo-3 (data not shown). Whether bile acid can directly modulate voltage-activated Ca^{2+} channel is currently not known and remains to be determined.

Bile acids in the present study showed biphasic effects depending on the concentrations applied, which may explain some conflicting [e.g. hypotension (Bomzon et al, 1984) and hypertension (Ohkubo et al, 1984; Thomas et al, 1991)] clinical manifestations of bile acids malabsorption. One possibility is that increase in $[Ca^{2+}]_i$ by bile acids activates ionic conductances such as Ca^{2+}-activated K^+ ($I_{K(Ca)}$) or Ca^{2+}-activated Cl^- (I_{Cl}) which elicits outward currents at depolarized membrane potentials, shifting the membrane potential toward a negative range, and eventually leads to relaxation of pre-contracted muscle. Supporting this hypothesis, TCA (750 μM) activated K^+ and Cl^- conductances in association with increased $[Ca^{2+}]_i$ in cultured colonic cells (Devor et al, 1993). However, nonspecific effects by high concentration of bile acids also cannot be ruled out. Regardless, at close to physiological concentrations bile acids more likely will excite than inhibit the intestinal smooth muscle contractility.

Effects of canine bile shown in this study were inconsistent in that it depolarized and activated I_{NSC} in isolated myocytes (0.5~2 %, Fig. 3-5), while the same concentration inhibited muscle contraction (Fig. 1B & 2C). One explanation can be that canine bile used for each experiment might differ in its composition of individual components and their concentrations. If each component of bile acids regulates the cell function in different manners with different affinity, dilutes of these mixture would result in wide variety of effects. Furthermore, it is possible that canine bile contains unknown component that inhibits smooth muscle contractility.

The discrepancy of bile effects between tissues and isolated myocytes suggests that bile acids may also influence cells other than smooth muscle cells, which can be only visible at a tissue level. For example, bile acids may stimulate interstitial cell of Cajal (ICC) which is a potential pacemaker cell of gastrointestinal tract (Lee & Sanders, 1993). Excitation of ICC may release a substance that can inhibit smooth muscle cell function. Such hypothesis is supported by a previous study where an increase in $[Ca^{2+}]_i$ in isolated ICC released nitric oxide that inhibited nearby smooth muscle cells (Publicover et al, 1993). Another possibility is that bile acids may also stimulate secretory cells in intestine to release paracrine substances such as arachidonic acid and its metabolites prostaglandin E (PGE, Combettes et al, 1989). Prostaglandins appears to be released even under physiological condition, and PGE and PGI in particular can inhibit electrical and mechanical activity of canine gastrointestinal muscle (Sanders, 1984). Bile effects on the cells other than smooth muscle cells will help us to better understand physiological and pathophysiological roles that bile acid may play *in vivo*.

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