

Full Length Research Paper

Effect of cholic acid on colonic motility in mice

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The role of bile acids on the gastrointestinal motility are contradictory, especially the role of mast cells mediators in this effect. Thus, cholic acid (CA) was examined for its *in vitro* action on the motility of the mice colon using different doses of CA (0.3, 30, 50, 100, 200, 300 and 500 μ M). The contractile activity of the colon segment was recorded as changes in intraluminal pressure under isovolumetric conditions. The mean amplitude of the peristaltic motor complexes and the frequency (interval) of phasic contractions were determined. In other experiments, to study the CA mode of action, tissues were preincubated with 5-HT₃ antagonist (Granisetron hydrochloride), 5-HT₄ antagonist (GR113808), H₁ antagonist (Pyrilamine maleate salt) and protease activated receptor (PAR1) antagonist (BMS-200261) prior to challenge with CA (300 μ M). CA inhibitory effect on contractile activity might be *via* its antagonistic action on the 5-HT₃, 5-HT₄, H₁ receptors and PAR1 with variable levels. In conclusion, CA perfusion, at certain concentration levels, induced significant physiological changes in colon motility that might propose its antagonistic action on the receptors of the mast cells neuromediators.

Key words: Cholic acid, colonic motility, receptor antagonists, peristaltic motor complexes.

INTRODUCTION

Bile acids (BAs) control intestinal motility and secretion, in addition to their role in the digestion and absorption of dietary fat. The primary bile acid, cholic acid (CA) is synthesized from cholesterol in hepatocytes, secreted into canaliculi, and stored in the gallbladder. After secretion into the intestine, CA is actively absorbed in the ileum. The small amounts of CA that normally reach the colon is deconjugated and dehydroxylated by bacteria to form the secondary BAs which are passively absorbed (Alemi et al., 2013).

Although BAs have marked effects on intestinal motility and secretion by poorly understood mechanisms. Diminished secretion of bile during cholestatic disease causes constipation, whereas excess delivery of bile due to impaired intestinal BA absorption causes diarrhea (Bunnett et al., 2012).

Irritable bowel syndrome (IBS) is an extremely common

disorder that affects up to 20% of the general population around the world (Astegiano et al., 2008). It is responsible for almost half of the referrals to gastroenterologists. Surprisingly, the cause of IBS is poorly understood and several pathophysiological mechanisms have been implicated. Bile acid malabsorption (BAM) is generally not regarded as a cause of IBS. However, recent improvements in the techniques employed for assessing BAM have demonstrated that it may promote and contribute to a low-grade mucosal inflammation in IBS (Camilleri et al., 2009) and diarrhea in patients with inflammatory bowel disease symptoms (IBD) (Surawicz, 2010). Although the cell types that mediate these actions of BAs have not been identified, studies using neurotoxins and neurotransmitter antagonists suggest that BAs control motility and secretion through effects on the enteric nervous system (Alemi et al., 2013).

Mast cell is a resident cell of several types of tissues and contains many granules rich in neuromediators such as histamine, serotonin and proteases. High circulating levels of BAs result in mast cell activation to release its neuromediators (Boyer et al., 2006) which are implicated in alterations in gastrointestinal motility during acute intestinal infection with pathogenic bacteria (Barbara et al., 2006; Wang et al., 2007). Serotonin is produced to stimulate enteric smooth muscle contraction *via* 5-HT₃ and 5-HT₄ receptors (Kim, 2009), while histamine activates enteric neurons and regulate intestinal motility (Umoren et al., 2013) *via* interaction with H₁ receptors (Liu et al., 2003). In other conditions mast cells are associated with impaired motility, often due to deleterious effects of mast cell proteases on the interstitial cells of cajal (Wang et al., 2007).

The H₁ receptor is a histamine receptor belonging to the family of rhodopsin like G-protein-coupled receptors. This receptor, which is activated by the biogenic amine histamine, is expressed throughout the body, to be specific, in smooth muscles, on vascular endothelial cells, in the heart, and in the central nervous system. Antihistamines, which act on this receptor, are used as anti-allergic drugs (Shimamura et al., 2011). Pyrilamine, also known as Mepyramine, is a first generation antihistamine, targeting the H₁ receptor (Parsons and Ganellin, 2006). It also has anticholinergic properties.

Serotonin or 5-hydroxytryptamine (5-HT) is found widely distributed throughout the gut and the central nervous system. In the gut, 5-HT is found mostly in mucosal enterochromaffin cells which are sensory transducers that release 5-HT to activate intrinsic (via 5-HT_{1P} and 5-HT₄ receptors) and extrinsic (via 5-HT₃ receptors) primary afferent nerves.

Serotonin 5-HT₄ receptors are in the alimentary tract (smooth muscle), urinary bladder, heart and adrenal gland as well as the central nervous system (Hegde and Eglén, 1996). The 5-HT₄ receptor appears to mediate both inhibition and activation of smooth muscle involving myogenic as well as neural actions (Wouters et al., 2007). In the alimentary tract, stimulation of 5-HT₄ receptors has a pronounced effect on smooth muscle tone, mucosal electrolyte secretion, and the peristaltic reflex (Hegde and Eglén, 1996). The 5-HT₄ receptors antagonist (GR113808) is a drug which acts as a potent and selective 5-HT₄ serotonin receptor antagonist (Kaumann, 1993). It is used in researching the roles of 5-HT₄ receptors in various processes (Mikami et al., 2008), and has been used to test some of the proposed therapeutic effects of selective 5-HT₄ agonists.

Protease-activated receptors (PAR) are a subfamily of related G protein-coupled receptors that are activated by cleavage of part of their extracellular domain. They are highly expressed in platelets, but also on endothelial cells, myocytes and neurons. There are four known protease-activated receptors or PARs, numbered from one to four (PAR 1-4). These receptors are members of the seven transmembrane G-protein-coupled receptor super family

and are expressed throughout the body.

Reports on the motor action of BAs on the gastrointestinal tract have been conflicting. The aim of this study is to examine the effect of CA as the primary BAs on colonic motility in mice and to characterize the role of mast cell mediators in this effect in controlling intestinal motility in mice.

MATERIALS AND METHODS

Experimental animals

Swiss male mice (27 to 33 g body weight) have been used in this study. Animals were caged (5/cage) *ad libitum* under standard conditions (light/dark cycle) in the animal house of the laboratory during the experiments. Mice were kept in the animal house for 2 weeks prior to the trial to customize the diet and the environmental conditions and their weight were monitored during this period by the investigators. Body weight of animals from all groups was monitored and measured during the whole period of the experiment. All experiments were approved by the Ethics Committee of King Fahad Medical Research Center, King Abdulaziz University.

Chemicals and drugs

CA, mast cell stabilizers, cholinergic and adrenergic blockers, mast cells mediator antibodies and antagonists were purchased from Sigma-Aldrich Corporation (St. Louis, MO USA).

The following drugs were used: CA, 5-HT₃ antagonist (Granisetron hydrochloride), 5-HT₄ antagonist (GR113808), H₁ antagonist (Pyrilamine maleate salt) and PAR1 receptor antagonist (BMS-200261).

All drugs were dissolved in distilled water, except CA which was dissolved in dimethylsulphoxide (DMSO, 0.1%). Drugs were stored at -20°C. Freshly diluted aliquots were maintained on ice during the course of the experiments and added to the bath in microliter volumes.

Tissue preparation for motility recordings

Randomly selected mice were killed by cervical dislocation. A mid-line laparotomy was performed and a segment of colon was rapidly excised and placed in gassed (95% O₂ and 5% CO₂) Krebs bicarbonate buffer solution (composition in mM: NaCl 117, KCl 4.7, NaHCO₃ 25, CaCl₂ 2.5, MgCl₂ 1.2, NaH₂PO₄·2H₂O 1.2 and D-glucose 11). Muscles tissue segments were cleared of any mesenteric connective tissue and the lumen flushed with Krebs solution. Tissues were prepared according to the method described by Abdu et al. (2002). Colon segments approximately 5 cm in length were prepared from each animal (four in total) and were mounted horizontally in separate 20 ml perfusion chambers. Tissues were maintained at 37°C, and perfused with Krebs solution at a rate of 5 ml/min and were allowed to equilibrate for at least 30 min before recording. The oral and aboral ends of each segment were secured to two metal catheters fixed at either end of the chamber and adjusted to maintain the segments at their resting length. For each segment, the oral end was connected to a perfusion pump for infusion of Krebs solution at a rate of 0.16 ml/min, and the aboral end was attached to a pressure transducer (NL108T2) to record contractile activity as changes in intraluminal pressure under isovolumetric conditions. The experimental setup was standardized by routinely infusing Krebs solution into the closed segment to an initial intraluminal pressure of 4 to 5 cm/H₂O. Regular aborally propagating waves of contraction (peristaltic

pressure waves) were developed under these conditions and could be maintained for several hours.

The output from the pressure transducers (motor complex of colon) of control and CA treated colon segments were monitored and analyzed by using a NeuroLog™ System (NL900D, Digitimer Ltd., Hertfordshire, UK) and data-acquisition system (Power1401, Cambridge Electronic Design Ltd., Cambridge, UK) which connected to a computer running Spike 2 version 4 software (Cambridge Electronic Design Ltd., Cambridge, UK), that displayed the two channel pressure recordings online and also stored the data for subsequent offline analysis to compare changes of amplitude and intervals of smooth muscle motility in different treatments.

Colon motility

Isolated colon segments were distended to 4 to 5 cm/H₂O to evoke peristaltic motor complexes (PMCs). Only preparations with regular maintained peristaltic motor complexes (PMCs) were used for subsequent experiments. PMCs were quantified in terms of their peak amplitude above baseline and were expressed as cm/H₂O, while duration and interval between them were expressed in seconds (s). The baseline and treated mean values of the amplitude of the PMCs and the frequency (interval) of phasic contractions were determined 15 min before and after challenge.

CA or the appropriate vehicle was added to the chambers 15 min after stopping perfusion. The recording continued for a further 15 min before washing out the drug and reinstating perfusion. Dose response curve was done for the primary BA (CA), for the following doses (0.3, 30, 50, 100, 200, 300 and 500 μM, n = 28, 4 mice for each dose).

Colon was pre-incubated with the following antagonists, 5-HT3 antagonist (Granisetron hydrochloride, 10 μM), 5-HT4 antagonist (GR113808, 10 μM), H1 antagonist (Pyrilamine maleate salt, 10 μM) and PAR1 receptor antagonist (BMS-200261, 20 μM) for 5 min after stopping perfusion and prior to challenge with CA (300 μM, n = 16, 4 mice for each antagonist). The recording continued for a further 15 min before washing out the drug.

Statistical analysis of data

All values are reported as mean (standard error of mean (SEM)). Data were statistically compared using "student's" *t* test paired, unpaired or Wilcoxon rank-sum test as appropriate. Data were also analyzed using one-way analysis of variance (ANOVA) with Dunnett's test for a multiple comparison. Probability of *P* < 0.05 was considered significant.

RESULTS

Dose response curve of CA

Luminal distension of isolated segments of mice colon evoked a regular pattern of contractile activity. The activity consisted of periodic increases in intraluminal pressure separated by relative intervals.

The dose response effect of CA on the intervals and amplitudes of the colon motility was done using different doses (0.3, 30, 50, 100, 200, 300 and 500 μM, n = 28, 4 mice for each dose).

By using 50, 100, 200, 300 and 500 μM of CA, there was a significant increase in the PMC intervals compared to the control values (*P* < 0.05, Figures 1 and 3), while

after administration of the 200, 300 and 500 μM, CA significantly decreased the PMC amplitudes (*P* < 0.05, Figures 1 and 4).

Effect of CA in the presence of pharmacological antagonists on PMC intervals

Treatment of colon segments with 300 μM of CA induced significant increase in the PMC intervals (101.6 ± 10 s) compared to the control values (90.8 ± 5 s, *P* < 0.05) (Figure 5). The same results were obtained after incubation of the colon with 10 μM of 5-HT3 antagonist (100.6 ± 19 s) (Figure 5A) and 10 μM of H1 antagonist (106.42 ± 23 s) (Figure 5C). Addition of CA (300 μM) following pre-incubation of colon segment with both blockers (5-HT3 and H1) augmented the interval values of PMCs significantly (122 ± 13 and 109.7 ± 20 s), respectively, compared to the control levels (90.8 ± 5 s, *P* < 0.05). It is important to mention that the elongation of the PMC intervals caused by the combination of (CA + 5-HT3) antagonist (122 ± 13 s) was significant compared to the values of CA alone (101.6 ± 10 s, *P* < 0.05) or 5-HT3 antagonist alone (100.6 ± 19 s, *P* < 0.05). Also, there was significant increase in the intervals of the PMC's of the colon treated with (CA + H1) compared to H1 alone (109.7 ± 20 s versus 106.42 ± 23 s, *P* < 0.05).

In contrast, 5-HT4 antagonist (10 μM, Figure 5B) and PAR1 receptor antagonist (20 μM, Figure 5D) significantly reduced the PMC intervals (70.6 ± 13 and 54.6 ± 3 s), respectively, compared to both control (90.8 ± 5 s, *P* < 0.05) and CA values (101.6 ± 10 s, *P* < 0.05). Colon tissues treated with the CA following treatment with both competitors (5-HT4, PAR1) showed the same noteworthy inhibitory effect on the PMC intervals (60.1 ± 11 and 55.7 ± 7 s), respectively, compared to the control (*P* < 0.05).

Effect of CA in the presence of pharmacological antagonists on PMC amplitudes

The response of the colon concerning the amplitudes of PMC to CA and the pharmacological antagonists is illustrated in Figures 6 A, B, C, D and 2. A significant inhibitory response to the PMC amplitudes were recorded following incubation of different segments of mice colon with CA 300 μM (70.5 ± 6 versus 56.2 ± 16 cm/H₂O).

5-HT3 antagonist 10 μM inhibited the amplitude to 53.3 ± 8 cm/H₂O compared to control (70.5 ± 6 cm/H₂O, *P* < 0.05). This inhibition was increased by the combination of CA+5-HT3 receptor antagonist to 38.9 ± 15 cm/H₂O, *P* < 0.05 (Figure 6A).

The addition of 5-HT4 antagonist (10 μM) alone or with CA into the colon segments (Figure 6B) showed a decrease in motility amplitudes (54.9 ± 5 and 56.9 ± 2 cm/H₂O, respectively) when compared with the control (70.5 ± 6 cm/H₂O, *P* < 0.05).

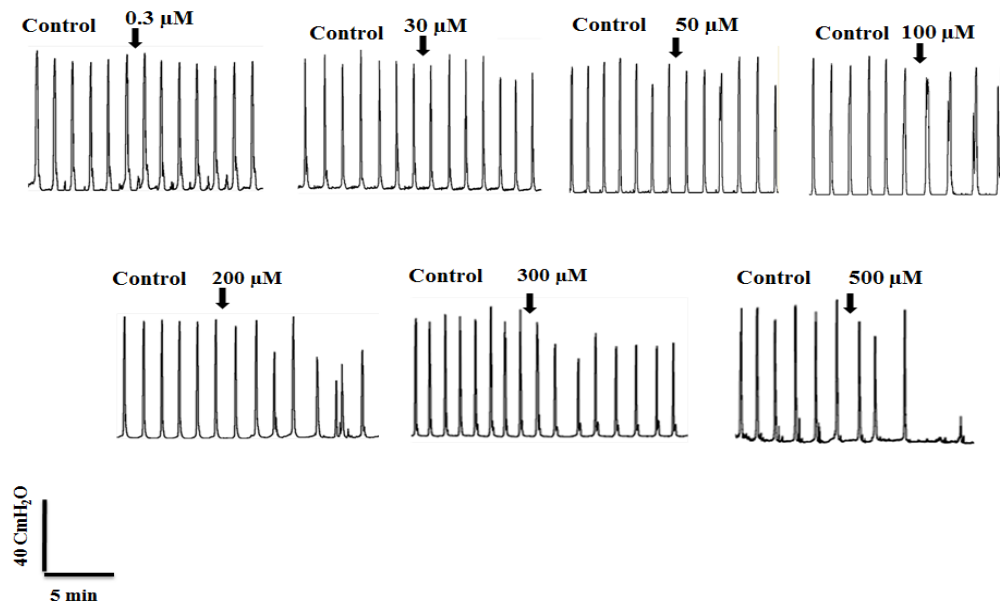


Figure 1. Dose response curve. Representative recording traces showing the effect of different doses (0.3, 30, 50, 100, 200, 300 and 500 μM) of CA on the intervals and amplitudes of the PMC of mice colon.

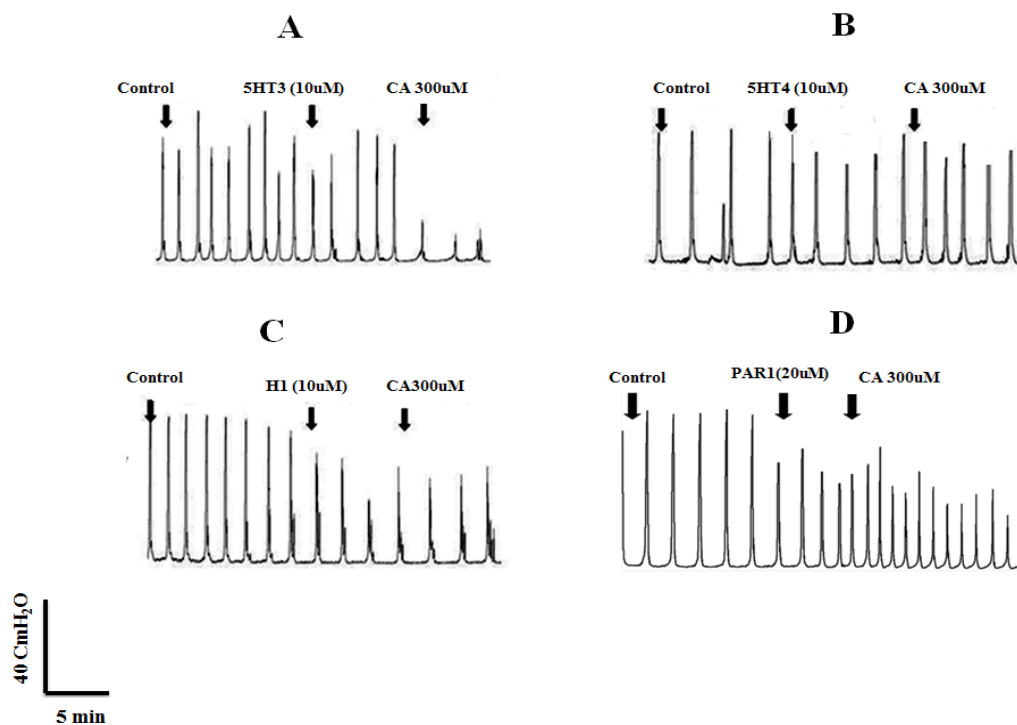


Figure 2. Effect of CA in the presence of pharmacological antagonists on colon motility. Representative recording traces showing the effect of intraluminal pressure on colon motility of mice. *In vitro* tissue preparations were preincubated with (5-HT₃ antagonist, 10 μM , A), (5-HT₄ antagonist, 10 μM , B), (H₁ antagonist, 10 μM , C) and (PAR1 receptor antagonist, 20 μM , D) prior to challenge with CA (300 μM).

The same inhibitory effect were noticed after applying 10 μM of H₁ antagonist (38.3 ± 7 cm/H₂O), and 20 μM PAR1 receptor antagonist (43.8 ± 10 cm/H₂O) and after

the combination of each blocker with CA (25.55 ± 7 cm/H₂O, Figure 6C) and (30.24 ± 3 cm/H₂O, Figure 6D), respectively compare to control (70.5 ± 6 cm/H₂O,

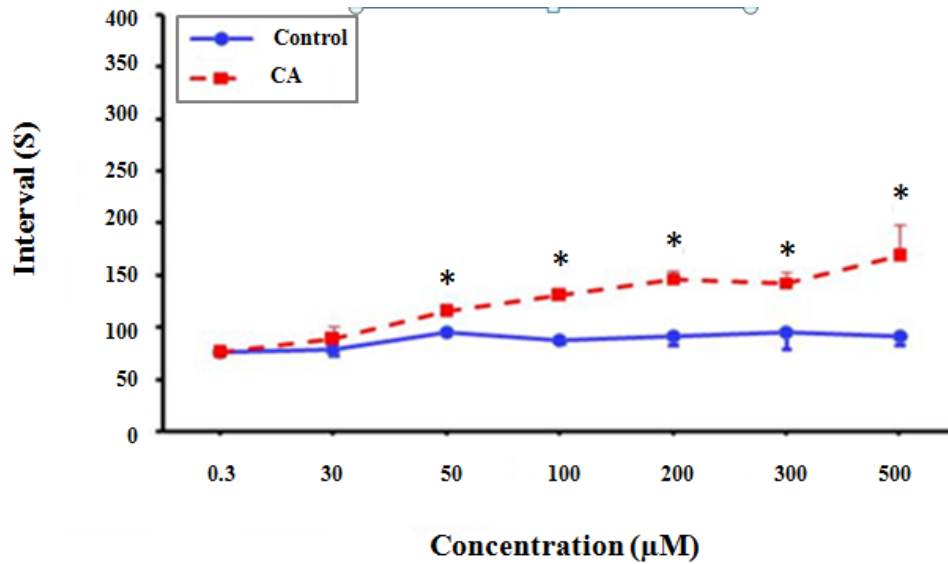


Figure 3. The effect of CA on PMC intervals. Effect of different doses (0.3, 30, 50, 100, 200, 300 and 500 µM) of CA on the PMC intervals. Values represents mean ± SE of 4 colon segments from different animals. Significantly different compared to the control group ($P < 0.05$).

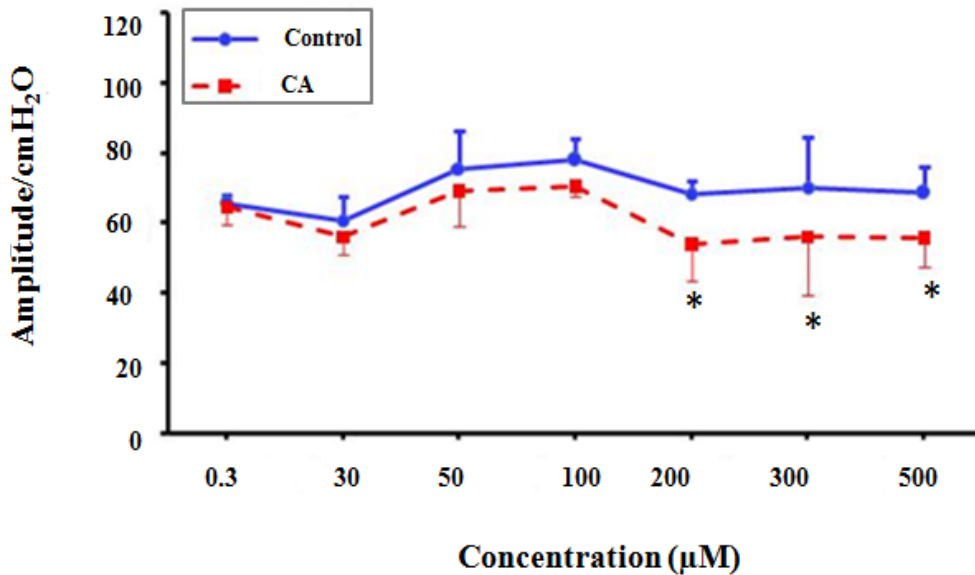


Figure 4. The effect of CA on PMC amplitudes. Effect of different doses (0.3, 30, 50, 100, 200, 300 and 500 µM) of CA on the PMC amplitudes. Values represents mean ± SE of 4 colon segments from different animals. Significantly different compared to the control group ($P < 0.05$).

$P < 0.05$).

DISCUSSION

BAs affect intestinal motility, although whether their actions are stimulatory or inhibitory is controversial. Early

in vivo studies showed that BAs either stimulate motility or have no effect (Feldman and Gibaldi, 1968; Kirwan et al., 1975 and Falconer et al., 1980). *In vitro* researches demonstrated that BAs inhibit contractions of rabbit and guinea pig intestine (Armstrong et al., 1993; Romero et al., 1993; Poole et al., 2010).

This study showed that CA significantly inhibited the

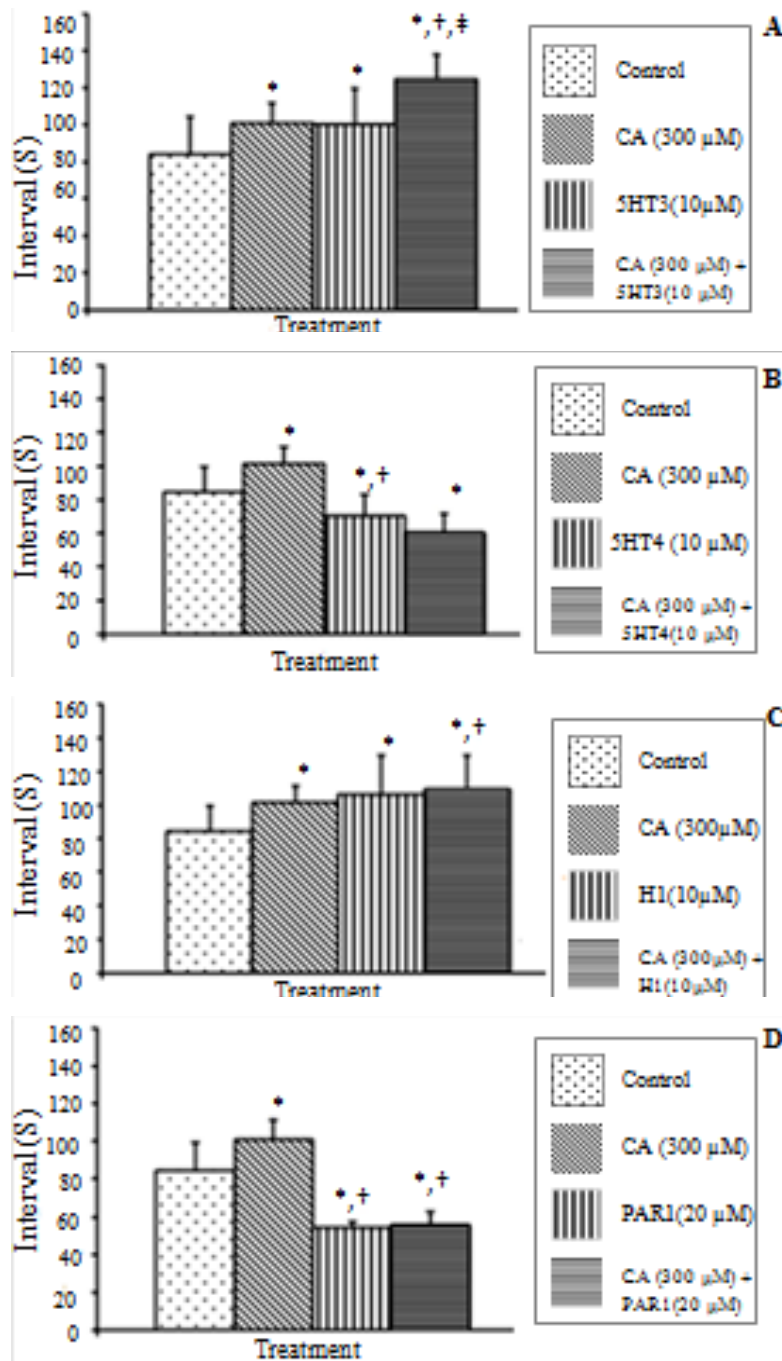


Figure 5. Effect of CA in the presence of pharmacological antagonists on the intervals of the PMC of mice colon. Tissue preparations were preincubated with (5-HT3 antagonist, 10 μM, A), (5-HT4 antagonist, 10 μM, B), (H1 antagonist, 10 μM, C) and (PAR1 receptor antagonist, 20 μM, D) prior to challenge with CA (300 μM). Values represents mean ± SE of 4 colon segments from different animals. *Significantly different compared to the control group, (P < 0.05). †Significantly different compared to the CA data, (P < 0.05). ‡Significantly different compared to the antagonist data, (P < 0.05).

PMC amplitudes and intervals in a dose dependent manner compared to the control. The effect of CA on these results was in agreement with the previous studies

since the infusion of BAs into the intestine delays transit, supporting an inhibitory role of CA (Van Ooteghem et al., 2002). Although in this study, the mechanisms by which

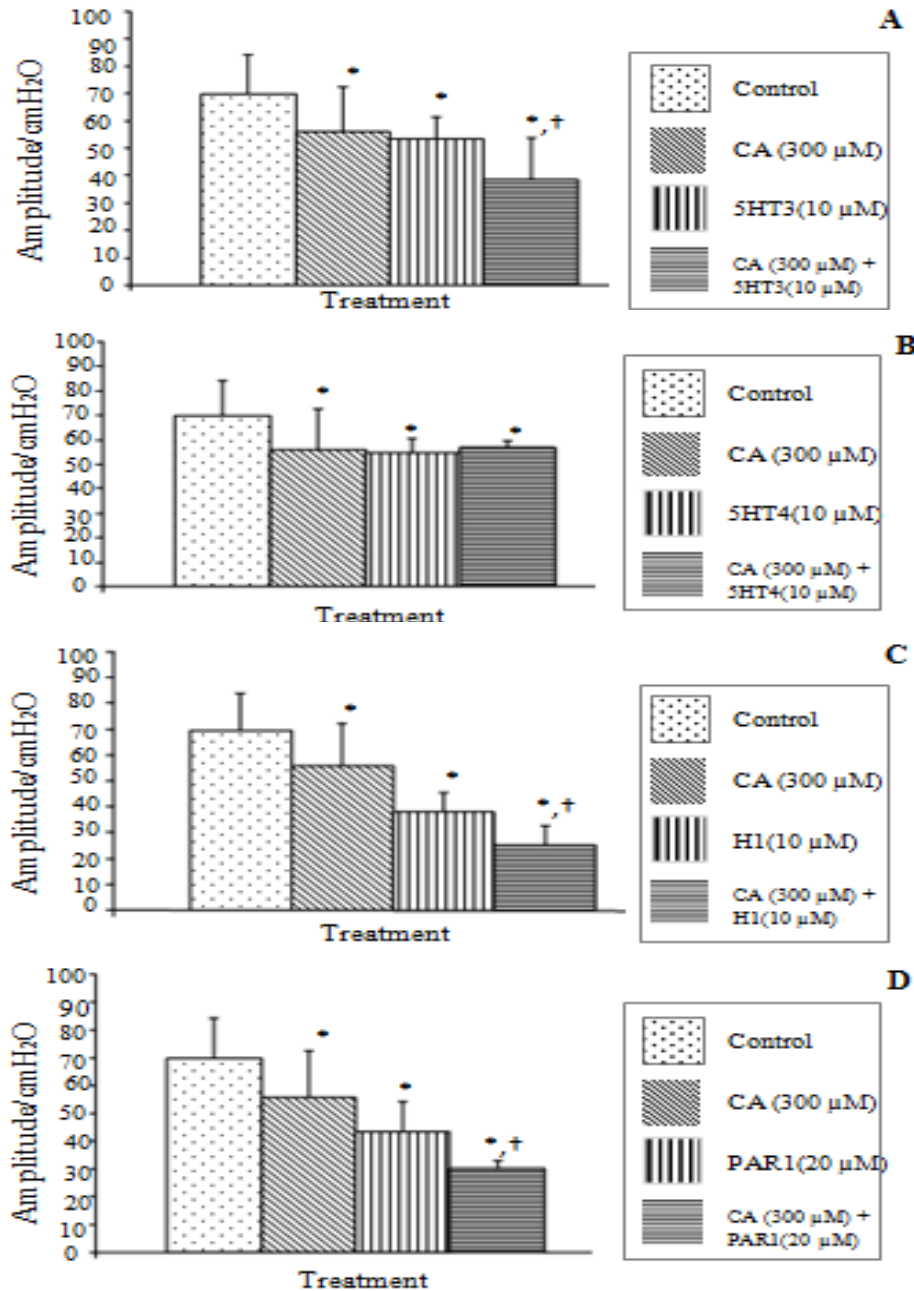


Figure 6. Effect of CA in the presence of pharmacological antagonists on the Amplitudes of the PMC of mice colon. Tissue preparations were preincubated with (5-HT3 antagonist, 10 μM, A), (5-HT4 antagonist, 10 μM, B), (H1 antagonist, 10 μM, C) and (PAR1 receptor antagonist, 20 μM, D) prior to challenge with CA (300 μM). Values represents mean ± SE of 4 colon segments from different animals. *Significantly different compared to the control group, ($P < 0.05$). †Significantly different compared to the CA data, ($P < 0.05$).

CAs inhibit intestinal function have not been investigated; recent researches that refer to recent discovery of receptors that mediate their effects confirmed our results (Lefebvre et al., 2009). However, the activation of GpBAR1 by BAs inhibits large intestinal motor function, which could be due to the effects of GpBAR1 activation which mediated through activation of cholinergic and

nitroergic interneurons (Keely, 2010). In these experiments, CA inhibited PMC amplitude after addition of 5-HT3 antagonist, 5-HT4 antagonist and H1 antagonist. These results were in agreement with previous studies which demonstrated that BAs are able to activate mast cells (Gelbmann et al., 1995; Fihn et al., 2003) and act *via* 5-HT3 receptor. Therefore, the inhibition of CA in the

presence of histamine antagonist on PMCs suggested that mast cells are playing an important role in mediating hyperexcitability and enteric neuron depolarization via their release of histamine from mast cell degranulation (Sand et al., 2009).

In these experiments, the protease inhibitors, PAR1 receptor antagonist also inhibited the PMCs. These data supported by Poole et al. (2010) who identified a novel mechanism for the well-known effects of BAs on intestinal motility. He found that BAs inhibit motility by a mechanism that is consistent with activation of GpBAR1 on inhibitory motor neurons that release nitric oxide (NO). However, in this study, the effect of PAR1 was controversial since CA increases motility in the presence of PAR1 antagonist. The justification for that is the mast cells are regulated by the release of NO (Sand et al., 2009). Therefore, increase motility in this study could be due to the lack of releases of NO during the application of CA, therefore, these results suggest new mechanism concerning the possibility of antagonistic action of CA on the receptors of the mast cells neuromediators and CA may also act on the mucosa to release factors that control motility.

The increase motility in this study supported also by Kirwan et al. (1975), who found that the abnormally high quantities of primary BAs in the colon cause diarrhea not only by inhibiting the absorption of water and electrolytes, but also by eliciting colonic motor activity.

This study showed that CA in the presence of PAR1 inhibited the amplitudes and is in agreement with Poole et al. (2010). BAs induced inhibitory effects on contractile activity were unaffected by atropine which indicates that muscarinic receptors are not involved in these responses. The same authors reported that a BA that is a potent GpBAR1 agonist inhibited motility of intestinal segments by a neurogenic and nitrergic mechanism.

In conclusion, this study had described that *in vitro* CA perfusion, at certain concentration levels, induced significant inhibitory changes in colon motility. The clear inhibitory effect of CA on PMC amplitudes in the presence of 5-HT3 and 5-HT4 and PAR1 antagonists suggests that CA may act through the release of mast cell neuromediators. This may propose its antagonistic action on the receptors in addition to the possible direct effect of the CA on the colon musculature.

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REFERENCES

- Abdu F, Hicks GA, Hennig G, Allen JP, Grundy D (2002). Somatostatin sst(2) receptors inhibit peristalsis in the rat and mouse jejunum. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 282(4):G624-633.
- Alemi F, Poole DP, Chiu J, Schoonjans K, Cattaruzza F, Grider JR, Bunnett NW, Corvera CU (2013). The receptor TGR5 mediates the prokinetic actions of intestinal bile acids and is required for normal defecation in mice. *Gastroenterology* 144(1):145-154.
- Armstrong DN, Krenz HK, Modlin IM, Ballantyne GH (1993). Bile salt inhibition of motility in the isolated perfused rabbit terminal ileum. *Gut* 34(4):483-488.
- Astegiano M, Pellicano R, Sguazzini C, Berrutti M, Simondi D, Reggiani S, Rizzetto M (2008). 2008 Clinical approach to irritable bowel syndrome. *Minerva. Gastroenterol. Dietol.* 54(3):251-257.
- Barbara G, Stanghellini V, De Giorgio R, Corinaldesi R (2006). Functional gastrointestinal disorders and mast cells: implications for therapy. *Neurogastroenterol. Motil.* 18(1):6-17.
- Boyer JL, Trauner M, Mennone A, Soroka CJ, Cai SY, Moustafa T, Zollner G, Lee JY, Ballatori N (2006). Upregulation of a basolateral FXR-dependent bile acid efflux transporter OSTalpha-OSTbeta in cholestasis in humans and rodents. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 290(6):G1124-1130.
- Bunnett N, Alemi F, Poole DP, Auwerx J, Schoonjans K, Grider JR, Corvera CU (2012). The TGR5 receptor mediates the effects of bile acids on intestinal motility in mice. *Gastroenterol.* 142(5):1-148.
- Camilleri M, Nadeau A, Tremaine WJ, Lamsam J, Burton D, Odunsi S, Sweetser S, Singh R (2009). Measurement of serum 7alpha-hydroxy-4-cholesten-3-one (or 7alphaC4), a surrogate test for bile acid malabsorption in health, ileal disease and irritable bowel syndrome using liquid chromatography-tandem mass spectrometry. *Neurogastroenterol. Motil.* 21(7):734-743.
- Falconer JD, Smith AN, Eastwood MA (1980). The effects of bile acids on colonic motility in the rabbit. *Q. J. Exp. Physiol. Cogn. Med. Sci.* 65(2):135-144.
- Feldman S, Gibaldi M (1968). Effect of bile salts on gastric emptying and intestinal transit in the rat. *Gastroenterology* 54(5):918-921.
- Fihn BM, Sjöqvist A, Jodal M (2003). Involvement of enteric nerves in permeability changes due to deoxycholic acid in rat jejunum *in vivo*. *Acta. Physiol. Scand.* 178(3):241-250.
- Gelbmann CM, Scheingart CD, Thompson SM, Hofmann AF, Barrett KE (1995). Mast cells and histamine contribute to bile acid-stimulated secretion in the mouse colon. *J. Clin. Invest.* 95(6):2831-2839.
- Hegde SS, Eglen RM (1996). Peripheral 5-HT4 receptors. *FASEB. J.* 10(12):1398-1407.
- Keely SJ (2010). Missing link identified: GpBAR1 is a neuronal bile acid receptor. *Neurogastroenterol. Motil.* 22:711-717.
- Kaumann AJ (1993). Blockade of human atrial 5-HT4 receptors by GR113808. *Br. J. Pharmacol.* 110(3):1172-1174.
- Kim HS (2009). 5-Hydroxytryptamine4 receptor agonists and colonic motility. *J. Smooth. Muscle Res.* 45(1):25-29.
- Kirwan WO, Smith AN, Mitchell WD, Falconer JD, Eastwood MA (1975). Bile acids and colonic motility in the rabbit and the human. *Gut* 16(11):894-902.
- Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B (2009). Role of bile acids and bile acid receptors in metabolic regulation. *Physiol. Rev.* 89:147-191.
- Liu S, Hu HZ, Gao N, Gao C, Wang G, Wang X, Peck OC, Kim G, Gao X, Xia Y, Wood JD (2003). Neuroimmune interactions in guinea pig stomach and small intestine. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 284(1):G154-164.
- Mikami T, Sugimoto H, Nagane R, Ohmi T, Saito T, Eda H (2008). Contribution of active and inactive states of the human 5-HT4d receptor to the functional activities of 5-HT4-receptor agonists. *J. Pharmacol. Sci.* 107(3):251-259.
- Parsons ME, Ganellin CR (2006). Histamine and its receptors. *Br. J. Pharmacol.* 147(Suppl 1):S127-135.
- Poole DP, Godfrey C, Cattaruzza F, Cottrell GS, Kirkland JG, Pelayo JC, Bunnett NW, Corvera CU (2010). Expression and function of the bile acid receptor GpBAR1 (TGR5) in the murine enteric nervous system. *Neurogastroenterol. Motil.* 22(7):814-825.
- Romero F, Frediani-Neto E, Paiva TB, Paiva AC (1993). Role of

- Na⁺/Ca²⁺ exchange in the relaxant effect of sodium taurocholate on the guinea-pig ileum smooth muscle. *Naunyn. Schmiedebergs. Arch. Pharmacol.* 348(3):325-331.
- Sand E, Themner-Persson A, Ekblad E (2009). Mast cells reduce survival of myenteric neurons in culture. *Neuropharmacology* 56(2):522-530.
- Shimamura T, Shiroishi M, Weyand S, Tsujimoto H, Winter G, Katritch V, Abagyan R, Cherezov V, Liu W, Han GW, Kobayashi T, Stevens RC, Iwata S (2011). Structure of the human histamine H1 receptor complex with doxepin. *Nature* 475(7354):65-70.
- Surawicz CM (2010). Mechanisms of diarrhea. *Curr. Gastroenterol. Rep.* 12(4):236-241.
- Umoren EB, Obembe AO, Osim EE (2013). Ulcerogenic and intestinal motility/transit stimulating actions of nevirapine in albino Wistar rats. *J Physiol Biochem.*, in press (available at <http://www.ncbi.nlm.nih.gov/pubmed/23536414>).
- Van Ooteghem NA, Van Erpecum KJ, Van Berge-Henegouwen GP (2002). Effects of ileal bile salts on fasting small intestinal and gallbladder motility. *Neurogastroenterol. Motil.* 14(5):527-533.
- Wang XY, Zarate N, Soderholm JD, Bourgeois JM, Liu LW, Huizinga JD (2007). Ultrastructural injury to interstitial cells of Cajal and communication with mast cells in Crohn's disease. *Neurogastroenterol. Motil.* 19(5):349-364.
- Wouters MM, Farrugia G, Schemann M (2007). 5-HT receptors on interstitial cells of Cajal, smooth muscle and enteric nerves. *Neurogastroenterol. Motil.* 19(Suppl 2):5-12.