

Mechanism of Action of Cholecystokinin on Colonic Motility in Isolated, Vascularly Perfused Rat Colon

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Background/Aims

It is generally believed that cholecystokinin (CCK) stimulates colonic motility, although there are controversial reports. It has also been suggested that postprandial peptide YY (PYY) release is CCK-dependent. Using a totally isolated, vascularly perfused rat colon, we investigated: (1) the roles of CCK and PYY on colonic motility, (2) to determine if CCK modulates PYY release from the colon to influence the motility and (3) to clarify whether the action of CCK and PYY on colonic motility is mediated via the influence of cholinergic input.

Methods

An isolated whole rat colon was used. Luminal pressure was monitored via microtip catheter pressure transducers from proximal and distal colon. After a control period, CCK-8 or PYY was administered intraarterially with or without an anti-PYY serum, loxiglumide or atropine at 12, 60 and 240 pM. Each dose was given for a period of 15-minute and the contractile response was expressed as % changes over basal. PYY concentration in the portal effluent was determined by radioimmunoassay.

Results

Exogenous CCK-8 increased colonic motility which paralleled the increase in PYY release in the portal effluent. Exogenous PYY also significantly increased colonic motility although it was less potent than CCK. The stimulating effect of CCK-8 was significantly inhibited by an anti-PYY serum, and was completely abolished by loxiglumide, and almost completely abolished by atropine.

Conclusions

CCK increases colonic motility via CCK₁ receptor and it is mediated partly by PYY. Cholinergic input is required for the increased motility by either PYY or CCK.

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Key Words

Cholecystokinin; Colon; Peptide YY

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Introduction

Cholecystokinin (CCK) was indentified in 1966 as a 33 amino acid peptide, extracted from porcine intestine.¹ There are 2 cell types producing CCK: (1) endocrine cells of the small intestine and (2) various neurons in the gastrointestinal tract and central nervous system. CCK is a peptide known to act both as a circulating hormone and as a neurotransmitter.^{2,3}

CCK stimulates gallbladder contraction and pancreatic enzyme secretion, and inhibits gastric emptying which facilitates the nutrients digestion and absorption from gut. It was reported that CCK stimulates colonic motility *in vitro*⁴ as well as *in vivo*,⁵⁻⁷ although there have been controversial reports.^{8,9}

CCK and gastrin comprise a family of gastrointestinal peptides that share an identical carboxyl-terminal pentapeptide sequence. Sulfation of the tyrosine (Tyr) residue at position 7 from the carboxyl terminus of CCK is critical for biological activity. The characteristic CCK-like activity depends on the sulfated Tyr residue at the seventh position. If the Tyr residue is not sulfated, or if another amino acid residue is present at this location, the peptide behaves as a gastrin analogue and loses its CCK-like potency.

The biological actions of CCK are mediated by 2 distinct receptors, the originally denoted CCK-A (alimentary type) and CCK-B (brain type), based on their anatomical location. They are now termed CCK₁ and CCK₂, respectively, because of the evidences indicating overlapping areas of location.¹⁰

Peptide YY (PYY), a 36 amino acid peptide, isolated from the porcine duodenum, has been known to be present in endocrine cells, pancreas and brain.^{11,12} PYY is found mainly in mucosal endocrine cells of the ileum, colon and rectum.¹²⁻¹⁴ In rats, it was shown to increase colonic motility,¹⁵ whereas it was reported to either increase or decrease small intestinal motility depending on species.^{13,16,17} It has been suggested that the release of PYY in postprandial state is mediated by CCK, and PYY was shown to be released from rat colon when CCK was administered intraarterially in an isolated, vascularly perfused rat colon.¹⁸

The major portion of neurons in human small and large intestines are cholinergic. A number of cells resembling enteroendocrine cells are found in the epithelium of the small and large bowels which contain choline acetyltransferase, a marker of cholinergic neurons.¹⁹

In the present study, using a totally isolated, vascularly perfused rat colon, we investigated: (1) the roles of CCK and PYY

on colonic motility, (2) to determine if CCK modulates PYY release from the colon to influence the motility and (3) to clarify whether the action of CCK and PYY on colonic motility is mediated via the influence of cholinergic input.

Materials and Methods

Materials

Bovine serum albumin (BSA) and dextran were purchased from Sigma Chemical (St. Louis, MO, USA) and atropine sulfate from American Reagent (Shirley, NY, USA). Loxiglumide was a gift from the Rotta Research Laboratories (Milan, Italy). CCK-8 was purchased from Squibb Diagnostics (Princeton, NJ, USA) and PYY from Peninsula (Belmont, CA, USA). PYY antiserum was prepared in University of Rochester (Rochester, NY, USA) as described previously.²⁰ Briefly, anti-PYY serum was produced in a New Zealand white rabbit by repeated immunization with PYY-BSA conjugate. The antiserum is highly specific for PYY. Except for a small crossreaction with neuropeptide Y (1%) and avian pancreatic polypeptide (0.01%), it did not cross-react with other unrelated gut peptides at concentration of 70 ng/mL in the assay tube.

Animal Preparation

All experiments described in this report were approved by Animal Care Committee of the Chungbuk National University and University of Rochester. The operative procedure was similar to that previously described by Cuber et al for the isolated, vascularly perfused duodenojejunum²¹ and ileum of the rat.²² Male Sprague-Dawley rats weighing between 250 and 300 g were fasted for 48 hours with free access to tap water before surgery. Under anesthesia with intraperitoneal injection of xylazine 10 mg/kg and zolazepam 50 mg/kg, the abdomen was opened by a midline incision. The stomach, spleen and small intestine were removed after ligation of the supplying blood vessels. The whole colon was freed of its visceral and retroperitoneal fixations. A polyethylene cannula (0.58 mm ID, 0.96 OD) was then inserted in superior mesenteric artery and another one (1.52 mm ID, 1.90 OD) into the portal vein, respectively. The arterial perfusion started immediately at a rate of 1.2 mL/min with Krebs solution containing 0.1% BSA and 3% dextran. The mixture was continuously gassed with 95% O₂ and 5% CO₂ and warmed at 37°C. The preparation was removed approximately 8 cm in length and placed in a water bath at 37°C. A rubber cannula (5.0

mm ID, 6.5 mm OD) was inserted into both ends of the proximal and distal colon to drain the luminal secretion. The loop was then flushed out once or twice with 10 mL prewarmed 0.15 M NaCl gently. Microtip catheter pressure transducers (2 mm diameter, Millar Inst Inc, Houston, TX, USA) were placed in 2.0 cm apart through the ends of the proximal and distal colon, separately (Fig. 1).

Experimental Procedure

Intraluminal pressure was monitored continuously via microtip catheter pressure transducers. The pressure changes were recorded with a Hewlett Packard 7754B system (Hewlett Packard Co, Palo Alto, CA, USA). From the recording, motility index was calculated by multiplying the amplitude by the duration of each contractile wave for 5-minute period and expressed as % changes over basal period. All records were analyzed by one observer. Portal venous perfusate was collected as 15 minute fractions in ice-chilled glass tubes and frozen at -20°C for subsequent determination of PYY level by the radioimmunoassay method described previously.²⁰

Effect of exogenous cholecystokinin-8 on colonic motility

After 30-minute basal period, CCK-8 at 12, 60 and 240 pM were administered with each dose given for 15 minutes, starting with the lowest dose which was increased in stepwise fashion, while portal venous effluent was collected and frozen for future radioimmunoassay of PYY.

Effect of exogenous peptide YY on colonic motility

After 30-minute basal period, PYY at 12, 60 and 240 pM were administered with each dose given for 15 minutes, starting with the lowest dose which was increased in stepwise fashion.

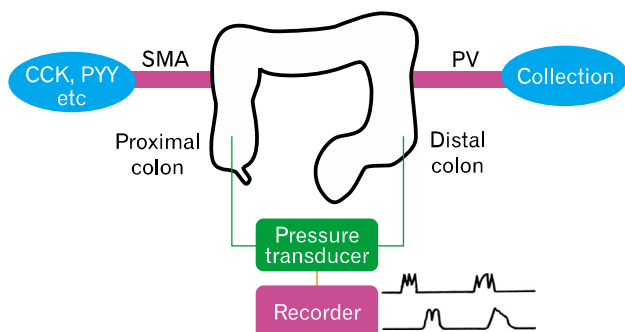


Figure 1. Schematic illustration of system used in an isolated vascularly perfused rat colon. CCK, cholecystokinin; PYY, peptide YY; SMA, superior mesenteric artery; PV, portal vein.

Effect of rabbit anti-peptide YY serum, loxiglumide and atropine on colonic motility stimulated by cholecystokinin-8

Effect of rabbit anti-peptide YY serum on colonic motility stimulated by cholecystokinin-8. The effect of CCK-8 at 240 pM was studied under the influence of a rabbit anti-PYY serum or a normal rabbit serum, given in 0.3 mL each. Portal venous perfusate was collected and frozen for radioimmunoassay of PYY.

Effect of loxiglumide on colonic motility stimulated by cholecystokinin-8. Under the influence of loxiglumide (10^{-4} M), the effect of CCK-8 at 240 pM on colonic motility was studied. Portal venous perfusate was collected and frozen for radioimmunoassay of PYY.

Effect of atropine on colonic motility stimulated by cholecystokinin-8. Under the influence of atropine (10^{-5} M), the effect of CCK-8 at 240 pM on colonic motility was studied.

Effect of atropine on colonic motility stimulated by peptide YY. Under the influence of atropine (10^{-5} M), the effect of PYY at 240 pM on colonic motility was studied.

Statistical Methods

The data was expressed as mean \pm SE. Statistical analysis was performed using the SPSS for Windows version 10 (SPSS Inc, Chicago, IL, USA). Correlation analysis was used to determine the effect of CCK or PYY on colonic motility according to concentration. Wilcoxon rank-sum test was used to evaluate the inhibition of each drugs against stimulation of CCK or PYY on colonic motility. A P -value of < 0.05 was considered statistically significant.

Results

Effect of Exogenous Cholecystokinin-8 on Colonic Motility

CCK-8 dose-dependently stimulated the colonic motility. Both in the proximal and distal colon, the percentage changes over basal motility indices in response to 3 different doses of CCK-8 (12, 60 and 240 pM) were significantly increased to $59\% \pm 20\%$ and $24\% \pm 6\%$, $67\% \pm 26\%$ and $79\% \pm 28\%$ and $112\% \pm 22\%$ and $134\% \pm 52\%$, respectively, which were statistically significant (Fig. 2, 9A and 10A). Interestingly, after CCK-8 at doses of 12, 60 and 240 pM, integrated PYY release (fmol/15 min) from portal effluent was significantly increased

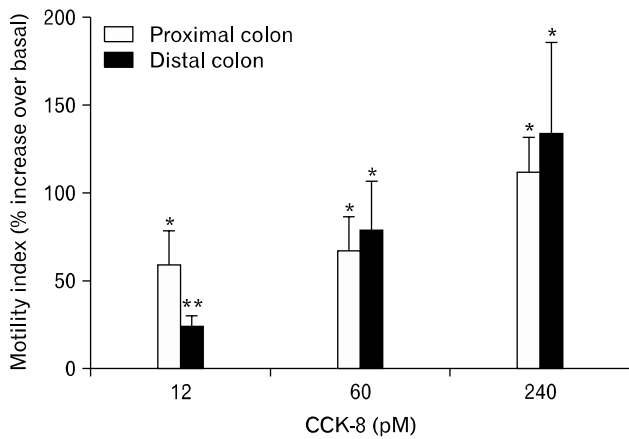


Figure 2. Effect of intraarterial infusion of cholecystinin-8 (CCK-8) on the colonic motility. Data were presented as % increases over the basal period in response to CCK-8 at doses of 12, 60 and 240 pM. Each bar represents mean \pm SE of 7 experiments. * $P < 0.05$, ** $P < 0.01$ vs basal values.

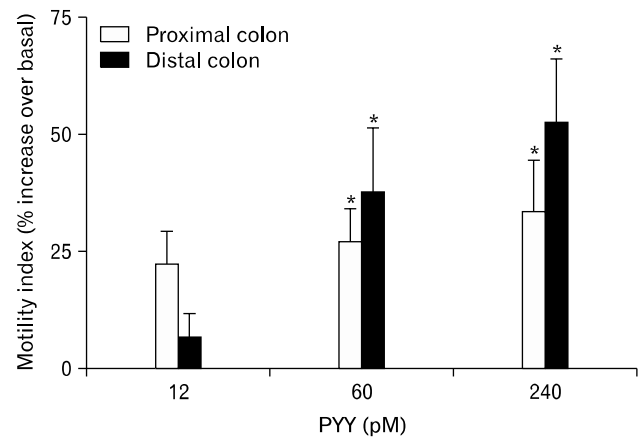


Figure 4. Effect of intraarterial infusion of peptide YY (PYY) on the colonic motility. Data were expressed as % increase over basal period in response to PYY at doses of 12, 60 and 240 pM. Each bar represents mean \pm SE of 6 experiments. * $P < 0.05$ vs basal values.

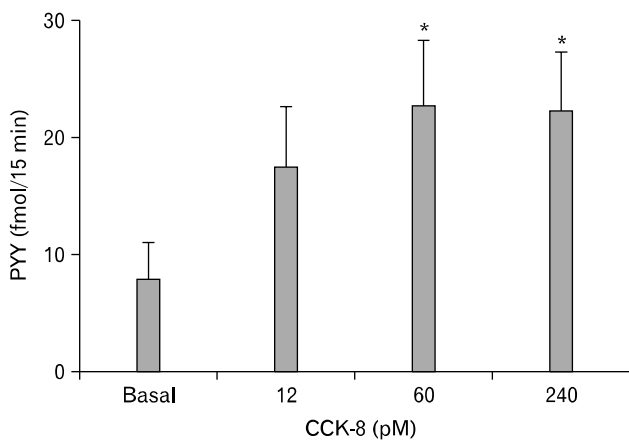


Figure 3. Integrated release of peptide YY found in the portal effluent in response to intraarterial infusion of cholecystinin-8 at doses of 0, 12, 60 and 240 pM. CCK-8, cholecystinin-8; PYY, peptide YY. Each bar represents mean \pm SE of 6 experiments. * $P < 0.05$ vs basal values.

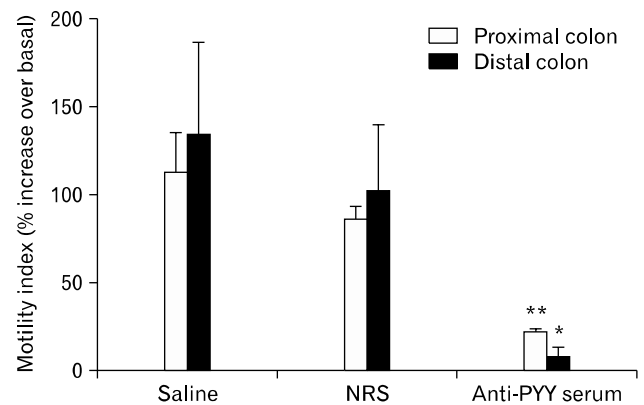


Figure 5. Effect of cholecystinin-8 (CCK-8) at 240 pM, on the colonic motility under the influence of saline, normal rabbit serum (NRS) or rabbit anti-peptide YY serum. PYY, peptide YY. Each bar represents mean \pm SE of 5-7 experiments. * $P < 0.05$, ** $P < 0.01$ vs CCK-8 + NRS.

from 8.0 ± 3.1 to 17.5 ± 5.3 , 22.8 ± 5.6 and 22.3 ± 5.1 , respectively (Fig. 3). The increase in colonic motility paralleled the increase in PYY release from the portal effluent.

Effect of Exogenous Peptide YY on Colonic Motility

In the proximal colon, the increases in the motility over basal values in response to 3 different doses of PYY (12, 60 and 240 pM) were $22\% \pm 7\%$, $27\% \pm 7\%$ and $33\% \pm 11\%$, respectively, which were statistically significant. In the distal colon, the mo-

tility index over basal values dose-dependently increased to $6\% \pm 5\%$, $37\% \pm 13\%$ and $52\% \pm 13\%$, respectively, which were also statistically significant (Fig. 4, 9B and 10B). Thus, PYY significantly and dose-dependently increased colonic motility both in the proximal and distal colon, although the magnitude of increase achieved by PYY was significantly less potent than that by CCK-8 at the doses employed.

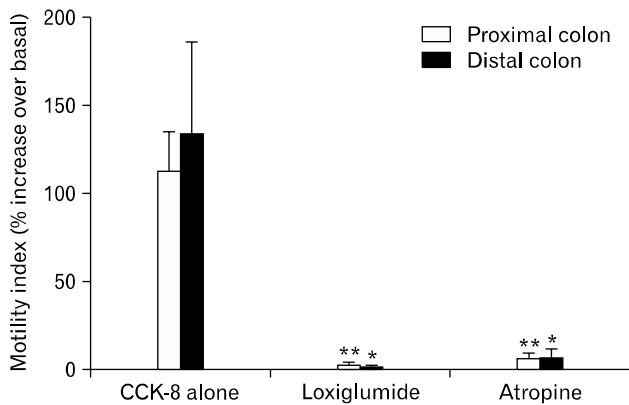


Figure 6. Effect of loxiglumide (10^{-4} M) and atropine (10^{-5} M) on cholecystokinin-8 (CCK-8, 240 pM) stimulated colonic motility. Each bar represents mean \pm SE of 7 experiments. * $P < 0.05$, ** $P < 0.01$ vs CCK-8 alone.

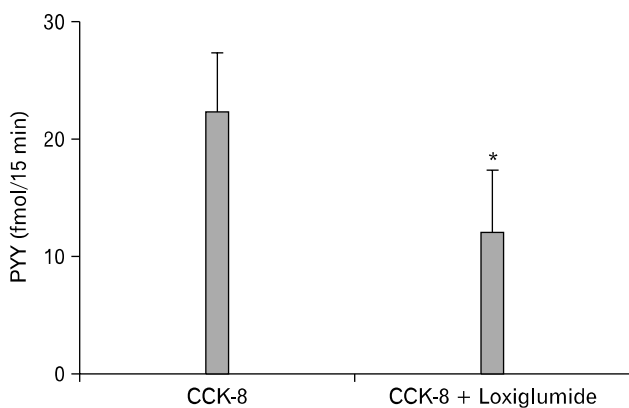


Figure 7. Effect of loxiglumide (10^{-4} M) on peptide YY release in portal effluent in response to cholecystokinin-8 (CCK-8, 240 pM) infusion. PYY, peptide YY. Each bar represents mean \pm SE of 7 experiments. * $P < 0.05$ vs CCK-8 alone.

Effect of Rabbit Anti-Peptide YY Serum, Loxiglumide and Atropine on Colonic Motility Stimulated by Cholecystokinin-8

Effect of rabbit anti-peptide YY serum on colonic motility stimulated by cholecystokinin-8

The infusion of a rabbit anti-PYY serum resulted in a profound decrease in the motility index both in the proximal and distal colon ($21.9\% \pm 1.5\%$ and $8.1\% \pm 5.0\%$, $P < 0.05$), whereas it was not influenced by a normal rabbit serum (Fig. 5, 9C, 9D, 10C and 10D). PYY antibody completely abolished the release of portal PYY stimulated by CCK-8 (data not shown).

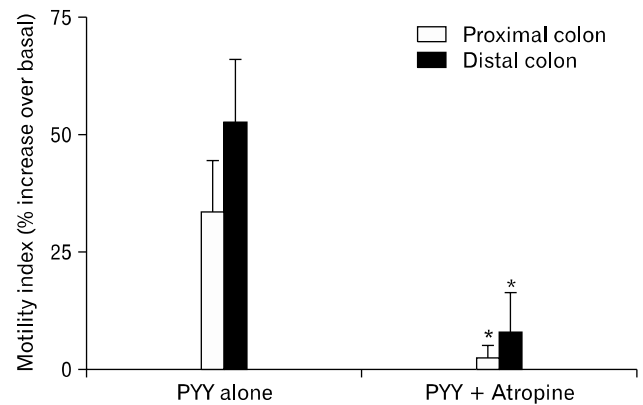


Figure 8. Effect of atropine (10^{-5} M) on peptide YY (PYY, 240 pM) stimulated colonic motility. Each bar represents mean \pm SE of 6 experiments. * $P < 0.05$ vs PYY alone.

Effect of loxiglumide on colonic motility stimulated by cholecystokinin-8

Under 10^{-4} M of loxiglumide background, the CCK action to stimulate colonic motility was almost completely abolished and the representing motility indices were $2.4\% \pm 2.1\%$ and $1.5\% \pm 1.0\%$, above basal respectively from the proximal colon and distal colon ($P < 0.05$) (Fig. 6, 9E and 10E). PYY release in portal perfusate was also decreased by loxiglumide from 22.3 ± 5.1 fmol/15 min during CCK alone to 12.0 ± 5.4 fmol/15 min after pretreatment with loxiglumide (Fig. 7).

Effect of atropine on colonic motility stimulated by cholecystokinin-8

After atropine (10^{-5} M), the CCK action to stimulate colonic motility was almost completely abolished revealing motility indices from the proximal and distal colon; $5.9\% \pm 3.8\%$ and $6.5\% \pm 5.4\%$ above basal (proximal colon, $P < 0.05$; distal colon, $P < 0.05$ vs 240 pM of CCK) (Fig. 6, 9F and 10F).

Effect of atropine on colonic motility stimulated by peptide YY

The stimulation by PYY of colonic motility was almost completely blocked by atropine in the proximal colon and distal colon ($2.6\% \pm 2.6\%$ and $8.2\% \pm 8.2\%$; $P < 0.05$) (Fig. 8, 9G and 10G).

Discussion

The gut endocrine cells are highly sensitive to ischemia and, therefore, their preserved ability to secrete hormones suggests perfused organ mobility. The isolated perfused rat colon is a use-

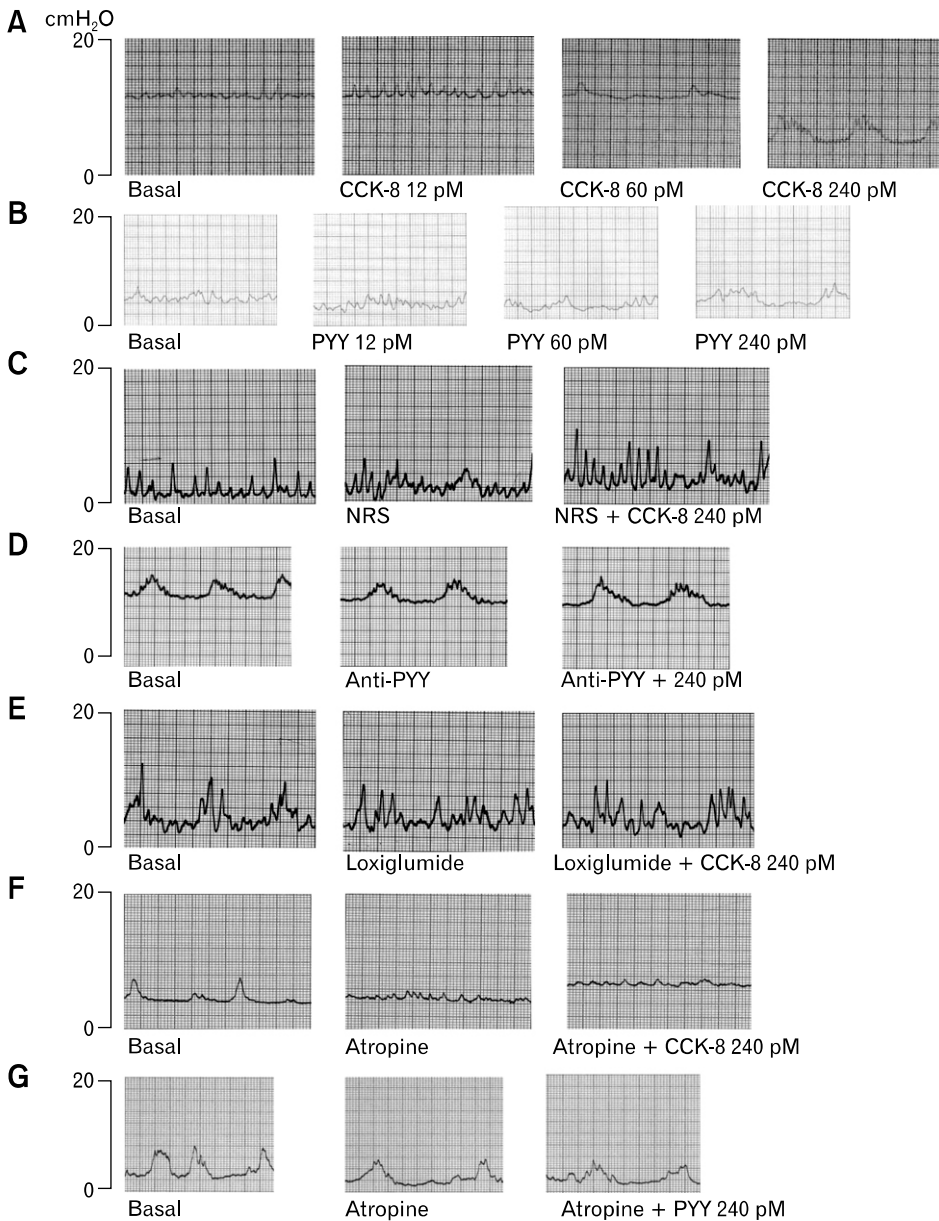


Figure 9. Representative traces response of isolated rat colon to intraarterial infusion of agents in proximal colon. Response of rat colon to intraarterial infusion of cholecystokinin-8 (CCK-8) (A) and peptide YY (PYY) (B) in proximal colon. The effect of normal rabbit serum (C), anti-PYY serum (D), loxiglumide (E) and atropine (F) on proximal colonic motility stimulated by CCK-8. The effect of atropine (G) on proximal colonic motility stimulated by PYY. NRS, normal rabbit serum.

ful tool for the investigation of colonic endocrine function and secretion.²³

CCK was first discovered in 1928 based on the ability of intestinal extracts to stimulate gallbladder contraction in dogs, and was later identified in 1966 from porcine intestine due to its recognition as a potent stimulant in pancreatic enzyme secretion.¹

CCK-8 has been reported to stimulate *in vitro* colonic muscle strips,^{4,24} and intact colon of canine²⁵ and human,^{6,7} whereas it was also reported to inhibit colonic motility in humans.^{8,9} In our study, the motor activity of isolated vascularly perfused rat colon, including both right and left colon, as measured by intraluminal

pressure changes, was stimulated by exogenous CCK-8.

Loxiglumide is a potent and specific CCK₁ receptor antagonist which blocks the CCK action on colon, gallbladder, pancreas and stomach in both experimental animals and humans.^{3,26} Although CCK₁ and CCK₂ receptors are present both in central nervous system and various peripheral organs, the CCK₂ receptor is predominantly present in central nervous system. In the intestine, as is in most regions of the gastrointestinal tract, CCK receptors on smooth muscle cells are CCK₁ type. It has long been known the gastrin and CCK₂ receptors to be similar. The CCK₁ receptor has an approximately 1,000-fold greater affinity for CCK

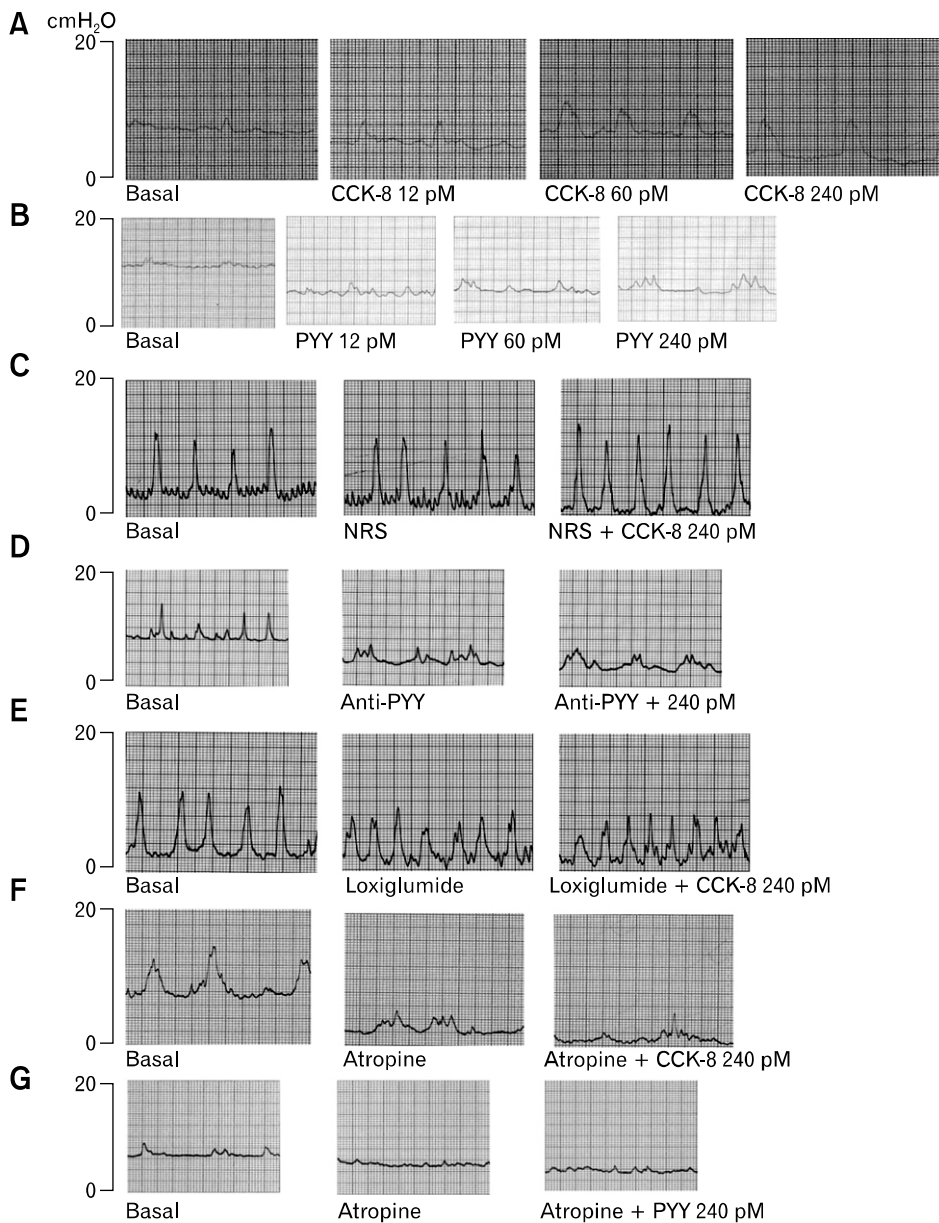


Figure 10. Representative traces response of isolated rat colon to intraarterial infusion of agents in distal colon. Response of rat colon to intraarterial infusion of cholecystokinin-8 (CCK-8) (A) and peptide YY (PYY) (B) in distal colon. The effect of normal rabbit serum (C), anti-PYY serum (D), loxiglumide (E) and atropine (F) on distal colonic motility stimulated by CCK-8. The effect of atropine (G) on distal colonic motility stimulated by PYY. NRS, normal rabbit serum.

than for gastrin, while the CCK₂ receptor has the same high affinity for both CCK and gastrin. CCK₁ receptor responds to sulfated CCK with a 1,000-fold greater potency than non-sulfated CCK. These receptors are characteristically activated by sulfated CCK much more potently than by desulfated CCK or sulfated and desulfated gastrin, and are blocked by the benzodiazepine derivative, L364,718.^{26,27} It has long been known that CCK₁ receptor antagonist is able to shorten colonic transit time.²⁸ On the other hand loxiglumide has been shown to interfere with the gastrocolonic reflex and ileal motility and is able to selectively slow colonic transit time in patients suffering from IBS.²⁹ In the pres-

ent study, the stimulating effect of CCK-8 was completely abolished by loxiglumide.

One of the study provided data regarding the distribution of CCK₁ receptors in human colon by using receptor autoradiography, and it was shown that the main target of CCK was the myenteric plexus, which predominantly occupied by CCK₁ receptors. In addition, CCK₁ receptors were distributed at a moderate-to-low density in the longitudinal muscle, while the circular muscle had no CCK₁ receptors.³⁰ These data suggest that CCK affects colonic motility by fundamentally 2 different pathways: acting on neurons in the myenteric plexus and directly on the smooth mus-

cle cells.

In the ileum of guinea pig, CCK was shown to stimulate local neuronal release of acetylcholine at the neuromuscular junction.³¹ Intraarterial CCK-8 was also shown to produce circular muscle contraction of the canine proximal colon that is mediated by stimulation of specific CCK receptors which stimulate the release of acetylcholine from cholinergic neurons.²⁵ In one study, motility patterns were compared between healthy volunteers and IBS patients with abdominal pain and frequent defecation or diarrhea. The motility index, the frequency of high-amplitude propagating complexes, and also the responses to CCK were significantly greater in this subset of IBS patients.³² The effect of CCK were profoundly inhibited by both loxiglumide and atropine.

The effect of PYY on the colonic motility depends on species. In the guinea pig and cat, PYY was shown to inhibit both small intestinal and colonic motility. In the canine, PYY stimulate small intestinal motility. In the rat, PYY inhibit small intestinal motility and stimulate colonic motility.^{13,15-17} In our study, PYY increased colonic motility in the proximal and distal colon.

PYY is not confined to distal intestinal endocrine cells in the dog but is also an enteric neuropeptide with maximal concentrations being present in the intestinal myenteric plexus.¹⁶ It was shown to stimulate circular muscle contractions of the isolated perfused canine ileum by enhancing acetylcholine release.³³ Electrical stimulation of the vagus nerve in dogs resulted in release of PYY. This data suggests that food-stimulated PYY secretion is dependent on ganglionic transmission and an atropine-blockable postganglionic parasympathetic pathway.³⁴ The action of PYY appears to heavily depend on the local release of acetylcholine. This implies that its action is mediated via the release of acetylcholine by PYY or it may require local cholinergic tone.

Postprandial PYY release is CCK dependent in dogs.³⁵ Plasma PYY increased with fat, but plasma PYY level was lower following CCK receptor antagonist. CCK serves as a foregut signal linking fat in the proximal gut with the release of distal gut PYY.³⁶ Release of PYY by fat in the proximal but not distal gut depends on an atropine-sensitive cholinergic pathway.³⁷ When proximal gut contact with fats, the PYY is released via an indirect mechanism (CCK and cholinergic pathways); whereas in distal gut, the PYY is released via a direct mechanism (exposure of PYY releasing cells to fat).³⁶ In our study, the effect of CCK-8 on increasing colonic motility is parallel with the increase in PYY release from colon. Moreover, the PYY has shown to increase colonic motility in proximal and distal colon as well, although it is not as potent as CCK-8 at the doses employed. It was of consid-

erable interest to note that the CCK-stimulated colonic motility was profoundly inhibited by both the anti-PYY serum, and loxiglumide, accompanied by complete or significant inhibition of the release of PYY stimulated by CCK. These results suggest that CCK-8 induced colonic motility is significantly mediated by PYY.

The pancreatic polypeptide (PP) family consists of 3 peptides; PP, PYY and neuropeptide Y. All 3 peptides are found both in the central nervous system and in peripheral tissue, and they show a characteristic distribution in the digestive tract.^{13,38} The antiserum used in the present study was highly specific for PYY except for a small crossreaction with neuropeptide Y (1%), and avian PP (0.01%). As described previously, radioimmunoassay for PYY that we used was specific for PYY.²⁰ It should be noted that the amount of PYY released after CCK-8 is relatively small, but effect of immunoneutralization of PYY on CCK-stimulated colonic motility was profound. This may be attributed to a possibility of PYY to exert as a paracrine or neurocrine messenger. It has been reported that enteric nervous system contains large amount of PYY in the dog.¹⁶ Since both neuropeptide Y and PP stimulate the colonic motility, it is possible that the effect of either 1 of these 2 in small quantity could have been blocked by the anti-PYY serum because of its cross reaction within the 2 peptides.

We conclude that CCK-8 may increase colonic motility via CCK₁ receptor and its action is partly mediated by PYY with the requirement for cholinergic input in increasing the motility by either PYY or CCK.

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