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Original Article

The Effect of Deoxycholic Acid on Secretion and Motility in the Rat and Guinea Pig Large Intestine

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Background/Aims
Bile acid is an important luminal factor that affects gastrointestinal motility and secretion. We investigated the effect of bile acid on secretion in the proximal and distal rat colon and coordination of bowel movements in the guinea pig colon.

Methods
The short-circuit current from the mucosal strip of the proximal and distal rat colon was compared under control conditions after induction of secretion with deoxycholic acid (DCA) as well as after inhibition of secretion with indomethacin, 1,2-bis (*o*-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid (an intracellular calcium chelator; BAPTA), and tetrodotoxin (TTX) using an Ussing chamber. Colonic pressure patterns were also evaluated in the extracted guinea pig colon during resting, DCA stimulation, and inhibition by TTX using a newly developed pressure-sensing artificial stool.

Results
The secretory response in the distal colon was proportionate to the concentration of DCA. Also, indomethacin, BAPTA, and TTX inhibited chloride secretion in response to DCA significantly ($P < 0.05$). However, these changes were not detected in the proximal colon. When we evaluated motility, we found that DCA induced an increase in luminal pressure at the proximal, middle, and distal sensors of an artificial stool simultaneously during the non-peristaltic period ($P < 0.05$). In contrast, during peristalsis, DCA induced an increase in luminal pressure at the proximal sensor and a decrease in pressure at the middle and distal sensors of the artificial stool ($P < 0.05$).

Conclusions
DCA induced a clear segmental difference in electrogenic secretion. Also, DCA induced a more powerful peristaltic contraction only during the peristaltic period.
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Key Words
Colon; Deoxycholic acid; Gastrointestinal motility; Peristalsis; Secretion

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Introduction

A high concentration of bile acids can induce watery diarrhea in the human large colon. Chronic diarrhea is caused by unabsorbed bile acids in cases of ileal resection up to 100 cm,[1] and agents that sequester bile acid cause a reduction in diarrhea without interfering with the metabolism of fat or bile acid.[2] Several mechanisms are involved in the development of diarrhea secondary to unabsorbed bile acids. Bile acids have a direct secretory effect on colonic enterocytes,[3] and indirectly induce a secretory response by stimulating mast cells or intrinsic neural arcs.[4,5]

In addition, the introduction of bile acids into the human sigmoid colon and rectum stimulates colonic motility,[6] and chenodeoxycholate significantly accelerates colon transit time in healthy subjects.[7] A failure to reabsorb bile acid was also suggested to be a cause of diarrhea owing to excess bile acids entering the colon.[8] Recently, bile acids have been suggested to play an important role in irritable bowel syndrome with diarrhea. Idiopathic adult onset bile acid malabsorption is not rare in irritable bowel syndrome with predominant diarrhea (IBS-D),[9] and increased bile acid biosynthesis is associated with IBS-D.[10,11] The role of bile acid in the development of gastrointestinal symptoms has become more important than ever.

However, in previous studies, segmental heterogeneity[12] has not been considered in bile acid induced secretion experiments, and in terms of colonic motility, peristaltic movement was evaluated only by measuring muscular contractility, not by determining changes in intracolonic pressure along a peristaltic wave.[13] Therefore, in the present study, we aim to evaluate the existence of segmental heterogeneity of colonic secretion to deoxycholic acid (DCA), and the actual influence of DCA on peristalsis using a newly-developed sensor system. DCA is the most prominent secondary bile acid in human beings.[14]

Materials and Methods

Animals

Thirty-six female Sprague-Dawley rats (300-400 g) were used in the secretion study. Rats were euthanized by intravenous injection of sodium pentobarbital (45 mg/kg) and ketamine (15 mg/kg). All animal protocols were performed in accordance with Animal Experiment Guidelines of Kangbuk Samsung Hospital and approved by the Animal Care Committee. The colon was immediately removed, opened longitudinally, and washed with oxygenated Krebs solution (NaCl 125 mM, KCl 5.9 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 1.2 mM, $NaHCO_3$ 15.5 mM, NaH_2PO_4 1.2 mM, glucose 11.5 mM; pH 7.4). Then, the serosa and muscle were removed under light microscopy to obtain a stripped epithelium. To evaluate the secretory responses of the proximal and distal colon, colonic segments within 2-3 cm from the ileocecal valve and 4-5 cm from the anus were used, respectively.

To evaluate peristalsis, guinea pigs were used instead of rats, since most experiments on intestinal peristalsis using artificial pellets and isolated colon were performed with guinea pig colon. Six male guinea pigs (250-400 g) were used in this experiment. They were sacrificed by CO_2 inhalation overdose and exsanguination. Then, the abdominal cavity was opened and an approximately 10 cm segment of the distal colon was removed. After removal of stools inside the colon, the colonic segment was placed immediately in an organ bath with Krebs solution. In our preliminary study, peristaltic movement could rarely be seen in the proximal colon of guinea pigs. Therefore, the distal colon (where the proximal end of the colonic segment was defined as being 10 cm away from the anus) was used to evaluate peristalsis.

Secretion in the Proximal and Distal Colonic Mucosa Using an Ussing Chamber

Stripped epithelium was mounted in a tissue holder with a window size of 2 mm. Because proximal and extracellular solutions were continuously perfused at a rate of 10-20 mL/min, the effects of various drugs could be evaluated sequentially in the same tissue. The pH was adjusted to 7.4 and the bath solution was heated with a water jacket. All experiments were performed at a temperature of 37°C. The time between the removal of the epithelium and the mounting in the Ussing chamber was about 30 minutes, during which time it was maintained in a oxygenated phosphate buffered solution at 4°C. The experiment was performed under open circuit conditions and the transepithelial voltage (V_{te}) value, which refers to the serum side of the epithelium, was used. The transepithelial resistance (R_{te}) was determined by the application of short (1 second) current pulses (change [Δ] at $I = 0.5\ \mu A$). The voltage deviation obtained when no tissue was present in the chamber was subtracted from the voltage deviation obtained when the chamber was present. After an equilibration period of 30 minutes, chloride secretion was evoked by treatment of DCA 0.5 mM on the luminal and basolateral sides. In the next step, to evaluate the secretory mechanism, calcium-dependent chloride secretions were blocked by 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid (BAPTA), and cAMP and calcium-dependent chloride secretions were blocked

by indomethacin, while secretion was stimulated by DCA. Lastly, secretion was measured after the addition of tetrodotoxin (TTX).

Quantitative real-time polymerase chain reaction for G protein-coupled bile acid receptor 1

G protein-coupled bile acid receptor 1 (GpBAR1) is the bile acid receptor that is expressed in rat colonic epithelium, enterochromaffin cells, and myenteric neurons in the colon.[14,16] The polymerase chain reaction (PCR) amplification was performed in a LightCycler 480 Real-Time PCR System (Roche Applied Science, Indianapolis, IN, USA) in 96 well plates. PCR mix contained 2 μL cDNA template, 2 μL of primer mix and 10 μL of LC480 SYBR Green I Master Mix from the LC480 SYBR Green I Master kit (Roche). Plate layout design was maintained for triplicates of the samples according to rat GpBAR1 (forward 5'-cactgcccttctctctgtcc-3'; reverse 5'-agttcaggtccagttacgc-3') and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward 5'-ggtgctgagtatgtcgtgga-3'; reverse 5'-gtggttcacacccatcacaa-3'), 3 wells for negative. The PCR protocol included 1 cycle of polymerase activation for 10 minutes at 95°C, 45 cycles of amplification with 10 seconds denaturation at 95°C, 1 minute annealing at 55°C, and 5 seconds extension at 72°C in each cycle and 1 cycle for melting curve analysis with 10 seconds denaturation at 95°C, 1 minute annealing at 60°C and a melting step at 98°C at 0.2°C/sec ramp rate. Each sample was run in triplicate.

Peristaltic Evaluation of Guinea Pig Large Intestine Using an Artificial Stool with Serial Capacitive Sensors

Development of a pressure-sensing artificial stool

To mimic real guinea pig excrement, we designed an artificial pellet (4 × 9 mm in size and the surface area of the proximal and distal segments was 4.7 mm^2, while that of the middle segment was 5.05 mm^2) and attached it to the packaged sensor. The artificial pellet was made of polylactic acid and formed by 3-dimensional printing (Fig. 1A). The proposed pressure sensor was designed with careful consideration of the structure and motility mechanism of the guinea pig large intestine. Three pressure sensors were mounted, one each on the proximal, middle, and distal portions of the artificial pellet, in order to provide some redundancy in the size and shape of the artificial guinea pig feces. The capacitance of a prototype sensor was recorded as 2.5-3.0 pF. This capacitance value was later converted to a count value using a lab-fabricated data conversion system. The sensitivity of the pressure sensor was recorded as below

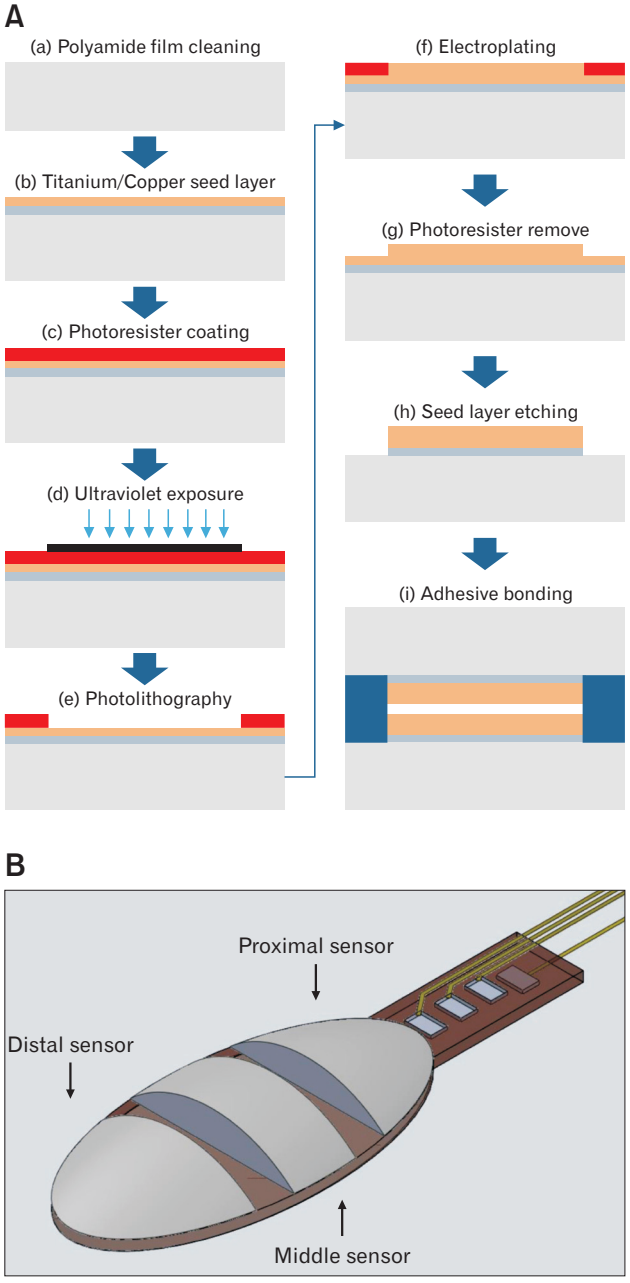

Figure 1. Fabrication sequence for the sensor and schematic of pressure-sensing artificial stool. (A) [a] Polyamide was chosen for the substrate with consideration of microfabrication and biocompatibility. [b] The surface was covered by titanium/copper (Ti/Cu, 20/300 nm) films by sputtering for seed layer in the electroplating process. The electrode for capacitor sensors was then shaped by photolithography and formed by Cu electroplating as shown in [c] and [e]. Then, the unnecessary photoresister and seed layer was removed by a solvent and a metal etchant ([f] to [h]). Those electrode formation processes were done on the bottom and upper substrates. [i] To achieve hermeticity of the pressure sensors, the bottom and upper substrates were directly bonded by the assistance of an adhesive film (OCA film; Shin-Chang, Dongducheon, Korea). (B) Location of proximal, middle, and distal sensors on the ovoid shaped artificial stool.

1 mmHg per atmospheric pressure (Fig. 1B). Finally, tests for the reproducibility and accuracy of measurements were done using an evaluation system (High precision pressure regulator CPC3000; Mensor San Marcos, Texas, USA), vacuum chamber, network analyzer (HP, 8753E; Palo Alto, CA, USA), and switch module (PXIe-1082; National Instruments; Austin, Texas, USA).

In vitro experiments using pressure-sensing artificial stool

After an equilibration period of 30 minutes in an organ bath, artificial stool was inserted into the oral side of the colonic segment (10 cm in length), and then 1, 10, and 100 μM DCA were added to the tissue bath. Changes in intraluminal pressure and frequency of colonic contraction were analyzed for 10-15 minutes after the introduction of the drugs. Frequency was calculated using a Fourier series (MATLAB program; MathWorks, Natick, MA, USA) and is expressed in Hertz. In addition, 100 μM DCA and 1 μM TTX were applied to the tissue bath and motor responses were recorded. The distal colon was used for the study of peristalsis since peristalsis rarely occurs in the proximal colon. All measured values are expressed as the change in pressure from baseline (pressure at rest).

Statistical Methods

All data are expressed as means ± standard deviations. Two-tailed Student's *t* tests and ANOVA tests were utilized for the comparison of statistical differences. A *P*-value of < 0.05 was considered to be significant.

Results

Secretory Responses of the Proximal and Distal Colon to Bile Acids

Electrical properties following luminal and basolateral addition of deoxycholic acid

In the distal colon, the luminal addition of DCA induced an increase in short-circuit current (I_{sc}) values in a concentration-dependent manner. However, the increase in delta I_{sc} after the addition of DCA 0.5 mM on the basolateral side was more significant as compared to the secretory responses on the luminal side (ΔI_{sc} = 49.76 ± 43.83 and 4.62 ± 6.83 μA·cm^{-2} for the proximal and distal colon, respectively; $P < 0.001$, n = 8) (Fig. 2A). When DCA was added to the luminal and basolateral sides of the proximal colon, no significant changes in I_{sc} were seen (n = 8) (Fig. 2B).

Segmental differences of secretory responses following basolateral addition of deoxycholic acid

When we compared secretory responses between segments, significant differences were detected in I_{sc} after the basolateral addition of 0.5 mM DCA between the values measured in the proximal and distal colon (ΔI_{sc} = 49.76 ± 43.83 and 11.45 ± 15.38 μA·cm^{-2} for the proximal and distal colon, respectively; $P < 0.05$, n = 8).

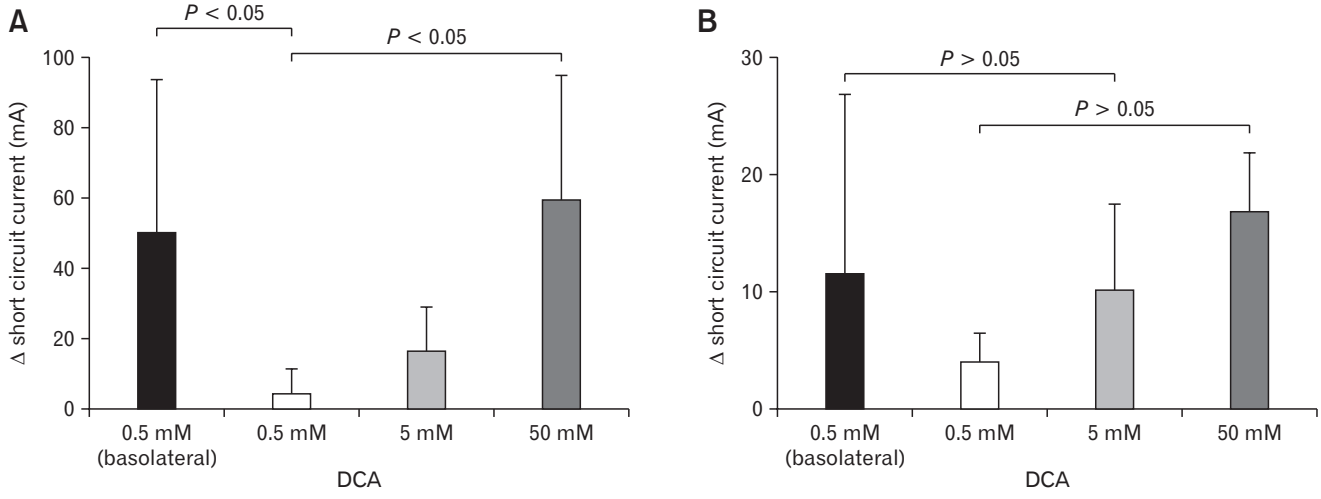

Figure 2. Secretory response to the luminal and basolateral addition of deoxycholic acid (DCA). (A) In the distal colon, the luminal addition of DCA induced an increase in short-circuit current (I_{sc}) values in a concentration dependent manner (n = 8), and the increase in secretion after the basolateral addition of DCA 0.5 mM was more significant than the secretory responses on the luminal side. (B) When DCA was added to the luminal and basolateral side in the proximal colon, only slight increase of I_{sc} was noted (n = 8).

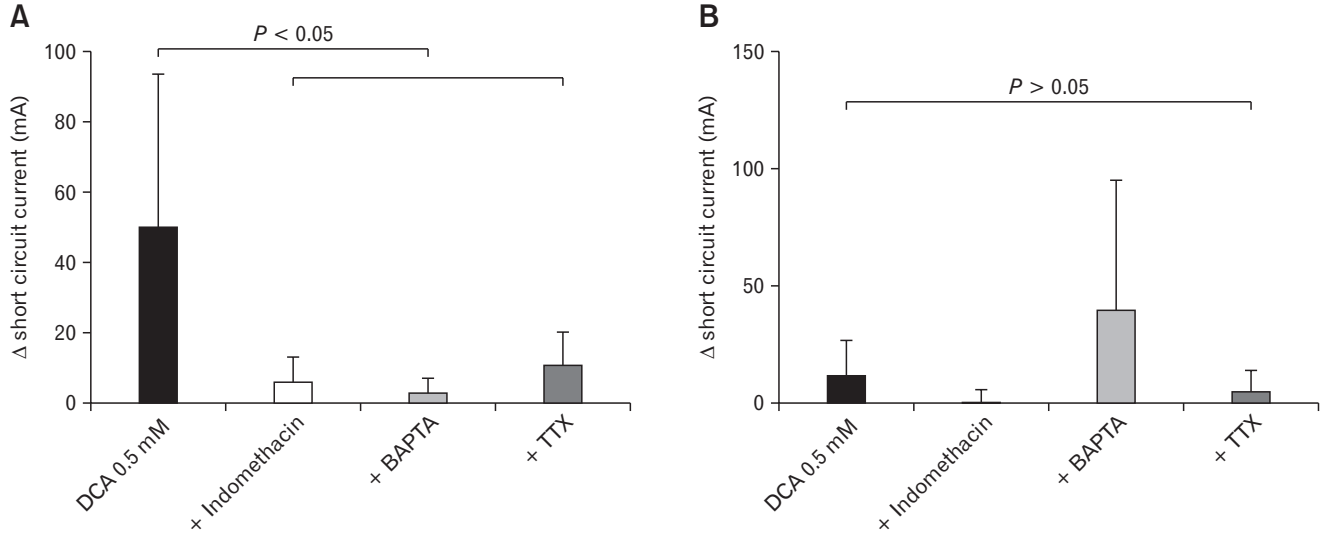

Figure 3. Effect of pretreatment with indomethacin, 1,2-bis(*o*-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid (BAPTA) and tetrodotoxin (TTX) on secretory response of the proximal and distal colon. (A) The basolateral addition of indomethacin, BAPTA and TTX resulted in the abolition of responses to deoxycholic acid (DCA) in the distal colon (n = 6), (B) but not in the proximal colon.

Effects of indomethacin, BAPTA, and tetrodotoxin after addition of deoxycholic acid in the proximal and distal colon

In the distal colon, the basolateral addition of indomethacin, BAPTA, and TTX resulted in an abolition of responses to DCA. This decrease in delta I_{sc} values was found to be significant ($P < 0.05$ for the application of indomethacin, BAPTA, and TTX, n = 6) (Fig. 3A). However, in the proximal colon, pretreatment with indomethacin, BAPTA, and TTX did not have an effect on the secretory responses to DCA ($P > 0.05$) (Fig. 3B).

Quantitative real-time polymerase chain reaction analysis of G protein-coupled bile acid receptor 1

The levels of GpBAR1 expression in the proximal and distal colon were determined using qRT-PCR, with the values normalized to the corresponding GAPDH gene transcription values. The distribution of GpBAR1 was similar in both colonic segments (Fig. 4).

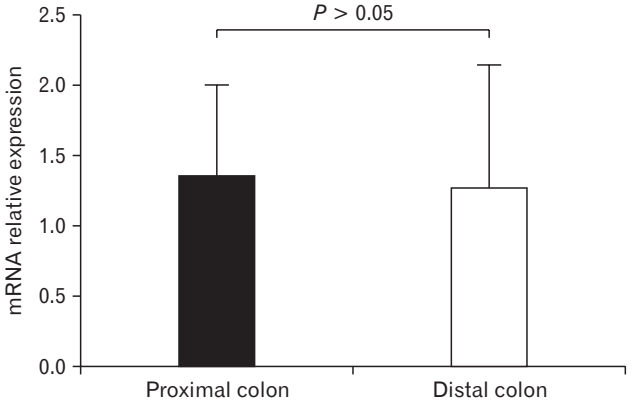

Figure 4. Expression levels of G protein-coupled bile acid receptor 1 (GpBAR1) between proximal and distal colon. The distribution of GpBAR1 was similar in both colon segments (n = 5).

Motor Effects of Bile Acid on the Distal Colon

Changes in contractility and frequency: during peristaltic and non-peristaltic period

During the non-peristaltic period, the addition of 1 μmol DCA into the colonic lumen induced a significant increase in pressure at the proximal, middle, and distal sensors of the artificial stool, and the pressure change at the distal sensor was greater than that at the proximal or middle sensors (n = 5, $P < 0.01$) (Fig. 5A). The increase in pressure at all sensors on an artificial stool was concentration-dependent (Fig. 5B). However, DCA had no effect on the frequency of contraction at the proximal, middle, and distal sensors (Fig. 5C).

However, during the peristaltic period, 1 μmol DCA caused a significant increase in pressure only at the proximal sensor of an artificial stool (Fig. 6A), while a notable decrease in pressure at the middle and distal sensors was detected (n = 4, $P < 0.05$) (Fig. 6B and 6C). There was no change in the frequency of contraction at any of the sensors on the artificial stool (n = 4, $P > 0.05$).

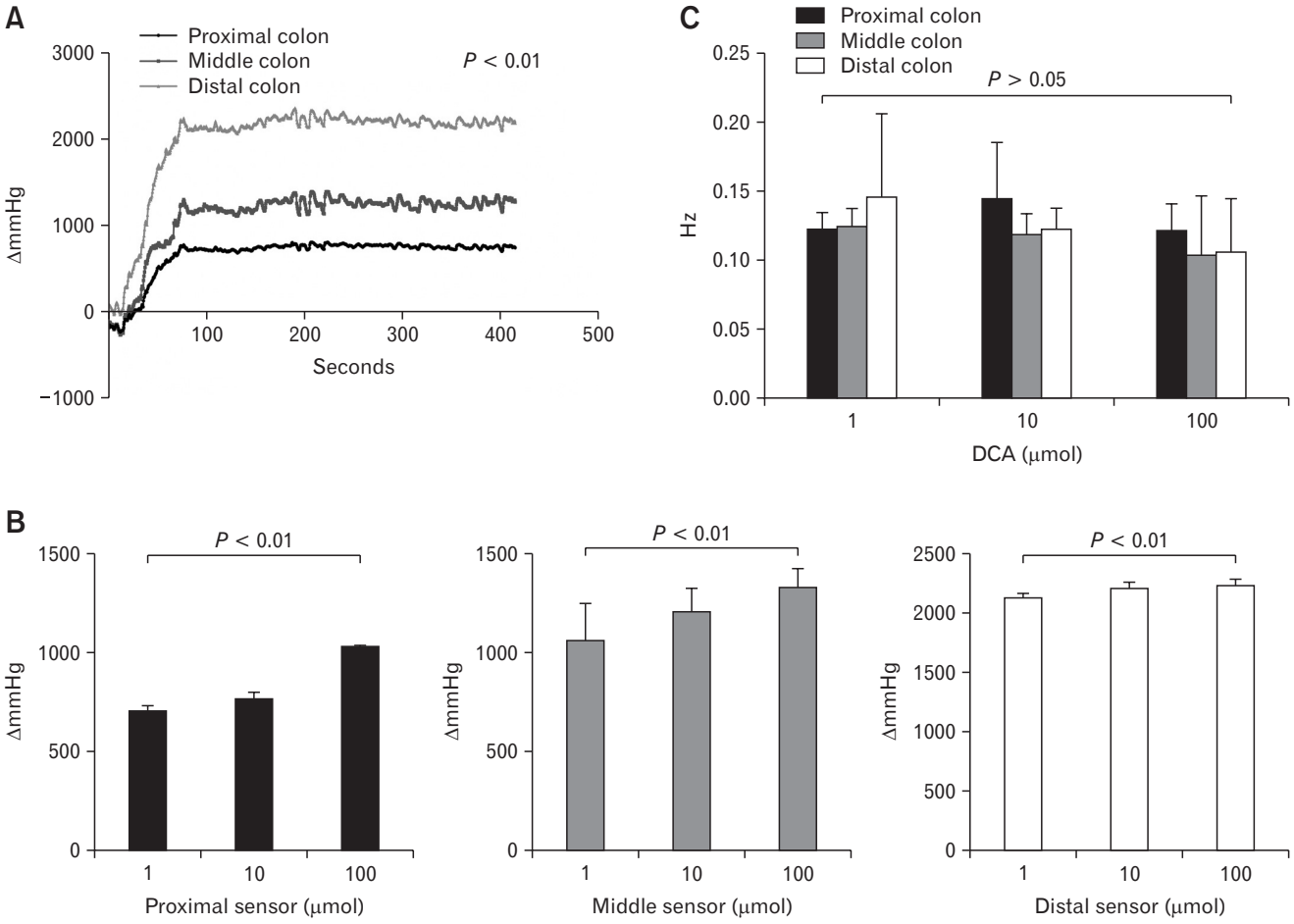

Figure 5. Pressure changes after the addition of deoxycholic acid (DCA) during non-peristaltic period. (A) The addition of DCA 1 μmol into the colonic lumen induced a significant increase in pressure at all sensors of an artificial stool, and pressure change at the distal sensor was greater than those in the proximal and middle sensors (n = 5). (B) Increase in pressure at all sensors was concentration dependent. (C) DCA had no effect on the frequency of contraction at the proximal, middle, and distal sensors of an artificial stool.

Change in contractility with the addition of tetrodotoxin during 100 μmol deoxycholic acid-induced contraction

At first, pretreatment with TTX significantly increased pressure at the proximal and distal sensors of an artificial stool (n = 5, $P < 0.01$) (Fig. 7). However, this change gradually disappeared over time.

Discussion

In this study, we showed that DCA induced a significant secretory response in the distal colon, and secretion was inhibited by indomethacin, BAPTA, and TTX. However, in the proximal colon, DCA caused only a slight secretory response and secretion was not inhibited by indomethacin, BAPTA, or TTX. Furthermore, during the non-peristaltic period, application of DCA induced a concentration-dependent increase in pressure at the proximal, middle and distal parts of an artificial stool. However, when DCA was added during peristalsis, pressure at the proximal part was significantly increased while pressure at the distal and middle parts was notably decreased compared with control pressure. Addition of TTX did not inhibit colonic contractility at first, but colonic contractility tended to decrease slowly as time passed.

We also observed that the secretory response induced by basolateral addition of DCA was 10 to 100 times more prominent than that caused by luminal addition of DCA in the distal colon; this result is consistent with that of previous reports.[17] Actually, efficient ileal conservation, together with rapid bacterial modification of bile acids entering the colon, results in the aqueous concentration of

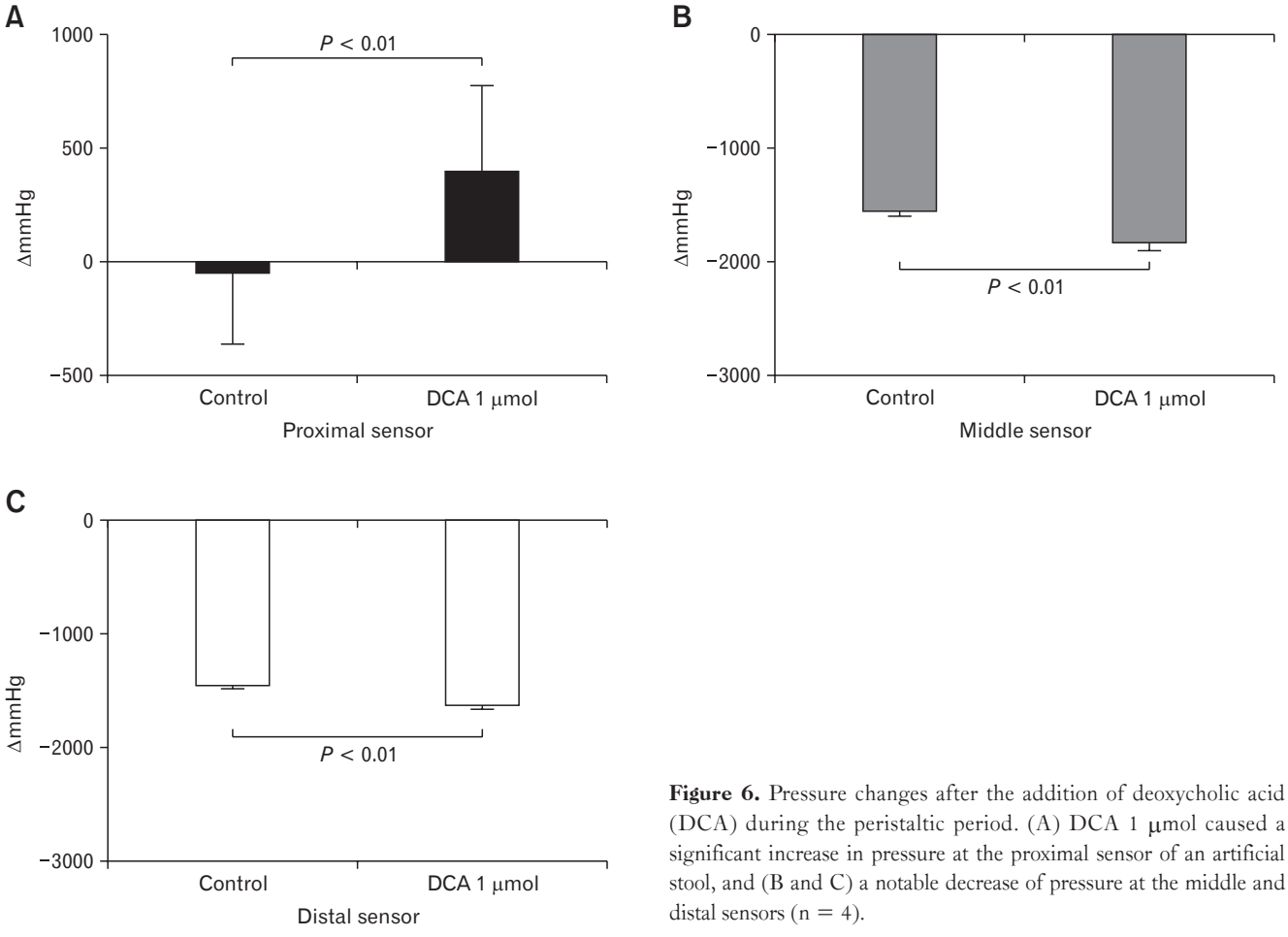

Figure 6. Pressure changes after the addition of deoxycholic acid (DCA) during the peristaltic period. (A) DCA 1 μmol caused a significant increase in pressure at the proximal sensor of an artificial stool, and (B and C) a notable decrease of pressure at the middle and distal sensors (n = 4).

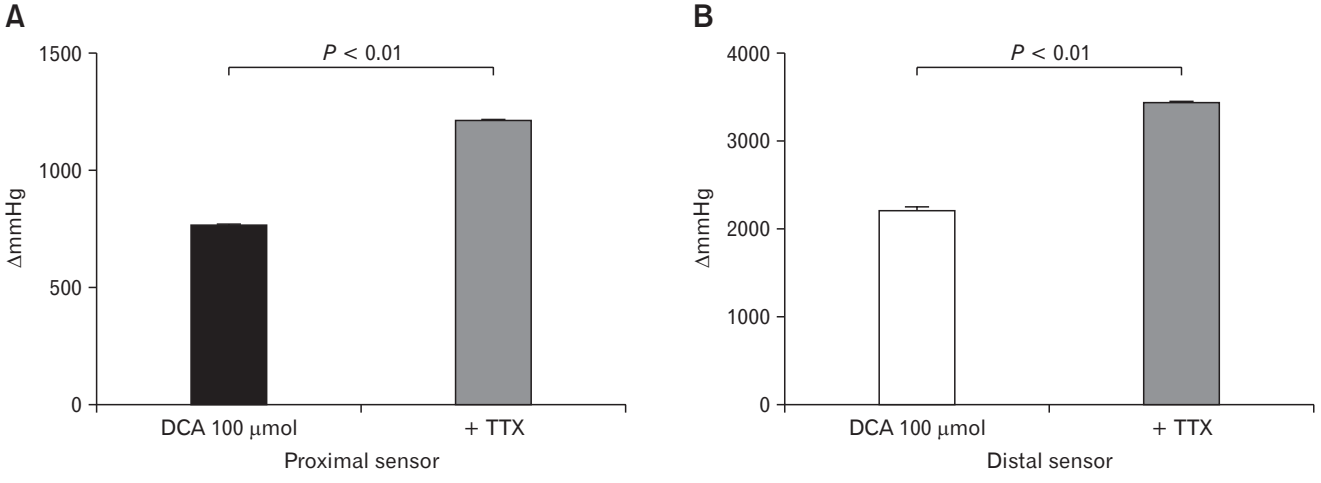

Figure 7. Pressure changes on the distal sensors (deoxycholic acid [DCA] 100 μmol + tetrodotoxin [TTX]). At first, pretreatment of TTX significantly increased pressure at the proximal (A) and distal sensors (B) of an artificial stool. However, this change gradually disappeared over time (n = 5).

bile acids in the colon being quite low, < 1 mM.[18] The circulating DCA pool is only about 0.66 g (1.7 mM).[19] Therefore, the physiologic concentration of luminal DCA might not have an effect on the secretory response at all. Alternatively, if DCA could enter the basolateral side of the colonic epithelium, perhaps because of inflammation or mucosal breakage, the possibility of inducing a secretory response would be much higher.[17]

Application of DCA induced a marked increase in I_{sc} in the proximal and distal colon, and the increase in I_{sc} in the distal colon was greater than that in the proximal colon. This segmental heterogeneity in the secretory responses is consistent with the results of previous reports. The distal segments of the rat colon were more sensitive to both carbachol and forskolin than were the proximal segments[20] and similar results were reported in the human ascending colon and rectum.[12] In terms of secretory mechanisms, our results are in line with those of previous studies; stimulation of a colonic mucosal cAMP system is strongly implicated in mediating DCA-induced colonic secretion,[21] and DCA probably activates Ca^{2+}-regulated K^+ conductance and acts on non-epithelial cells to activate Cl^- secretion indirectly[17] However, some of our results appear to contradict those of previous reports. Activation of GpBAR1 rapidly decreased basal chloride secretion and attenuated the chloride-mediated secretory response to a cholinergic agent.[16] This discrepancy may be due to differences in agonist action on GpBAR1. Ursodeoxycholic acid and lithocholic acid attenuate colonic epithelial secretory function[22,23] In contrast, DCA activates ion channels in colonocytes and acts on non-epithelial cells to activate chloride secretion indirectly.[17] Furthermore, pretreatment with TTX changed the secretory responses to DCA, confirming that the nervous system is associated with secretion induced by DCA. Considering that the effects of GpBAR1 activation are independent of the ENS,[16] DCA may induce secretory responses in the colon by a dual mechanism consisting of GpBAR1 activation and neuronal activity. In the proximal colon, addition of basolateral DCA only induced a slight increase in I_{sc} and pretreatment with indomethacin, BAPTA, or TTX did not change the electrical properties. This indicates that the proximal colon is not responsible for the secretory response to DCA. A similar result was found in the human proximal colon.[12] However, there is also a possibility that the lack of secretory response to DCA in the proximal colon might lead to low sensitivity of proximal colon to the BAPTA and TTX.

DCA had a different effect on colonic contractility according to whether peristalsis did or did not occur. During the non-peristaltic period, DCA induced a significant increase in pressure at the proximal, middle, and distal parts of an artificial stool simultaneously. This pattern of increased contractility did not accelerate propulsion of the artificial stool. However, during the peristaltic period, DCA caused faster movement of an artificial stool by increasing pushing strength and decreasing resistance at the distal part of the artificial stool. This result was not dependent upon the way DCA was administered (intraluminally or extraluminally). These results are partly consistent with a previous report that mucosal application of DCA stimulated ascending contraction and descending relaxation of the colonic circular muscle, thus inducing peristalsis.[14] Bile acids are known to activate GpBAR1, which is expressed by enterochromaffin cells and intrinsic primary afferent neurons, and the release of 5-hydroxytryptamine and calcitonin gene-related peptide, the major transmitters of the afferent limb of the peristaltic reflex.[13]

In terms of the efficacy of DCA on the peristaltic reflex, DCA was suggested to stimulate migrating action potential complexes (MAPC) in the colon, and the increase in MAPC activity is dependent on intact cholinergic and alpha adrenergic neurons.[24] Also, full-thickness segments of mouse colon stimulated by various GpBAR1 agonists induced peristalsis in mice.[14] However, an increase in the peristaltic reflex in previous reports was estimated by whole-gut transit time and defecation frequency, not by the actual number of peristaltic movements. Shortened whole-gut transit time does not necessarily indicate an increased frequency of the peristaltic reflex. In the present study, DCA did not increase the frequency of peristaltic movements, and instead only strengthened the propulsive power on the artificial stool. Considering these results, the decreased transit times seen in a previous study might be caused by faster movement of an artificial stool instead of by increased frequency of the peristaltic reflex.

Luminal bile acids exert region-specific actions in the intestine. They inhibit motility of the small intestine,[25] while stimulating motility in the large intestine.[26] Segmental heterogeneity also exists inside the large intestine. The peristaltic reflex could be easily induced only in the distal colon of the guinea pig, but not in the proximal colon. This heterogeneity may be caused by differences in neuronal innervation rather than by differences in the distribution of GpBAR1, because the distribution of GpBAR1 was similar in both colonic segments. Furthermore, rat colon was not suitable for the evaluation of peristalsis, since artificial stools rarely induce the peristaltic reflex in the rat colon. For that reason, guinea pig colon was used in the present study. Thus, species diversity was also shown through our experiments.

One interesting finding of this study is that DCA induced increased contractility after pretreatment with TTX. This result was contrary to our expectations. Possible explanations are as follows.

First, rhythmic phasic contractions of the human sigmoid colon are not affected by TTX. This lack of effect of TTX demonstrated the non-neuronal origin of rhythmic phasic contractions[27] and an unbalanced effect of TTX on excitatory and inhibitory enteric motor neurons could increase colonic contractility for a short time. Actually, first-stage gastrointestinal symptoms after TTX intoxication include nausea, vomiting, diarrhea, and abdominal pain, rather than paralytic ileus. Secondly, the experiment was not long enough to identify a relaxation response after pretreatment with TTX. Owing to the viability of a resected colon, 2 hours was the maximum experimental time.

In conclusion, DCA induced a clear segmental difference in electrogenic secretion. Also, DCA caused a general increase in the pressure of spontaneous colonic contractions during the non-peristaltic period. However, during the peristaltic period, DCA induced a more powerful peristaltic movement in the distal colon.

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Conflicts of interest: None.

Author contributions: Nam Hee Kim: wrote the manuscript; Jung Ho Park: planned the study; and Jae-soon Park and Yeun-Ho Joung: made sensors.

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