ORIGINAL ARTICLE

혈류가 유지된 채 분리된 흰 쥐 결장에 대한 Urocortin 1의 영향

유일영, 이승호, 김기배, 이희승, 장종순, 연명호, 한정호, 윤순만, 채희복, 박선미, 윤세진

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The Effect of Urocortin 1 on Motility in Isolated, Vascularly Perfused Rat Colon

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Background/Aims: Urocortin 1, a corticotropin-releasing factor related peptide, increases colonic motility under stressful conditions. We investigated the effect of urocortin 1 on colonic motility using an experimental model with isolated rat colon in which the blood flow and intestinal nerves were preserved. Furthermore, we assessed whether this effect was mediated by adrenergic or cholinergic nerves.
Methods: Colonic motility was measured in the proximal and distal parts of resected rat colon. The colon resected from the peritoneum was stabilized, and then urocortin 1 (13.8, 138, 277, and 1,388 pM) was administered via a blood vessel. Motility index was measured in the last 5 min of the 15 min administration of urocortin 1 and expressed as percentage change from baseline. Subsequently, the change in motility was measured by perfusing urocortin 1 in colons pretreated with phentolamine, propranolol, hexamethonium, atropine, or tetrodotoxin.
Results: At concentrations of 13.8, 138, 277, and 1,388 pM, urocortin 1 increased the motility of proximal colon (20.4±7.2%, 48.4±20.9%, 67.0±25.8%, and 64.2±20.9%, respectively) and the motility of distal colon (3.3±3.3%, 7.8±7.8%, 71.1±28.6%, and 87.4±32.5%, respectively). The motility induced by urocortin 1 was significantly decreased by atropine to 2.4±2.4% in proximal colon and 3.4±3.4% in distal colon ($p < 0.05$). However, tetrodotoxin, propranolol, phentolamine, and hexamethonium did not inhibit motility.
Conclusions: Urocortin 1 increased colonic motility and it is considered that this effect was directly mediated by local muscarinic cholinergic receptors. **(Korean J Gastroenterol 2015;65:283-290)**

Key Words: Urocortins; Corticotropin-releasing factor; Rats; Cholinergic receptors; Muscarinic receptors

INTRODUCTION

It has been reported that in mammals, corticotropin-releasing factor (CRF) increases the secretion of adrenocorticotropic hormone by stimulating the pituitary-adrenal axis under stressful conditions, and this effect is mediated by the CRF receptor.[1,2] Since then, urocortin (Ucn), another ligand of the CRF receptor, has been identified. Another subtype of Ucn was subsequently identified, Ucn 1 was originally identified as Ucn, Ucn 2 and Ucn 3 were later identified on the basis of their different affinities for the CRF receptor.[3,4] The Ucns bind to CRF 1 receptor or CRF 2 receptor; different Ucns bind to different CRF receptors, having different binding affinities and actions.[5,6]

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Ucn 1 is a peptide consisting of 40 amino acids. It activates a signaling pathway by binding to CRF 1 receptor or CRF 2 receptor in the same manner as CRF but with a higher affinity.[7] CRF and Ucn 1 have been observed prominently in the central nervous system (CNS), but they are also found in peripheral tissues. Their role in the heart, skin, and immune system has been identified, but not much is known of their role in the gastrointestinal tract.[8-12]

Studies have reported that when centrally administered, Ucn 1 inhibited gastric emptying and stimulated colonic propulsive motor function.[13-16] Other studies have reported that when peripherally injected, Ucn 1 exerted effects that were similar to those observed when it was administered centrally.[17,18] However, the exact mechanism by which Ucn 1 acts when it is peripherally administered remains unclear. It is known that gastrointestinal motility is regulated by three factors (myogenic, neural, and hormonal factors). Of the three factors, neural regulation is mediated by a complex entanglement of the myenteric plexus and the autonomic nervous system. Various peptides have been identified as gastrointestinal neurotransmitters. It is known that the endogenous and autonomic nervous systems affect the secretion and inhibition of these peptides.

Kimura et al.[19] investigated the action of peripherally injected Ucn 1 in colon muscle strips. Whether the results of those experiments can be reproduced *in vivo* remains uncertain. This is because the myenteric nerves and many parts of the autonomic nervous system lying next to the intestinal tract are lost in the colonic muscle strips. To identify the complex mechanism of gastrointestinal motility, an experimental model that includes the sympathetic, parasympathetic, and enteric nervous systems, as well as various neurotransmitters secreted from these nervous systems, and the neurotransmitter receptors are required. The purpose of this study was to investigate the change in colonic motility induced by Ucn 1 in the proximal and distal colon resected from rats, using an experimental model where the blood flow and intestinal nerves of the colon were preserved and extrinsic neural and hormonal effects were excluded. In addition, this study also aimed to investigate the mechanism of Ucn 1 by observing the change in colonic motility under conditions where the adrenergic and cholinergic nerves and nerve conduction were blocked.

SUBJECTS AND METHODS

1. Animal preparation

All experiments described in this report were approved by the Animal Care Committee of Chungbuk National University. Male Sprague-Dawley rats weighing between 250 and 300 g were fasted for 48 h with free access to tap water before surgery. The operative procedure was similar to that previously described by Cuber et al.[20,21] for the isolated, vascularly perfused duodenojejunum and ileum of rat. 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 (inner diameter 0.58 mm, outer diameter 0.96 mm) was then inserted into the superior mesenteric artery. Arterial perfusion was started immediately at a rate of 1.2 mL/min with Krebs solution containing 0.1% bovine serum albumin (Amresco, Solon, OH, USA) and 3% dextran (Sigma Chemical Co., St. Louis, MO, USA). The mixture was continuously gassed with 95% O_2-5% CO_2 and warmed at 37°C. A rubber cannula (inner diameter 5.0 mm, outer diameter 6.5 mm) was inserted into both ends of the proximal and distal colon to drain the luminal secretion. The loop was then gently flushed out once or twice with 10 mL prewarmed 0.15 M NaCl. Microtip catheter pressure transducers (2 mm diameter; Millar Instruments Inc., Houston, TX, USA) were placed 2.0 cm from the ends of both the proximal and distal colon (Fig. 1).

2. Experimental protocols

Intraluminal pressure was continuously monitored using microtip catheter pressure transducers and recorded with a data acquisition system (ML846 PowerLab 4/25; AD Instruments, Sydney, Australia). The motility index (MI) was calculated by multiplying the amplitude by the duration of each contractile wave during the last 5 min in each 15 min interval and expressed as percentage changes over the basal period. All records were analyzed by one observer.

1) Effect of exogenous Ucn 1 on colonic motility

After a 30 min basal period, Ucn 1 was administered at dose of 13.8, 138, 277, and 1,388 pM, infused consecutively
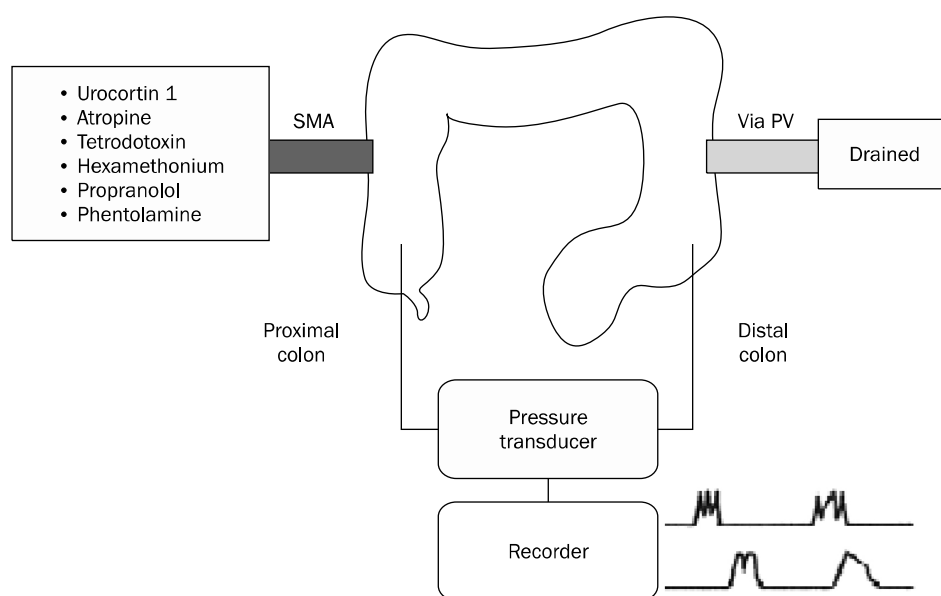
Fig. 1. Schematic illustration of the system used in the isolated vascularly perfused rat colon.
SMA, superior mesenteric artery; PV, portal vein.

every 15 min in 5 rats.

2) The effect of adrenergic and, cholinergic receptors, and nerve conduction blockade on Ucn 1-stimulated colonic motility

Drugs including Ucn 1 were simultaneously infused for 15 min in 5 rats. MI was measured for the last 5 min, when the drug effect reached its peak. The Ucn 1 concentration that caused the greatest colon contraction were used in the proximal and distal colon.

(1) Effect of phentolamine on Ucn 1-stimulated colonic motility

Phentolamine is a reversible non-selective alpha-adrenergic antagonist. After infusion with phentolamine mesylate (10^{-5} M; Reyon Pharm Co., Ltd., Seoul, Korea), the effect on colonic motility of Ucn 1 at 277 pM in the proximal colon and at 1,388 pM in the distal colon was measured.

(2) Effect of propranolol on Ucn 1-stimulated colonic motility

Propranolol is a non-selective sympatholytic beta-adrenergic antagonist. After infusion with propranolol HCl (10^{-5} M; Union Korea Pharm Co., Ltd., Seoul, Korea), the effect on colonic motility of Ucn 1 at 277 pM in the proximal colon and at 1,388 pM in the distal colon was measured.

(3) Effect of hexamethonium on Ucn 1-stimulated colonic motility

Hexamethonium is a ganglionic receptor antagonist that acts in the autonomic ganglia by binding mostly to nicotinic acetylcholine receptors, and not the acetylcholine-binding site itself. After infusion with hexamethonium bromide (10^{-3} M; Sigma Chemical Co.), the effect on colonic motility Ucn1 at 277 pM in the proximal colon and at 1,388 pM in the distal colon was measured.

(4) Effect of atropine on Ucn 1-stimulated colonic motility

Atropine is a competitive antagonist of muscarinic acetylcholine receptors. After infusion with atropine sulfate (10^{-5} M; Jeil Pharmacy, Seoul, Korea), the effect on colonic motility of Ucn 1 at 277 pM in the proximal colon and at 1,388 pM in the distal colon was measured.

(5) Effect of tetrodotoxin on Ucn 1-stimulated colonic motility

Tetrodotoxin (TTX) blocks the action potentials in nerves by binding to voltage-gated, fast sodium channels in the nerve cell membranes, essentially preventing affected nerve cells from firing by blocking the channels involved in the process. After infusion with TTX (10^{-6} M; Tocris Cookson Inc., Ballwin, MO, USA), the effect on colonic motility of Ucn 1 at 277 pM in the proximal colon and at 1,388 pM in the distal colon was measured.

3. Statistical methods

Statistical analysis was performed using IBM SPSS Statistics for Windows version 21.0 (IBM Co., Armonk, NY, USA). The data were presented as mean±standard error. The Wilcoxon signed rank test was performed to compare the differences in Ucn 1-stimulated motility in the proximal and dis-

tal colon and to test whether Ucn 1-stimulated motility was inhibited or not. The statistical significance level was set at $p < 0.05$.

RESULTS

1. Effect of exogenous Ucn1 on colonic motility

When injected into an artery at the increasing concentrations of 13.8, 138, 277, and 1,388 pM, Ucn 1 (n=5) significantly increased colonic contractility in proximal colon; the mean percentage changes of MI from baseline motility showed 20.4±7.2%, 48.4±20.9%, 67.0±25.8%, and 64.2± 20.9%, respectively in the proximal colon. In the distal colon, however, the mean percentage changes of MI from baseline showed 3.3±3.3%, 7.8±7.8%, 71.1±28.6%, and 87.4±32.5%,

respectively; the changes were significant only with 277 pM and 1,388 pM (Fig. 2, 3A, and 4A).

2. The effect of adrenergic, and cholinergic receptors, and nerve conduction blockade on Ucn 1-stimulated colonic motility

MI was obtained following administration of 277 and 1,388 pM of Ucn 1, which caused the greatest colon contraction in the proximal and distal colon, respectively (Table 1).

After the infusion of phentolamine, the percentage change of MIs were modified, but not significantly from 67.0±25.8% (Ucn 1 only) to 133.0±57.9% (Ucn 1 + phentolamine) in the proximal colon and from 87.4±32.5% (Ucn 1 only) to 69.06±29.5% (Ucn 1 + phentolamine) in the distal colon.

After the infusion of propranolol, MIs were modified but not
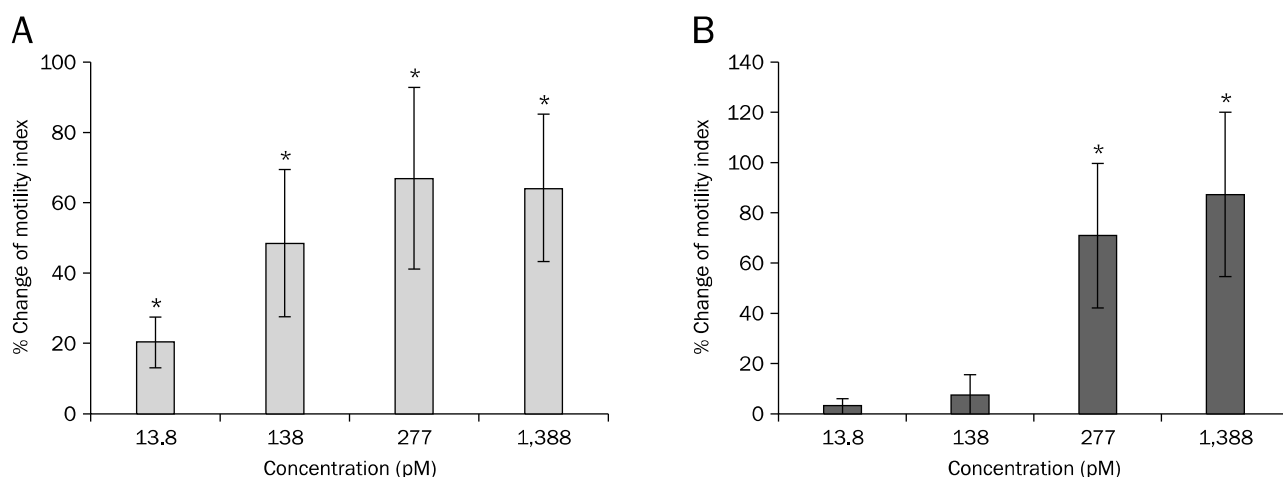
Fig. 2. Effect of intra-arterial infusion of urocortin 1 on the colonic motility. Data are expressed as the percentage change of the motility index over the basal period in response to various concentrations of urocortin 1. (A) Urocortin 1 significantly increased motility in the proximal colon at concentrations of 13.8, 138, 277, and 1,388 pM. (B) Urocortin 1 significantly increased motility in the distal colon at concentrations of 277 and 1,388 pM. Each bar represents the mean±standard error from 5 experiments. *$p < 0.05$ vs. basal values.
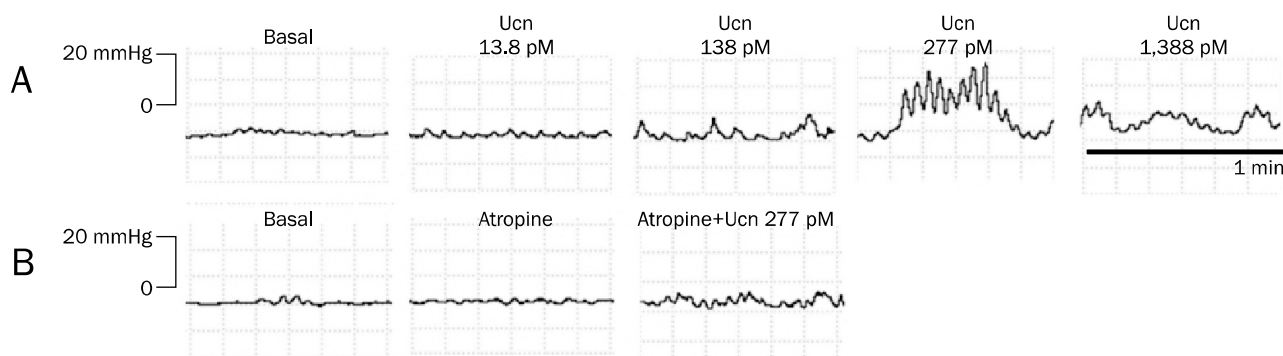
Fig. 3. Representative tracings of the effect of urocortin 1 (Ucn 1) alone (A) and atropine (B) on the proximal colonic motility stimulated by Ucn 1. The stimulatory effect was significantly inhibited by atropine.
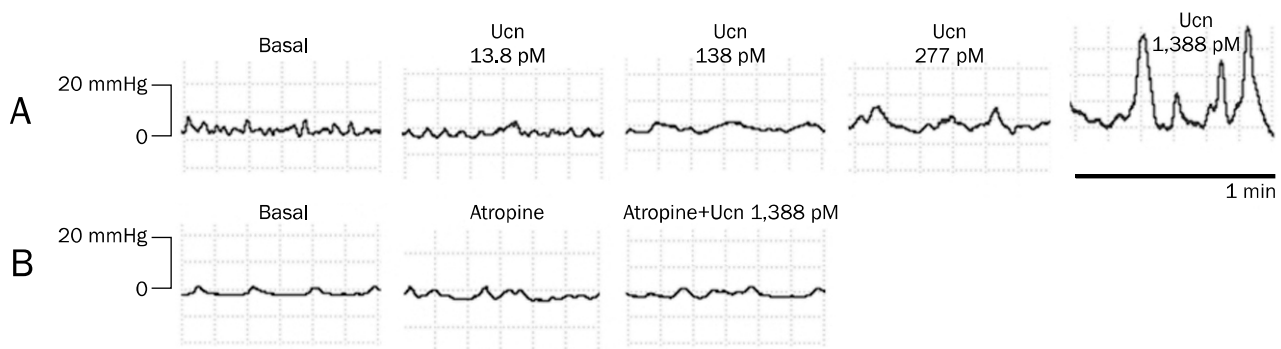
Fig. 4. Representative tracings of the effect of urocortin 1 (Ucn 1) alone (A) and atropine (B) on the distal colonic motility stimulated by Ucn 1. The stimulatory effect was significantly inhibited by atropine.

Table 1. The Effects of Urocortin 1 (Ucn 1) and Various Substances on Colonic Motility

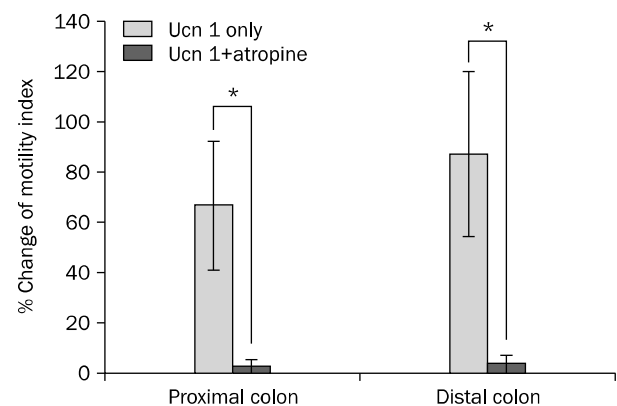| Colon	Ucn 1 dose (pM)	Percentage change of MI
Proximal colon	277	67.0±25.8
	277 + PL	133.0±57.9
	277 + PP	49.1±35.4
	277 + HM	188.0±59.8
	277 + AT	2.4±2.4*
	277 + TTX	51.2±22.2
Distal colon	1.388	87.4±32.5
	1.388 + PL	69.1±29.5
	1.388 + PP	37.0±21.0
	1.388 + HM	114.4±57.4
	1.388 + AT	3.7±3.7*
	1.388 + TTX	66.8±29.1

Values are presented as mean±standard error.
MI, motility index; PL, phentolamine; PP, propranolol; HM, hexamethonium; AT, atropine; TTX, tetrodotoxin.
*p < 0.05 *vs.* Ucn 1 alone.

Fig. 5. Effect of atropine on colonic motility stimulated by urocortin 1 (Ucn 1). The stimulatory effect of Ucn 1 on the proximal and distal colonic motility was significantly inhibited by pretreatment with atropine. Data are expressed as the percentage change of motility index over the basal period. *p < 0.05 *vs.* Ucn 1 only.

significantly from 67.0±25.8% (Ucn 1 only) to 49.1±35.4% (Ucn 1 + propranolol) in the proximal colon and from 87.4±32.5% (Ucn 1 only) to 37.0±21.0% (Ucn 1 + propranolol) in the distal colon.

After the infusion of hexamethonium, MIs were modified, but not significantly, from 67.0±25.8% (Ucn 1 only) to 188.0±59.8% (Ucn 1 + hexamethonium) in the proximal colon and from 87.4±32.5% (Ucn 1 only) to 114.4±57.4% (Ucn 1 + hexamethonium) in the distal colon.

After the infusion of atropine, the stimulating effect of Ucn 1 on colonic motility was inhibited. MIs were decreased from 67.0±25.8% (Ucn 1 only) to 2.4±2.4% (Ucn 1 + atropine) in the proximal colon and from 87.4±32.5% (Ucn 1 only) to 3.7±3.7% (Ucn 1 + atropine) in the distal colon, which were statistically significant (p < 0.05) (Fig. 5, 3B and 4B). After

the infusion of TTX, MIs were modified, but not significantly, from 67.0±25.8% (Ucn 1 only) to 51.2±22.2% (Ucn 1 + TTX) in the proximal colon and from 87.4±32.5% (Ucn 1 only) to 66.8±29.1% (Ucn 1 + TTX) in the distal colon.

DISCUSSION

The Ucns and CRF bind to the same receptor and they are similar in structure and function. However, the affinity of the Ucns for the receptor is different from that of CRF and although both CRF and the Ucns are found in the CNS and peripheral nervous system, their distributions are partially overlap. Thus, it is considered that Ucns have different functions and mechanisms from those of CRF and, as such, there is increasing interest in this peptide.[22]

Ucn 1 binds to both CRF 1 receptor and CRF 2 receptor. Ucn 2 and Ucn 3 bind only to the CRF 2 receptor. Martinez et al.[22]

reported that when peripherally injected, Ucn 1 reduced colonic transit time whereas Ucn 2 and Ucn 3 resulted in no response, indicating that Ucn 1 could be responsible for increasing colonic motility. Gourcerol et al.[23] reported that concomitant intraperitoneal injection of CRF and Ucn 2 reduced Fos expression in the colonic myenteric plexus compared with injection of CRF alone. This suggests a possible mechanism whereby Ucn 1 and CRF receptor 1 stimulate colonic motility and Ucn 2 and CRF 2 receptor mediate the inhibition of colonic motility.

Compared to previous studies, our study has a distinct feature. This is the first study about the effect of peripherally infused Ucn 1 on colonic motility in a setting that is similar setting to *in vivo* conditions. Kimura et al.[19] reported that in an experiment using rat distal colonic muscle strips, CRF and Ucn 1 increased contractility and this effect was inhibited when selective CRF 1 receptor antagonists, atropine, TTX, 5-HT$_3$ antagonists, and 5-HT$_4$ antagonists were used together with CRF and Ucn 1. Based on this, the effect of Ucn 1 is thought to be mediated by CRF 1 receptor and cholinergic and serotonergic neurotransmission. This experiment, however, was under different conditions as muscle strips were used. Our experiment, more closely resembled *in vivo* conditions as it was performed with preservation of the blood flow and intestinal nerves. In addition, factors such as perfusion pressure, intravenous secretion of lactate dehydrogenase, glucose consumption, lactic acid elimination, and oxygen consumption were preserved in order to prevent ischemic injury of the colonic mucosa. The resected colon tissue showed microscopic changes of ischemia after 2 h of perfusion. This experiment was completed within 2 h, therefore, the potential of ischemic damage to the colonic mucosa was minimized.

When injected via the superior mesenteric artery, Ucn 1 significantly increased the MI both in the distal and proximal colon compared with the pre-injection MI. In the proximal colon, the MI increased at all concentrations compared with the pre-injection, and the highest MI observed was with 277 pM. In the distal colon, however, in the MI increased significantly only at higher concentrations of 277 and 1,388 pM, showing the greatest MI change at 1,388 pM. Although there were differences in the response between the proximal and distal colon, the cause of differences remains unclear.

In this study, Ucn 1 did not inhibit motility when the colon was pretreated with phentolamine as an adrenergic alpha receptor blocker, propranolol as an adrenergic non-selective beta receptor blocker, hexamethonium as a nicotinic cholinergic receptor blocker, or TTX as a neural blocker, but motility was significantly inhibited when the colon was pretreated with atropine as a muscarinic cholinergic receptor blocker in both the distal and proximal colon.

This indicates that the effect of Ucn 1 is mediated by endogenous muscarinic cholinergic receptors of the nerves, which is consistent with the study by Kimura et al.[19] in which distal colon muscle strips were used. In the study by Kimura et al.,[19] however, Ucn 1 did not increase motility when the colon was pretreated with TTX, as was the case with atropine, therefore, the effect of Ucn 1 was reported to be mediated by TTX-sensitive neurons. However, in our study, only atropine could inhibit Ucn 1-stimulated motility. TTX blocks Na$^+$ channels by binding to the exterior pore loop of cells and is very useful for experiments intended to investigate the action of neurotransmitters.[24-26] In other recent studies, it has been reported that TTX increased colonic motility and this effect was mediated by the blockade of tonic inhibitory neuronal regulation.[27] Unlike the experiment by Kimura et al.,[19] our experiment was performed while the intestinal nerves were preserved; therefore, it is believed that tonic inhibitory neurons were more available compared with colonic muscle strips. It is considered that this blockade of tonic inhibitory neurons might have offset the TTX-mediated effect of Ucn 1. Another possibility for why TTX did not inhibit the action of Ucn 1 is that if Ucn 1 promotes acetylcholine secretion in the colon tissue, or activates a muscarinic current through direct stimulation of muscarinic cholinergic receptors of the colonic smooth muscle cells, the action of Ucn 1 may not be affected by TTX. Further studies of the actions of Ucn 1 and TTX are needed.

The importance of CRF and the Ucns, which are stress-related peptides, is increasing because they may provide a clue to the treatment of various diseases that are considered stress-related.[28-31] The gastrointestinal tract is very sensitive to stress and chronic stress is considered one of the important factors in the pathophysiology of irritable bowel syndrome (IBS). Studies have reported that stress increases visceral hypersensitivity and may lead to IBS.[32-35]

As our study included a small number of specimens, further studies with larger numbers are required, and studies on

selective CRF 1 receptor antagonists, CRF 2 receptor antagonists, 5-HT$_3$ antagonists, 5-HT$_4$ antagonists, and nitro-L-arginine methyl ester are suggested in order to more fully understand the mechanism of the effect of Ucn 1 on colonic motility.

In summary, Ucn 1 increased colonic motility and it is considered that this effect was directly mediated by local muscarinic cholinergic receptors but not by tetrodotoxin-sensitive nerve conduction.

REFERENCES

1. Bale TL, Vale WW. CRF and CRF receptors: role in stress responsivity and other behaviors. Annu Rev Pharmacol Toxicol 2004;44:525-557.

2. Owens MJ, Nemeroff CB. Physiology and pharmacology of corticotropin-releasing factor. Pharmacol Rev 1991;43:425-473.

3. Hauger RL, Grigoriadis DE, Dallman MF, Plotsky PM, Vale WW, Dautzenberg FM. International Union of Pharmacology. XXXVI. Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands. Pharmacol Rev 2003;55:21-26.

4. Weninger SC, Dunn AJ, Muglia LJ, et al. Stress-induced behaviors require the corticotropin-releasing hormone (CRH) receptor, but not CRH. Proc Natl Acad Sci USA 1999;96:8283-8288.

5. Hsu SY, Hsueh AJ. Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nat Med 2001;7:605-611.

6. Lewis K, Li C, Perrin MH, et al. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc Natl Acad Sci USA 2001;98:7570-7575.

7. Donaldson CJ, Sutton SW, Perrin MH, et al. Cloning and characterization of human urocortin. Endocrinology 1996;137:2167-2170.

8. Takahashi K, Totsune K, Sone M, et al. Regional distribution of urocortin-like immunoreactivity and expression of urocortin mRNA in the human brain. Peptides 1998;19:643-647.

9. Okosi A, Brar BK, Chan M, et al. Expression and protective effects of urocortin in cardiac myocytes. Neuropeptides 1998;32:167-171.

10. Slominski A, Roloff B, Curry J, Dahiya M, Szczesniewski A, Wortsman J. The skin produces urocortin. J Clin Endocrinol Metab 2000;85:815-823.

11. Singh LK, Boucher W, Pang X, et al. Potent mast cell degranulation and vascular permeability triggered by urocortin through activation of corticotropin-releasing hormone receptors. J Pharmacol Exp Ther 1999;288:1349-1356.

12. Bamberger CM, Wald M, Bamberger AM, Ergün S, Beil FU, Schulte HM. Human lymphocytes produce urocortin, but not corticotropin-releasing hormone. J Clin Endocrinol Metab 1998; 83:708-711.

13. Chen CY, Million M, Adelson DW, Martínez V, Rivier J, Taché Y. Intracisternal urocortin inhibits vagally stimulated gastric motility in rats: role of CRF(2). Br J Pharmacol 2002;136:237-247.

14. Kihara N, Fujimura M, Yamamoto I, Itoh E, Inui A, Fujimiya M. Effects of central and peripheral urocortin on fed and fasted gastroduodenal motor activity in conscious rats. Am J Physiol Gastrointest Liver Physiol 2001;280:G406-G419.

15. Martínez V, Taché Y. Role of CRF receptor 1 in central CRF-induced stimulation of colonic propulsion in rats. Brain Res 2001;893:29-35.

16. Martínez V, Wang L, Rivier J, Grigoriadis D, Taché Y. Central CRF, urocortins and stress increase colonic transit via CRF1 receptors while activation of CRF2 receptors delays gastric transit in mice. J Physiol 2004;556:221-234.

17. Maillot C, Million M, Wei JY, Gauthier A, Taché Y. Peripheral corticotropin-releasing factor and stress-stimulated colonic motor activity involve type 1 receptor in rats. Gastroenterology 2000;119:1569-1579.

18. Martínez V, Wang L, Rivier JE, Vale W, Taché Y. Differential actions of peripheral corticotropin-releasing factor (CRF), urocortin II, and urocortin III on gastric emptying and colonic transit in mice: role of CRF receptor subtypes 1 and 2. J Pharmacol Exp Ther 2002;301:611-617.

19. Kimura T, Amano T, Uehara H, et al. Urocortin I is present in the enteric nervous system and exerts an excitatory effect via cholinergic and serotonergic pathways in the rat colon. Am J Physiol Gastrointest Liver Physiol 2007;293:G903-G910.

20. Cuber JC, Vilas F, Charles N, Bernard C, Chayvialle JA. Bombesin and nutrients stimulate release of CCK through distinct pathways in the rat. Am J Physiol 1989;256:G989-G996.

21. Cuber JC, Herrmann C, Kitabgi P, et al. Neuromedin-N is not released with neurotensin from rat ileum. Endocrinology 1990;126:1584-1592.

22. Martinez V, Wang L, Million M, Rivier J, Taché Y. Urocortins and the regulation of gastrointestinal motor function and visceral pain. Peptides 2004;25:1733-1744.

23. Gourcerol G, Wu SV, Yuan PQ, et al. Activation of corticotropin-releasing factor receptor 2 mediates the colonic motor coping response to acute stress in rodents. Gastroenterology 2011;140:1586-1596.e6.

24. Terlau H, Heinemann SH, Stühmer W, et al. Mapping the site of block by tetrodotoxin and saxitoxin of sodium channel II. FEBS Lett 1991;293:93-96.

25. Gershon MD. Effects of tetrodotoxin on innervated smooth muscle preparations. Br J Pharmacol Chemother 1967;29:259-279.

26. Narahashi T. Mechanism of action of tetrodotoxin and saxitoxin on excitable membranes. Fed Proc 1972;31:1124-1132.

27. Okuno Y, Kondo T, Saeki A, Uchida E, Teraoka H, Kitazawa T. Colon-specific contractile responses to tetrodotoxin in the isolated mouse gastrointestinal tract. Auton Autacoid Pharmacol 2011;31:21-30.

28. Kim BJ, Jones HP. Implications of corticotropin releasing factor in targeted anticancer therapy. J Pharm Pract 2010;23:86-90.

29. Paez-Pereda M, Hausch F, Holsboer F. Corticotropin releasing factor receptor antagonists for major depressive disorder. Expert Opin Investig Drugs 2011;20:519-535.

30. Lowery EG, Thiele TE. Pre-clinical evidence that corticotropin-releasing factor (CRF) receptor antagonists are promising targets for pharmacological treatment of alcoholism. CNS Neurol Disord Drug Targets 2010;9:77-86.

31. Boonprasert P, Lailerd N, Chattipakorn N. Urocortins in heart failure and ischemic heart disease. Int J Cardiol 2008;127:307-312.

32. O'Malley D, Quigley EM, Dinan TG, Cryan JF. Do interactions between stress and immune responses lead to symptom exacerbations in irritable bowel syndrome? Brain Behav Immun 2011;25:1333-1341.

33. Elsenbruch S. Abdominal pain in irritable bowel syndrome: a review of putative psychological, neural and neuro-immune mechanisms. Brain Behav Immun 2011;25:386-394.

34. Lee OY. Psychosocial factors and visceral hypersensitivity in irritable bowel syndrome. Korean J Gastroenterol 2006;47:111-119.

35. Taché Y, Martinez V, Wang L, Million M. CRF1 receptor signaling pathways are involved in stress-related alterations of colonic function and viscerosensitivity: implications for irritable bowel syndrome. Br J Pharmacol 2004;141:1321-1330.