

# World Journal of *Gastroenterology*

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The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

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 Fax: +1-925-2238243  
 E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
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## Basic Study

**Corticotropin-releasing factor stimulates colonic motility  
via muscarinic receptors in the rat**

Kyung-Jo Kim, Ki Bae Kim, Soon Man Yoon, Joung-Ho Han, Hee Bok Chae, Seon Mee Park, Sei Jin Youn

Kyung-Jo Kim, Department of Gastroenterology, University of Ulsan College of Medicine, Asan Medical Center, Seoul 05535, South Korea

Ki Bae Kim, Soon Man Yoon, Joung-Ho Han, Hee Bok Chae, Seon Mee Park, Sei Jin Youn, Department of Internal Medicine, Chungbuk National University College of Medicine, Cheongju, Chungcheongbuk-do 28644, South Korea

**Author contributions:** Kim KJ and Youn SJ designed the research; Kim KJ, Kim KB, Yoon SM, Han JH, Chae HB and Park SM contributed substantially to the conception and design of the study, data acquisition and analysis and interpretation of the data; all the authors drafted the article and made critical revisions related to its intellectual content, and approved the final version to be published.

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**Correspondence to:** Sei Jin Youn, MD, Department of Internal Medicine, Chungbuk National University College of Medicine, 410 Seong bong-ro, Heungduk-gu, Cheongju, Chungcheongbuk-do 28644, South Korea. [sjyoun@chungbuk.ac.kr](mailto:sjyoun@chungbuk.ac.kr)

Telephone: +82-43-2696357

Fax: +82-43-2733252

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**Abstract****AIM**

To measure exogenous corticotropin-releasing factor (CRF)-induced motility of the isolated rat colon and to demonstrate the effect of pharmacologic inhibition on CRF-induced motility.

**METHODS**

The isolated vascularly-perfused rat colon was used. Luminal pressure was monitored *via* microtip catheter pressure transducers in the proximal and distal colon. At first, exogenous CRF was administered in a stepwise manner and the concentration of CRF yielding maximal colonic motility was selected. After recording basal colonic motility, hexamethonium, phentolamine, propranolol, atropine and tetrodotoxin were infused into the isolated colon. Initially, only the test drug was infused; then, CRF was added. The motility index was expressed as percentage change over basal level.

**RESULTS**

Administration of 1.4, 14.4, 144 and 288 pmol/L CRF progressively increased colonic motility in the proximal and distal colon. Infusion of atropine or tetrodotoxin reduced CRF-induced motility of both the proximal and distal colon, whereas hexamethonium, phentolamine and propranolol had no effect.

## CONCLUSION

CRF-induced colonic motility appears to be mediated by local cholinergic signaling *via* muscarinic receptors. Muscarinic receptors are potential targets for counteracting CRF-induced colonic hypermotility.

**Key words:** Gastrointestinal motility; Rats; Stress; Colon; Corticotropin-releasing factor

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**Core tip:** Corticotropin-releasing factor (CRF) has emerged as a key mediator of functional bowel disorders and the effects of stress and inflammation on the gastrointestinal tract. CRF-induced colonic motility is mediated by local cholinergic signaling *via* muscarinic receptors, and blocking muscarinic receptors is a potential way to prevent CRF-induced hypermotility of the colon.

Kim KJ, Kim KB, Yoon SM, Han JH, Chae HB, Park SM, Youn SJ. Corticotropin-releasing factor stimulates colonic motility *via* muscarinic receptors in the rat. *World J Gastroenterol* 2017; 23(21): 3825-3831 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i21/3825.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i21.3825>

## INTRODUCTION

Corticotropin-releasing factor (CRF), a 41-amino acid peptide of hypothalamic origin<sup>[1]</sup>, was initially identified as the main activator of the pituitary adrenal axis in stress<sup>[2]</sup>. It also plays a key role in endocrine, behavioral (anxiety/depression), autonomic (sympathetic activation) and immune responses to stress in the brain<sup>[3,4]</sup>.

In addition to the established role of CRF in the brain, both peripheral and central administration of CRF cause stress-like gastrointestinal (GI) motor responses, including stress-induced inhibition of gastric emptying and stimulation of colonic motor function<sup>[5]</sup>. Accordingly, CRF has emerged as a key mediator of functional bowel disorders and of the effects of stress and inflammation on the GI tract<sup>[6,7]</sup>.

Maillot *et al*<sup>[5]</sup> reported that peripheral administration of CRF stimulated colonic motility *via* peripheral CRF receptors, and this effect was antagonized by peripheral injection of CRF antagonists. There was also earlier evidence that CRF activated the colonic motility of isolated rat colons when it was perfused directly into the bath<sup>[8]</sup>. In contrast, Tsukamoto *et al*<sup>[9]</sup> suggest that peripheral administration of CRF activates the dorsal nucleus of vagi *via* central CRF receptors, resulting in stimulation of the vagal efferent and cholinergic transmission to the proximal colon.

We conducted the present study to measure the

concentration dependence of CRF-induced motility and to assess the influence of pharmacologic inhibitors on CRF-induced motility using the isolated vascularly perfused rat colon model.

## MATERIALS AND METHODS

### *Animals and ethical statement*

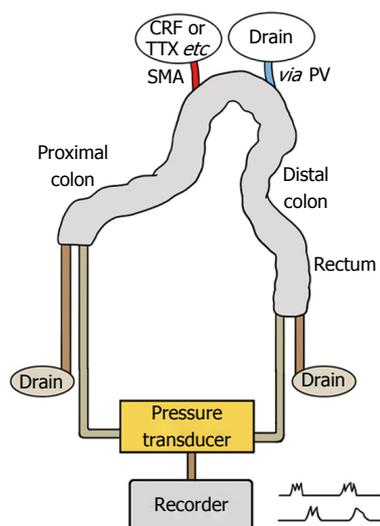
All experiments were approved by the Animal Care Committee of Chungbuk National University. Fifty male Sprague-Dawley rats, weighing between 250 g and 300 g, were starved and given free access to tap water for 48 h to clean out their bowels before surgery. The surgical procedures were similar to those described by Cuber *et al*<sup>[10,11]</sup> for isolation and vascular perfusion of the rat duodenojejunum<sup>[10]</sup> and ileum<sup>[11]</sup>. Anesthesia was introduced by intraperitoneal injection of xylazine 10 mg/kg and zolazepam 50 mg/kg, and the abdomen was opened *via* midline incision. The stomach, spleen and small intestine were removed after ligating the blood vessels supplying the area. The whole colon together with the superior mesenteric artery (SMA) and portal vein (PV) was freed of its visceral and retroperitoneal fixations and dissected. The rectum was excised at the level of the pelvic brim and the point at which the retroperitoneum reflects. The resected colon along with SMA and PV was transferred to wet gauze on a Petri dish at 37 °C. The cecum was removed. Two cm on the anal side of the proximal resected margin was defined as the proximal colon, and 2 cm on the oral side of the distal resected margin as the distal colon. A polyethylene cannula [0.58 mm in inner diameter (ID), 0.96 mm in outer diameter (OD)] was inserted into the SMA and another (0.58 mm in ID, 0.96 mm in OD) into the PV, and secured. Krebs solution containing 0.1% bovine serum albumin and 3% dextran was given immediately at a rate of 1.2 mL/min through the SMA. The solution was continuously gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and warmed at 37 °C.

A rubber cannula (5 mm ID, 6.5 mm OD) was then inserted and secured at both ends of the colon to drain the luminal secretion. The loop was gently flushed out once or twice with 10 mL prewarmed 0.15 mol/L NaCl. Figure 1 shows a schematic view of a rat colon comprising the proximal and the distal colon after removing the cecum and rectum.

### *Pharmacological intervention studies*

**Effects of exogenous CRF infusion on colonic motility:** Following a 30 min basal period, CRF (Sigma Chemical Co., St. Louis, MO, United States) was administered at doses of 1.4, 14.4, 144 and 288 pmol/L every 15 min to 6 male Sprague-Dawley rats.

**Effects of drugs on CRF-induced colonic motility:** To analyze the effects of CRF (used at a concentration



**Figure 1** Schematic illustration of the isolated vascularly-perfused rat colon. The isolated whole rat colon was placed in a temperature-controlled water bath and vascularly perfused with Krebs solution via the superior mesenteric artery. Luminal pressure was monitored via microtip catheter pressure transducers at the proximal and distal ends of the colon. Pressure changes were recorded with a data acquisition system. AT: Atropine sulfate; CRF: Corticotropin-releasing factor; PV: Portal vein; SMA: Superior mesenteric artery; TTX: Tetrodotoxin.

of 144 pmol/L in the distal colon and 288 pmol/L in the proximal colon) on colonic motility,  $10^{-5}$  mol/L phentolamine mesylate (Ciba, Basel, Switzerland),  $10^{-5}$  mol/L propranolol HCl (AstraZeneca, Cambridge, United Kingdom), hexamethonium bromide (Sigma Chemical Co.),  $10^{-6}$  mol/L tetrodotoxin (Tocris Cookson Inc., Ballwin, MO, United States) and  $10^{-5}$  mol/L atropine (atropine sulfate; Jeil Pharmacy, Suncheon, South Korea) were each given to 5 or 6 male Sprague-Dawley rats.

Arterial perfusion was commenced at a rate of 1.2 mL/min (72 mL/h) and each test drug was infused at a rate of 2 mL/h through a side channel, as described previously by Plaisancié *et al.*<sup>[12]</sup> and Mancinelli *et al.*<sup>[8]</sup> with some modifications. The effect of each test drug was examined as follows. First, baseline colonic motility was measured for 30 min; then, each test drug was infused separately for 15 min at a rate of 2 mL/h followed by 144 pmol/L CRF for the distal colon or 288 pmol/L CRF for the proximal colon, these being the concentrations of CRF with maximal effects on colonic motility.

#### Measurement of colonic motility and data analysis

Microtip catheter pressure transducers of 2 mm diameter (Millar Inc., Houston, TX, United States) were calibrated in accordance with the manufacturer's instructions. Briefly, to calibrate a pressure transducer in a channel, recording was started and two known pressures were applied to the transducer to obtain two voltages. Then, recording was stopped and the regions of the two readings were selected and Unit Conversions were chosen from the Channel Function pop-up

menu for the channel. Using the 2 Point Calibration mode, the area in the waveform corresponding to one pressure was selected and a value button was used to enter its value, with the known pressure entered into the box beside it on the right. This process was repeated for the other pressure, with the data entered in the unused row.

Microtip catheter pressure transducers (2 mm diameter; Millar Inc.) were placed separately 2.0 cm apart through the proximal and distal ends of the colon. Intraluminal pressure was continuously monitored with a microtip catheter pressure transducer and recorded with a data acquisition system (ML846 Powerlab 4/25; AD Instruments Inc., Victoria, Australia).

Motility indices (MIs) were automatically calculated with the Medical Measurement Systems computer program (ML846 Powerlab 4/25) by multiplying the amplitude of each contractile wave by its recorded duration for the last 5 min of each 15 min interval, and were expressed as percentage changes over basal level. All records were analyzed by the same observer.

#### Statistical analysis

Statistical analysis was performed using SPSS for Windows, Version 18 (SPSS Inc., Chicago, IL, United States). Data are presented as mean  $\pm$  SE. Kendall's rank correlation coefficient was used to measure the association between drug concentration and motility response. Because of the small sample size, continuous variables, such as "CRF" and "CRF and atropine", were compared by the Mann-Whitney *U* test. A *P* value of  $< 0.05$  was considered statistically significant.

## RESULTS

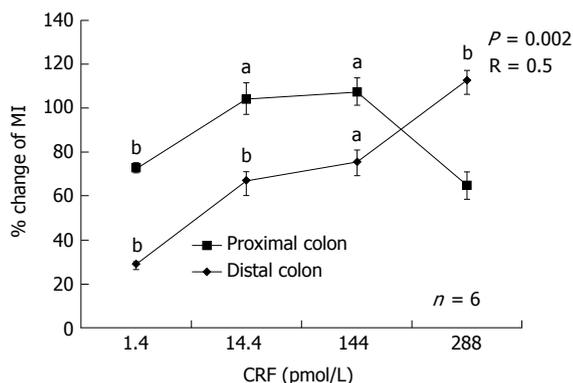
#### Mechanical response produced by exogenous CRF

Exogenous infusion of CRF increased colonic motility significantly ( $P < 0.05$ ) at concentrations of 1.4, 14.4, 144 and 288 pmol/L in the proximal colon ( $28.3\% \pm 5.5\%$ ,  $66.9\% \pm 18.2\%$ ,  $75.4\% \pm 27.1\%$  and  $112.5\% \pm 22.0\%$ , respectively) and in the distal colon ( $72.6\% \pm 10.6\%$ ,  $104.1\% \pm 34.7\%$ ,  $107.4\% \pm 31.4\%$  and  $64.7\% \pm 31.1\%$ , respectively) The mechanical responses induced by infusion of CRF tended to be stronger in the distal colon than in the proximal colon, although the difference was not statistically significant. A maximal response (a 112.5% increase in MI) was observed at 288 pmol/L CRF in the proximal colon, whereas a maximal response (107.4% increase) was observed in the distal colon at 144 pmol/L.

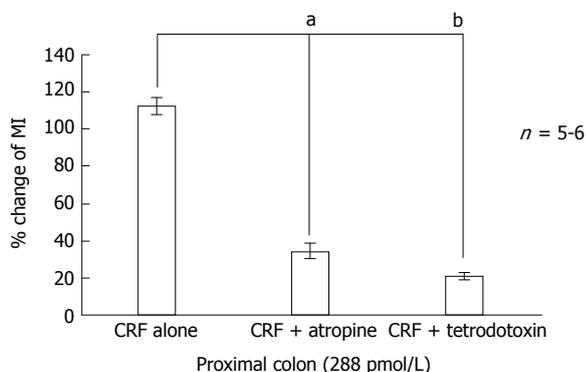
A concentration-response curve for the contractile effects of CRF was observed in the proximal colon only (Figure 2). The correlation coefficient was 0.5 ( $P = 0.002$ ).

#### Effect of tetrodotoxin on CRF-induced motility

The administration of tetrodotoxin ( $10^{-6}$  mol/L) markedly inhibited CRF-induced colonic motility. MIs



**Figure 2** Concentration-response curves for corticotropin-releasing factor-induced contractility in isolated vascularly perfused rat colons. CRF increased both proximal and distal colonic motility. Concentration-motility was observed only in the proximal colon. Data are expressed as percent change of MI over basal. Each data point represents the mean of 6 experiments. <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01. CRF: Corticotropin-releasing factor; MI: Motility index; R: Correlation coefficient.



**Figure 3** Effects of atropine and tetrodotoxin on corticotropin-releasing factor-induced colonic motility in the proximal colon. Atropine ( $10^{-5}$  mol/L) reduced MI from  $112.5\% \pm 22.0\%$  to  $34.3\% \pm 17.8\%$  (*P* < 0.05). Administration of tetrodotoxin ( $10^{-6}$  mol/L) reduced MI from  $112.5\% \pm 22.0\%$  to  $21.1\% \pm 9.8\%$  (*P* < 0.01). <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01. CRF: Corticotropin-releasing factor; MI: Motility index.

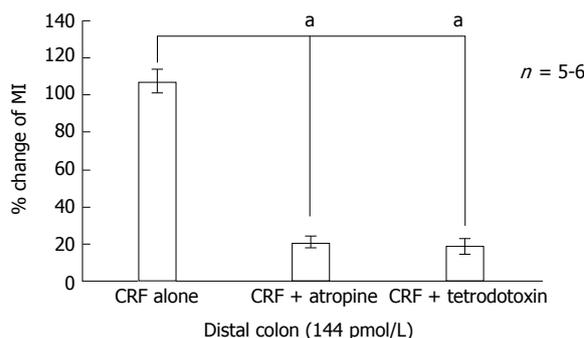
decreased from  $112.5\% \pm 22.0\%$  in the proximal colon and from  $107.3\% \pm 31.4\%$  in the distal colon to  $21.08\% \pm 9.8\%$  (*P* < 0.05) and  $18.62\% \pm 18.6\%$  (*P* < 0.01), respectively (Figures 3, 4, 5E and 6E).

**Effect of atropine on CRF-induced motility**

Infusion of atropine ( $10^{-5}$  mol/L) on its own did not alter basal MI but it markedly inhibited CRF-induced colonic motility. MIs decreased from  $112.5\% \pm 22.0\%$  in the proximal colon and  $107.3\% \pm 31.4\%$  in the distal colon to  $34.3\% \pm 17.8\%$  (*P* < 0.05) and  $20.5\% \pm 15.2\%$  (*P* < 0.01), respectively (Figures 3, 4, 5F and 6F).

**Effects of phentolamine, propranolol or hexamethonium on CRF-induced motility**

Since maximal responses were observed in the proximal colon at 288 pmol/L CRF and in the distal colon at 144 pmol/L, we selected these concentrations



**Figure 4** Effects of atropine and tetrodotoxin on corticotropin-releasing factor-induced colonic motility in the distal colon. Atropine ( $10^{-5}$  mol/L) reduced MI from  $112.5\% \pm 22.0\%$  to  $20.5\% \pm 15.2\%$  (*P* < 0.05). Tetrodotoxin ( $10^{-6}$  mol/L) reduced MI from  $112.5\% \pm 22.0\%$  to  $18.6\% \pm 18.6\%$  (*P* < 0.05). <sup>a</sup>*P* < 0.05. CRF: Corticotropin-releasing factor; MI: Motility index.

for assessing the effects of phentolamine, propranolol or hexamethonium on CRF-induced motility.

As shown in Figures 5B and 6B, phentolamine ( $10^{-5}$  mol/L), a reversible nonselective alpha-adrenergic antagonist, had no significant effect on colonic motility when 288 pmol/L CRF was applied to the proximal colon. Infusion of propranolol ( $10^{-5}$  mol/L), a nonselective beta-adrenergic antagonist, tended to reduce CRF-stimulated colonic motility (from a MI value of  $112.5\% \pm 22.0\%$  to  $65.9\% \pm 24.9\%$  in the proximal colon, and from  $107.3\% \pm 31.4\%$  to  $34.9\% \pm 15.2\%$  in the distal colon), although this effect was not statistically significant (Figures 5C and 6C). Furthermore, infusion of hexamethonium ( $10^{-3}$  mol/L), a nicotinic receptor antagonist, had no effect on CRF-induced colonic motility (Figures 5D and 6D).

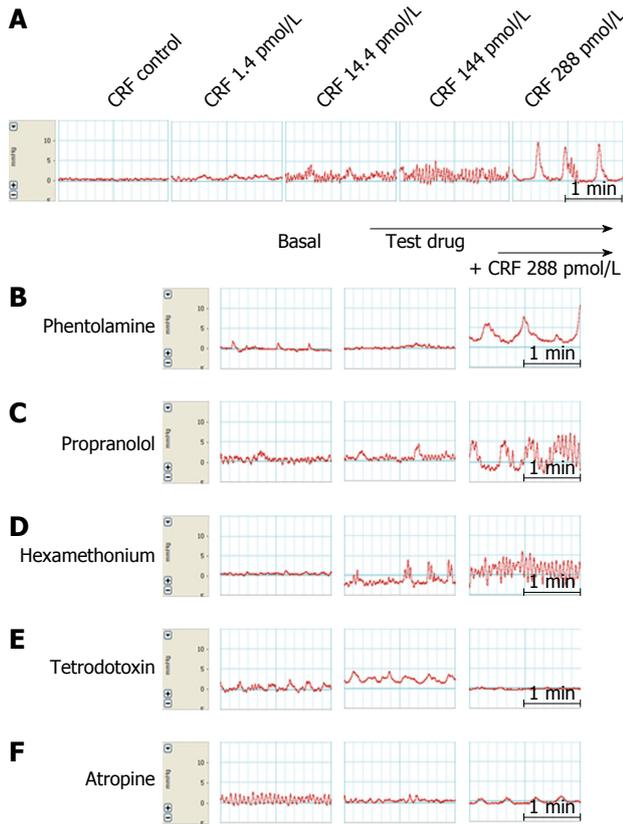
**DISCUSSION**

In the present study, administration of CRF *via* the superior mesenteric artery increased colonic motility in both the proximal and distal colon, and we obtained evidence that this effect of CRF was mediated by local cholinergic signaling *via* muscarinic receptors.

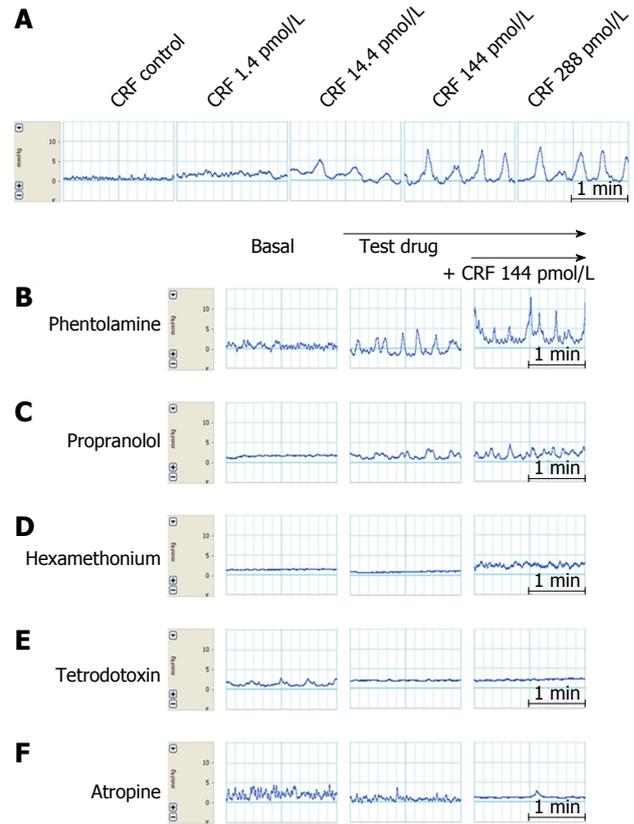
CRF stimulates the dorsal motor nucleus of the vagus nerve both *in vivo* and *in vitro*<sup>[13]</sup>, and may have a direct effect on the enteric nervous system by binding cholinergic and nitrenergic myenteric and submucosal neurons expressing CRF<sub>1</sub> receptors<sup>[3,14,15]</sup>. Maillot *et al*<sup>[5]</sup> have reported that vascularly-perfused CRF stimulated colonic myoelectric activity and peristalsis in a similar isolated rat colon model<sup>[8]</sup>.

For our experiments, we used the isolated rat colon, which may be an optimum model for investigating colonic motility<sup>[12,16]</sup>. Although the isolated bowel is free from control by the autonomic nervous system, the enteric nervous system and regulatory systems (endocrine, paracrine) remain functional.

The primary outcome of this study was the MI at each concentration of CRF. CRF increased the MI and tended to exert a more potent influence on the distal



**Figure 5** Representative tracings of the effects on proximal colonic motility of corticotropin-releasing factor alone (A), and of phentolamine (B), propranolol (C), hexamethonium (D), tetrodotoxin (E) and atropine (F). Atropine and tetrodotoxin significantly inhibited CRF-induced colonic motility. CRF: Corticotropin-releasing factor; TTX: Tetrodotoxin.



**Figure 6** Representative tracings of the effects on distal colonic motility of corticotropin-releasing factor alone (A) and phentolamine (B), propranolol (C), hexamethonium (D), tetrodotoxin (E) and atropine (F). Atropine and tetrodotoxin significantly inhibited CRF-induced colonic motility. CRF: Corticotropin-releasing factor; TTX: Tetrodotoxin.

colon than the proximal colon, although the difference was not statistically significant. It is possible that the number of contractions on the tracings affected the values of MI obtained. However, MI was automatically measured with the Medical Measurement Systems computer program and expressed as percentage change over basal levels.

Although the mechanical responses induced by infusion of CRF tended to be more potent in the distal colon in this study, a concentration-dependent MI increase was only observed in the proximal colon, and the correlation coefficient R was quite low. Since it is known that the CRF gene is more highly expressed in the distal rat colon<sup>[17]</sup>, a more vigorous response might have been expected in the distal colon. However, in our experiments, the difference in mechanical response to CRF between the distal and proximal colon was not significant, perhaps because of the small number of mice. Another explanation for the absence of a difference and the modest correlation between dose and response may have been a decline in CRF action at the highest doses in the distal colon due to an inhibitory effect of the CRF-R2 receptor<sup>[18]</sup>.

Fast rhythmic activities at around 15-20 per min in our experiments were recorded in tracings of both the proximal and distal colon before administration of the

test drugs. These activities seemed not to be influenced by phentolamine, propranolol, hexamethonium, atropine or tetrodotoxin (Figures 5 and 6). Therefore, we speculate that they may originate in the stellate interstitial cells of Cajal near the myenteric border<sup>[19]</sup>.

Hexamethonium, a nicotinic receptor antagonist<sup>[20]</sup>, had no effect on either basal colonic motility or CRF-induced motility. Atropine, a non-selective muscarinic receptor antagonist, is known to suppress colonic contractions. A specific antagonistic effect of atropine on CRF-induced motility in our experiments is shown by the fact that it suppressed CRF-induced hypermotility but did not alter basal motility. Taken together, our findings indicate that CRF does not act on nicotinic receptors in the myenteric ganglia but on muscarinic receptors.

Tetrodotoxin, a voltage-gated sodium channel blocker, abolished CRF-induced colonic hypermotility in both the proximal and distal colon<sup>[21]</sup>. Overall, we found that CRF action was inhibited by atropine and tetrodotoxin, but not by hexamethonium, phentolamine or propranolol. We conclude that CRF action on the colon requires local cholinergic input *via* the muscarinic receptors of intrinsic cholinergic neurons in the enteric nervous system.

Although the role of CRF in colonic motility is well-established, in this study we used a pharmacological

approach to assess the mechanisms of peripheral CRF-induced activation of colonic motility in rats using isolated colonic segments. Our data may help to further elucidate the role of CRF in gastrointestinal physiology and promote a novel approach to developing therapeutics for stress-related disorders of the gut.

However, our experiments have some noteworthy limitations and there are issues that remain to be addressed. Thus, we did not determine which of the CRF receptor subtypes was involved in the effects of CRF on the isolated colon. In addition, we did not show whether the increased MI translates into propulsive motility or not, and whether the contractile effects of CRF were propagative or not.

In conclusion, CRF stimulates colonic motility *via* peripheral CRF receptors, and CRF effects on the colon require local cholinergic signaling *via* muscarinic receptors. Although intrinsic cholinergic neurons in the enteric nervous system may contribute to this cholinergic input, further study is required to establish whether the cholinergic input is from the parasympathetic nerves innervating the colon or from intrinsic cholinergic neurons in the enteric nervous system.

## ACKNOWLEDGMENTS

All procedures performed in the studies involving animals were carried out in accordance with the ethical standards of the institution or practice at which the studies were conducted. This study was presented as an oral presentation at the 18<sup>th</sup> United European Gastroenterology Week, October 2010, in Barcelona, Spain.

## COMMENTS

### Background

Both peripheral and central administration of corticotropin-releasing factor (CRF) cause stress-like gastrointestinal motor responses, including stress-induced inhibition of gastric emptying and stimulation of colonic motor function.

### Research frontiers

There is no pharmacologic intervention study to reduce the action of CRF.

### Innovations and breakthroughs

The authors conducted the present study to measure exogenous CRF-induced motility according to the concentration and to demonstrate the influence of the pharmacologic inhibition on CRF-induced motility using an isolated vascularly perfused rat colon model.

### Applications

CRF stimulates colonic motility *via* peripheral CRF receptors, and CRF effects on the colon require local cholinergic signaling *via* muscarinic receptors.

### Terminology

A motility index (MI) was calculated with the Medical Measurement Systems computer program (ML846 Powerlab 4/25; AD Instruments Inc., Victoria, Australia) automatically by multiplying the amplitude of each contractile wave by the recorded duration for the last 5 min of each 15 min interval, and was

expressed as percentage change over basal levels.

## Peer-review

The theme of this paper is interesting and this is a very well-written manuscript and well-done basic research study. Please clarify and explore differences between the proximal and the distal colon. Some differences between distal and proximal colon may not have been significant because of number. Please clarify that. The R, although significant, is low, indicating only a moderate correlation.

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