Table 1. Comparison of the effects of acyl ghrelin, des-acyl ghrelin and obestatin on the gastroduodenal motility

	Acyl ghrelin		Des-acyl ghrelin		Obestatin	
	fasted motility	fed motility	fasted motility	fed motility	fasted motility	fed motility
Stomach	↑	↑	↓	–	–	↓
Duodenum	↑	↑	–	–	–	↓
Hypothalamic neuron	NPY		Urocortin-2		CRF, urocortin-2	
Brain receptor	Y2, Y4		CRF type 2		CRF type 1, type 2	
Vagal afferent pathway	+		–		+	

the brain receptor and disrupt the fasted motility in the antrum.

The centrally administered CRF type 2 receptor antagonist, but not the CRF type 1 receptor antagonist, blocked the effects of centrally and peripherally administered des-acyl ghrelin on gastric motility [9]. The density of c-*Fos*-positive cells in the PVN was significantly increased by intraperitoneal injection of des-acyl ghrelin compared to vehicle-injected controls [9]. These data suggest that peripherally administered des-acyl ghrelin may activate neurons in the PVN by crossing the BBB, and exert inhibitory effects on the antral motility via CRF type 2 receptor in the brain (fig. 2; table 1).

Regulation of Obestatin on the Gastroduodenal Motility

Most of the previous studies have shown the negative effects of obestatin on the GI motility; in those studies, however, only the gastric emptying or MMC cycle time has been used as indices for motor activity. We obtained the positive effects of obestatin on the gastroduodenal motility by analyzing the motor activity in fed and fasted states, and measuring the time taken to the initiation of phase III-like contractions in the antrum and duodenum of conscious rats [10].

Obestatin decreased the %MI of fed motility in the antrum and prolonged the time before the return of fasted motility in the duodenum [10]. IV injection of obestatin induced a significant increase in the number of c-*Fos*-positive cells in the PVN compared to saline-injected controls [10]. Immunofluorescence overlap staining showed that the PVN neurons activated by IV injection of obestatin contain CRF or urocortin-2 [10]. The inhibitory action of IV injection of obestatin on the motor activities in the antrum and duodenum were blocked by ICV injection of CRF type 1 and type 2 receptor antagonists, suggesting that both types of CRF receptors in the brain may mediate the action of peripherally injected obestatin on gastroduodenal motility [10]. Combined together, obestatin inhibits gastroduodenal motility in the fed state but not in the fasted state of conscious rats. In the brain, CRF- and urocortin-2-containing neurons might be activated by IV injection of obestatin, and at the level, CRF type 1 and type 2 receptors might be involved in the inhibitory action of obestatin on antral and duodenal motility. Vagal afferent pathways might be involved partially, but not entirely, in these actions of obestatin (fig. 2; table 1).

Conclusions

Acyl ghrelin, des-acyl ghrelin and obestatin are included in the endocrine cells in the stomach and regulate the upper GI motility by activating hypothalamic peptides [11, 12]. Since hypothalamic peptides are strongly affected by stress or anxiety, such brain-gut interaction seems to be important to understand the pathogenesis of functional disorder in the GI tracts.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

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Alteration of antral and proximal colonic motility induced by chronic psychological stress involves central urocortin 3 and vasopressin in rats

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Ataka K, Nagaishi K, Asakawa A, Inui A, Fujimiya M. Alteration of antral and proximal colonic motility induced by chronic psychological stress involves central urocortin 3 and vasopressin in rats. *Am J Physiol Gastrointest Liver Physiol* 303: G519–G528, 2012. First published May 25, 2012; doi:10.1152/ajpgi.00390.2011.—Because of the difficulties in developing suitable animal models, the pathogenesis of stress-induced functional gastrointestinal disorders is not well known. Here we applied the communication box technique to induce psychological stress in rats and then examined their gastrointestinal motility. We measured upper and lower gastrointestinal motility induced by acute and chronic psychological stress and examined the mRNA expression of various neuropeptides in the hypothalamus. Chronic psychological stress disrupted the fasted motility in the antrum and accelerated motility in the proximal colon. mRNA expression of AVP, oxytocin, and urocortin 3 was increased by chronic psychological stress. Intracerebroventricular (ICV) injection of urocortin 3 disrupted the fasted motility in the antrum, while ICV injection of Ucn3 antiserum prevented alteration in antral motility induced by chronic psychological stress. ICV injection of AVP accelerated colonic motility, while ICV injection of SSR 149415, a selective AVP V1b receptor antagonist, prevented alteration in proximal colonic motility induced by chronic psychological stress. Oxytocin and its receptor antagonist L 371257 had no effect on colonic motility in either the normal or chronic psychological stress model. These results suggest that chronic psychological stress induced by the communication box technique might disrupt fasted motility in the antrum via urocortin 3 pathways and accelerates proximal colonic motility via the AVP V1b receptor in the brain.

brain-gut axis; arginine vasopressin V1b receptor; communication box

PSYCHOLOGICAL STRESS, EXPOSURE to repeated stress-inducing stimuli for a long period, has been reported to trigger anxiety, depression, functional gastrointestinal disorders including irritable bowel syndrome, functional dyspepsia, and eating disorder (30). To investigate the pathogenesis of these stress-related disorders, various kinds of animal models including those of restraint stress, cold restraint stress, electrical foot shock stress, or water immersion stress have been used (20). However, since these models induce more physical than psychological stress on the animals, they might not accurately reflect human stress.

Recently, the water avoidance (WA) stress model has been developed. This model elicits a greater deal of psychological stress than physical stress, and the consequent effects on gastrointestinal (GI) motility and visceral hyperalgesia have been examined (4, 6, 22, 23). In the WA model, animals are placed on a floating board, which is an environment that induces psychological stress (29).

The communication box (CB) technique has also been developed to induce psychological stress in animals without the need for physical stimuli (39). CB experiments seem to be more effective than WA experiments because they elicit a stress response in the animals via a complex combination of visual, auditory, and olfactory stimuli.

In the present study, we aimed to examine the effects of acute and chronic psychological stress generated by the CB technique on upper and lower GI motility and investigate the brain mechanisms involved in stress-induced abnormality in GI motility. For the measurement of GI motility, we used a conscious rat model that enabled real-time recording of pressure waves under stress conditions (1, 2, 8, 11, 12). Changes in the expression of hypothalamic peptides under exposure to psychological stress were examined by quantitative RT-PCR. We further examined the candidates for specific peptides that alter upper or lower GI motility by intracerebroventricular (ICV) administration of peptide agonists or antagonists.

MATERIALS AND METHODS

Animals. Male Wistar rats (Japan SLC, Shizuoka, Japan) weighing 200–250 g at the start of the experiments were maintained under conditions of controlled temperature (22–24°C), humidity (44–46%), and a 12:12-h light/dark cycle (lights on 7:00–19:00). Food and water were available ad libitum. Animals were acclimated to the facility for 1 wk and handled daily for 10 min by the same investigator for at least 1 wk to prevent stress caused by the laboratory environment and subsequent experimental handling. All rats were used once for each experiment. All animal experiments were approved by the Institutional Animal Care and Use Committee at Sapporo Medical University School of Medicine.

Implantation of ICV cannula. Rats were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg; Kyoritsu Seiyaku, Tokyo, Japan) and implanted with a chronic guide cannula (25-guage; Eicom, Kyoto, Japan) into the right lateral ventricle using a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA). Stereotaxic coordinates were 0.8 mm posterior to the bregma, 1.5 mm right lateral to the midline, and 3.5 mm below the outer surface of the skull. The guide cannula was secured with dental cement and anchored by two stainless screws fixed on the dorsal surface of the skull. A dummy cannula (Eicom) was placed into each guide cannula and fixed with a screw cap (Eicom) to prevent occlusion. When ICV injection was given to conscious animals, the dummy cannula was replaced by a microinjection cannula (model AMI-5; Eicom), 1 mm longer than the guide cannula, connected to a polyethylene tube (PE-50; Clay Adams, Parsippany, NJ). At the end of the experiments, animals were euthanized by intraperitoneal injection of pentobarbital sodium (150 mg/kg), and the correct location of the ICV cannula was verified by a 10-μl injection of dye (0.05% cresyl violet).

Implantation of catheters for manometric recording. Rats were anesthetized with intraperitoneal injection of pentobarbital sodium (50

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mg/kg). For measurements of gastroduodenal motility, two open-tipped catheters (3-French, 1 mm ID; Atom, Tokyo, Japan) were placed into the gastric antrum and duodenum 4 cm distal to the pylorus. For measurements of colonic motility, two open-tipped catheters were placed proximal and distal to the colon, 3 cm and 13 cm from the ileocecal junction, respectively. The catheters were fixed by purse-string sutures, which were run subcutaneously to emerge at the back of the neck and secured to the skin. Each rat was allowed to recover in an individual cage for 7 days before the experiments.

Measurement of upper and lower GI motility. Seven days after cannula implantation, rats scheduled for measurement of gastroduodenal motility were deprived of food for 18 h before the experiment, while rats scheduled for measurement of colonic motility were allowed to eat. We measured the upper and lower GI motility of rats exposed to acute and chronic psychological stress. Gastroduodenal and colonic motility were measured by manometric methods (1, 2, 8, 11, 12) as shown in Fig. 1*A*. On the day of the experiments, two manometric catheters inserted into the GI tracts were connected to infusion swivels (model 375/D/20; Instech Laboratories, Plymouth Meeting, PA) to enable free movement, and each catheter was connected to a pressure transducer (model DX-100T; Nihon Koden Kogyo, Tokyo, Japan). The catheters were continuously infused with bubble-free distilled water at a rate of 1.5 ml/h by an infusion pump (model NE-1600; KD Scientific, Wantagh, NY). The pressure signals from the transducers were recorded and stored by a PowerLab system (ADInstruments Colorado Springs, Co). Phase III-like contractions in the antrum were defined according to previous studies (1, 11, 12), and the frequency was defined by the number of phase III-like contractions per 60 min. The percentage motility index (%MI) in the proximal and distal colon was calculated as follows: (area under the intraluminal pressure wave for 60 min during stress exposure or after ICV injection)/(area under the intraluminal pressure wave for 60 min before stress exposure or ICV injection) × 100 (2).

Psychological stress loading using the CB. The CB consists of nine compartments divided by transparent acrylic panels (Fig. 1*B*; 50 × 50 × 60 cm, model BS-CC01; BrainScience-Idea, Osaka, Japan). Five compartments have a grid floor of stainless steel rods (Fig. 1*B*) that is connected to an electric generator (model BS-5ES; BrainScience-Idea), and four compartments have the safety grid floor without the connection to the electric generator (Fig. 1*B*). Five rats were individually placed in the electrical foot shock compartments and given electric current (2 mA) for 10 s, delivered randomly an average of twice per min for 60 min. Four other rats were placed individually in the psychological stress compartments with the safety floor but surrounded by electrical foot shock compartments on three sides to receive visual, auditory, and olfactory stimuli from rats that received electrical foot shock. Stress stimulation produced by CB was performed for 1 h a day in the morning (10:00–11:00). Rats in the acute stress model received stimulation only once, but those in the chronic stress model received stimulation for five successive days. Measurement of GI motility was started 2 h before stress loading. Sham-treated controls were placed in each psychological stress compartment for 1 day or five successive days without any stimuli.

Quantitative real-time RT-PCR analysis. Rats were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg) and perfused with 0.1 M PBS immediately after psychological stress exposure at *day 5*. Hypothalamic tissues were taken from rats and dissociated to single-cell suspension by Neural Tissue Dissociation Kit (P) (model 130–092-628; Milteny Biotec) and gentleMACS Dissociator (model 130–093-235; Milteny Biotec). Total RNA was extracted from the cell suspension of hypothalamus using RNeasy Micro Kit (model 74004; Qiagen, Hilden, Germany), and cDNA was synthesized using SuperScript III First-Strand Synthesis System for RT-PCR (model 18080–051; Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Quantitative RT-PCR for AVP, orexin, amphetamine-regulated transcript, proopiomelanocortin, agouti-related protein, oxytocin (OXT), corticotoropin-releasing factor (CRF), neuropeptide Y, urocortin (Ucn), Ucn 2, and Ucn 3 was performed on the ABI prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) with Power SYBAR GREEN PCR Master Mix (model 4367659; Applied Biosystems). Relative mRNA expression was quantified by the $2^{-\Delta CT}$ method. Primer sequences are shown in Table 1. We considered significance when the alteration was more than twofold or less than one-half.

Experimental design. Manometric measurement of intraluminal pressure waves was started after a 1-h stabilization period and continued for 4 h. The effects of ICV injection of AVP (cat. no. V9879; Sigma, St Louis, MO), OXT (cat. no. 4084-v; Peptide Ins, Osaka, Japan), or Ucn 3 (cat. no. H-5828; Bachem, Bubendorf, Switzerland) on the GI motility were examined in normal rats, and a paired vehicle (5-μl saline) was injected ICV as a control. The effects of the following chemicals on chronic stress-induced alterations of GI motility were also examined: A nonpeptide AVP V1b receptor antagonist, SSR 149415 [(2S,4R)-1-{5-chloro-1-[(2,4-dimethoxyphenyl)sul-

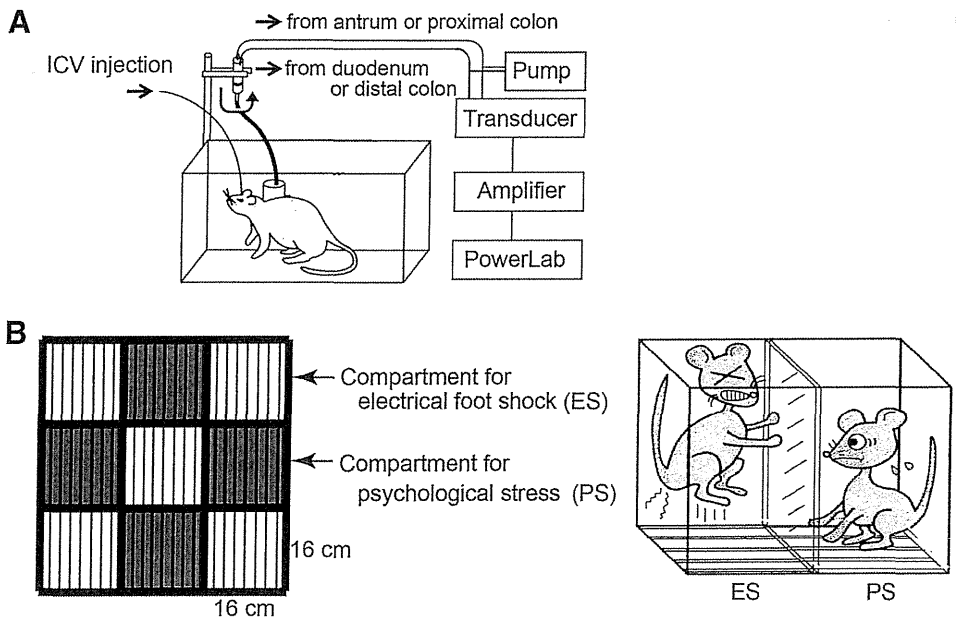

Fig. 1. Illustration of manometric measurements of gastrointestinal motility (*A*) and communication box experiments (*B*) in conscious rats. ICV, intracerebroventricular.

Table 1. *Primers for quantitative RT-PCR*

Gene		Forward Primer, 5′-/-3′	Reverse Primer, 5′-/-3′
AVP	NM_016992	ACCTCTGCCTGCTACTTCCAGA	ACACTGTCTCAGCTCCATGTCG
Orexin	NM_013179	GCAGCCTCTGCCCG	TCCGTGCAACAGTTCGTAGAGA
CART	NM_017110	TTGCAGATTGAAGCGCTGCAGGAA	GGACTTGGCCGTACTTCTTCTCAT
POMC	NM_012625	ACCGCAGAAAGATCGGTTGT	GGCAGACCGTGAGTTACGAG
AgRP	NM_033650	TCCACAGAACCGCGAGTCTC	CCCAAGCAGGACTCGTGC
OXT	NM_012996	TGGCCTACTGGCTCTGACCT	GGGAAGACACTTGCGCATATC
CRF	NM_031019	CCCATCTCTCTGGATCTCACCTT	CAGTTTCCTGTTGCTGTGAGCTT
NPY	NM_012614	GGGCTGTGTGGACTGACCC	GGTACCCCTCAGCCAGAATG
Ucn	NM_019150	CAGAGCAGAACCGCATCATATT	TCCAGTCAGAGTGTTCAGGGTAA
Ucn 2	NM_133385	CGTGTCATACTCTCCCTGGATG	ACGGGCTAGTATTTGGGCATTAG
Ucn 3	NM_001080208	ACAGATACCAATCCCAAGCACA	GCAAATTCTTGGCCTTGTCAAT
GAPDH	NM_017008	TGACTCTACCCACGGCAAGTT	GATGGGTTTCCCGTTGATGA

CART, amphetamine-regulated transcript; POMC, proopiomelanocortin; AgRP, agouti-related protein; OXT, oxytocin; CRF, corticotoropin-releasing factor; NPY, neuropeptide Y; Ucn, urocortin.

fonyl]-3-(2-methoxyphenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl}-4-hydroxy-N,N-dimethyl-2-pyrrolidinecarboxamide, Axon 1114; Axon Medchem, Groningen, Netherland]. A nonpeptide OXT receptor antagonist, L 371257 (1-{4-[(1-Acetyl-4-piperidinyl)oxy]-2-methoxybenzoyl}-4- [2-oxo-2H-3,1-benzoxazin-1(4H)-yl]) piperidine, 2410; Tocris Bioscience, Bristol, UK). These antagonists or a paired vehicle (5-μl DMSO) were injected ICV 10 min before the psychological stress procedure on *day 5*. Anti-Ucn3 antiserum (mouse, Y364, Yanaihara Institute, Shizuoka, Japan), or a paired 8-μl vehicle was also injected ICV 10 min before the psychological stress procedure on *day 5*.

Statistical analysis. All data were presented as means ± SE. The statistical significance between the control and experimental group was evaluated by the Student's *t*-test. Group differences were evaluated by one-way ANOVA followed by Newman-Keuls Multiple Comparison Test. A P value of < 0.05 was considered statistically significant.

RESULTS

Acute psychological stress did not alter upper GI motility. We examined the effects of psychological stress on the motility of the antrum and duodenum in the fasted state. The motility curves in the antrum and duodenum were recorded in rats exposed to a single psychological stress on *day1* (Fig. 2, *A* and *B*). The pressure waves consisted of a quiescent period, followed by series of strong contractions (phase III-like contractions indicated by arrowheads). The frequency of phase III-like contractions of the antrum and duodenum in the acute stress-loaded rats was 4.7 ± 0.9 h and 6.7 ± 0.7 h, respectively, and no difference was found compared with paired sham-treated controls; 5.3 ± 0.3 h in the antrum and 6.3 ± 0.3 h in the duodenum (Fig. 2*C*).

Chronic psychological stress suppressed antral motility. Rats were exposed to chronic psychological stress for 4 days, fasted for 18 h, and their motility curves were recorded on *day 5* before and during exposure to the psychological stress stimuli (Fig. 2*E*). The frequency of phase III-like contractions in the antrum in rats during exposure to psychological stress (3.0 ± 0.6 h) was significantly lower than that in the sham-treated controls (5.8 ± 0.3 h, $P < 0.05$) (Fig. 2*D*). The frequency of phase III-like contractions in the duodenum, on the other hand, was not different between psychologically stress-loaded rats (5.9 ± 0.7 h) and paired sham-treated controls (5.5 ± 0.3 h) (Fig. 2*F*).

Acute psychological stress did not alter colonic motility. The motility curves of the proximal and distal colon were recorded in rats exposed to acute psychological stress and paired sham-treated rats on *day 1* (Fig. 3, *A* and *B*). The %MI of the proximal and distal colon in rats exposed to acute psychological stress was 97.0 ± 5.5% and 94.8 ± 5.0%, respectively, and no difference was found when compared with paired sham-treated rats (93.6 ± 4.0% in proximal colon and 92.9 ± 5.0% in distal colon) (Fig. 3*C*).

Chronic psychological stress accelerated proximal colonic motility. The traces of colonic motility in the rats receiving chronic psychological stress procedure for 5 days (Fig. 3*E*) were compared with paired sham-treated controls (Fig. 3*D*). The %MI in the proximal colon during psychological stress (122.2 ± 6.2%) was significantly higher than that from paired sham-treated controls (92.6 ± 4.3%, $P < 0.05$). On the other hand, the %MI in the distal colon between psychological stress loaded rats (103.2 ± 3.3%) and paired sham-treated rats (101.7 ± 8.3%) was unchanged (Fig. 3*F*).

mRNA expressions in the hypothalamus of rats with chronic psychological stress. Changes in the mRNA expression of hypothalamic peptides induced by chronic psychological stress were examined. AVP, OXT, and Ucn 3 mRNA levels were significantly higher in rats exposed to chronic psychological stress at 2.8-, 2.8-, and 4.3-fold that of the paired sham-operated controls ($P < 0.05$) (Fig. 4). mRNA levels of other peptides were not changed (Fig. 4).

Effects of ICV injection of Ucn 3, AVP, or OXT on gastroduodenal motility and effects of ICV injection of Ucn 3 antiserum on the alteration of gastric motility induced by chronic psychological stress. Because the mRNA expressions for Ucn3, AVP, and OXT were increased in the hypothalamus of rats exposed to chronic psychological stress, we next examined whether central administration of Ucn 3, AVP, or OXT induced the alteration of upper GI motility in normal rats. When 3 nmol Ucn 3 was injected ICV, phase III-like contractions were blocked in the antrum as well as in the duodenum (Fig. 5*A*). ICV injection of 3-nmol Ucn 3 significantly decreased the frequency of contractions in the antrum (2.6 ± 0.4 h) compared with vehicle injection (4.8 ± 0.8 h, $P < 0.05$. Fig. 5*E*). ICV injection of 0.3-pmol AVP did not alter (Fig. 5*B*), while 1.5 pmol AVP significantly increased the frequency of phase III-like contractions in the antrum (6.5 ± 0.3 h, $P < 0.05$) and duodenum compared with that of vehicle-injected controls (Fig. 5, *C* and *E*). ICV injection of 1.5-pmol OXT did not alter the frequency of phase III-like contractions in the antrum (5.0 ± 0.4

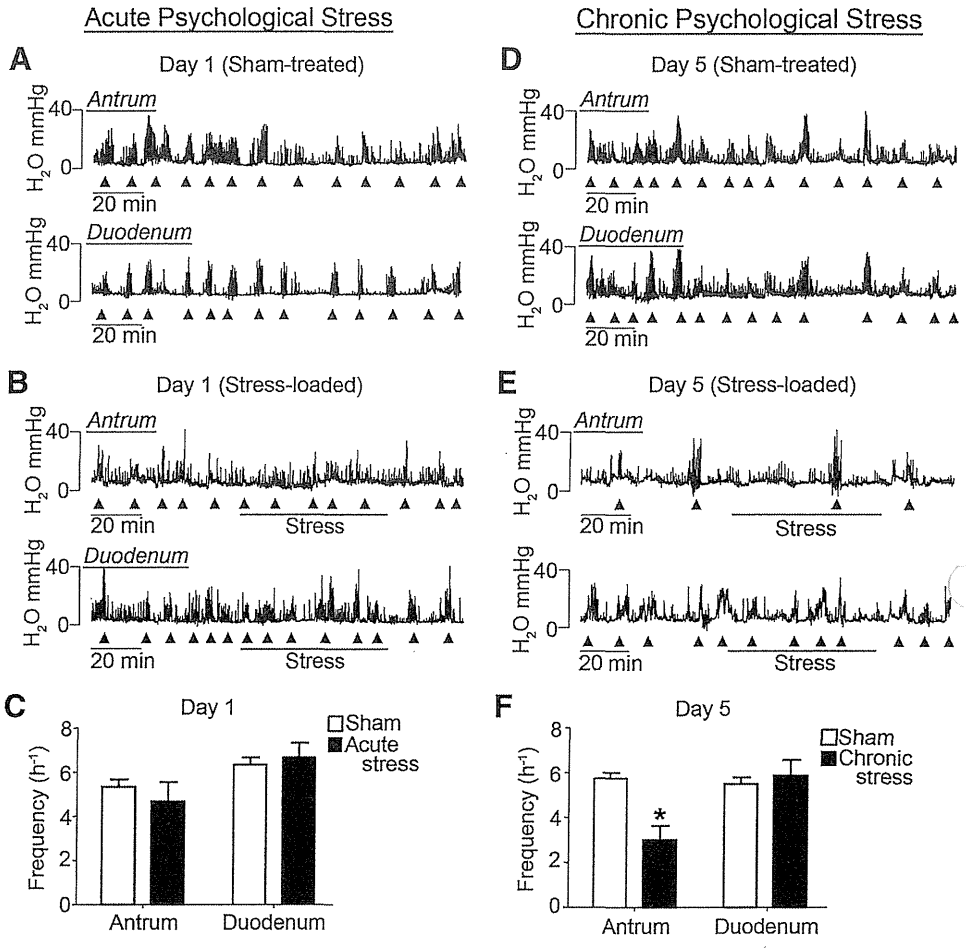

Fig. 2. Influences of acute (*day 1*) and chronic (*day 5*) psychological stress on the motor activity of the antrum and duodenum in fasted rats. Traces of motility curve in paired sham-treated control on *day 1* (*A*) or *day 5* (*D*) and acute (*B*) or chronic (*E*) stress-loaded rat were recorded. Frequency of phase III-like contractions (arrowheads) in the antrum and duodenum in paired sham-treated controls and acute (*C*) or chronic (*F*) stress-loaded rats were counted ($n = 4-8$). *$P < 0.05$ compared with paired sham-treated controls.

h; Fig. 5, *D* and *E*) and duodenum. We further examined whether ICV injection of Ucn 3 antiserum prevented a decrease in the frequency of phase III-like contractions in the antrum induced by chronic psychological stress. ICV injection of Ucn 3 antiserum at 10 min before psychological stress exposure on *day 5* reversed the decrease in the frequency of phase III-like contractions induced by chronic psychological stress from 2.1 ± 0.5 h to 4.1 ± 0.4 h ($P < 0.05$, Fig. 5, *F* and *G*).

Effects of ICV injection of Ucn 3, AVP, or OXT on colonic motility. We examined whether central administration of Ucn 3, AVP, or OXT induced the alteration of colonic motility in normal rats. ICV injection of 3-nmol Ucn 3 did not alter the %MI in both proximal and distal colon (97.9 ± 3.4% and 94.0 ± 4.14%, respectively, Fig. 6, *A, F,* and *G*). ICV injections of 0.3- and 1.5-pmol AVP significantly increased the %MI of proximal colon (125.2 ± 9.6% and 132.9 ± 6.7%, respectively, $P < 0.05$) compared with vehicle-injected controls (93.6 ± 4.0%, Fig. 6, *B, C,* and *F*). In the distal colon 0.3-pmol AVP did not alter (112.0 ± 7.8%), while 1.5-pmol AVP significantly increased the %MI (272.7 ± 8.6%) compared with vehicle-injected controls (92.9 ± 5.0%, Fig. 6, *B, C,* and *G*). ICV injection of 1.5-pmol OXT did not alter the %MI in both proximal and distal colon (110.6 ± 7.7% and 116.1 ± 4.7%, respectively, Fig. 6, *D, F,* and *G*). On the other hand, ICV injection of 100-pmol OXT significantly increased the %MI in the proximal colon (146.6 ± 10.4%, $P < 0.05$), but did not alter the %MI in distal colon (116.1 ± 4.7%, Fig. 6, *E, F,* and *G*).

Effects of ICV injection of SSR 149415 or L 371257 on the alteration of colonic motility induced by chronic psychological stress. We examined the involvement of endogenous AVP and OXT in the alteration of colonic motility induced by chronic psychological stress. AVP receptor antagonist SSR 149415 or OXT receptor antagonist L 371257 was injected ICV 10 min before the psychological stress exposure on *day 5*. Increase in the %MI of the proximal colon induced by psychological stress (144.0 ± 15.9%) was reversed by ICV injection of SSR 149415 (98.1 ± 6.8%, $P < 0.05$) but was not affected by ICV injection of L 371257 (157.4 ± 23.2%, Fig. 7, *A–C*). On the other hand, %MI in the distal colon induced by stress exposure (92.3 ± 13.1%) was not affected by ICV injection of SSR 149415 (98.1 ± 6.8%) or L 371257 (89.6 ± 3.2%, Fig. 7, *A, B* and *D*).

DISCUSSION

The present study demonstrates the effects of chronic psychological stress generated by the CB technique on GI motility in a conscious rat model. In previous studies, the CB experiment has been used to examine the effects of anxiety reaction (33) as well as food intake in rats (16, 36). These studies demonstrated that exposure to the CB increased plasma corticosterone (17) and brain neurotransmitters such as serotonin and dopamine in the amygdala and dorsal raphé nucleus (18, 43), as well as noradrenaline in the hypothalamus (40) in rats.

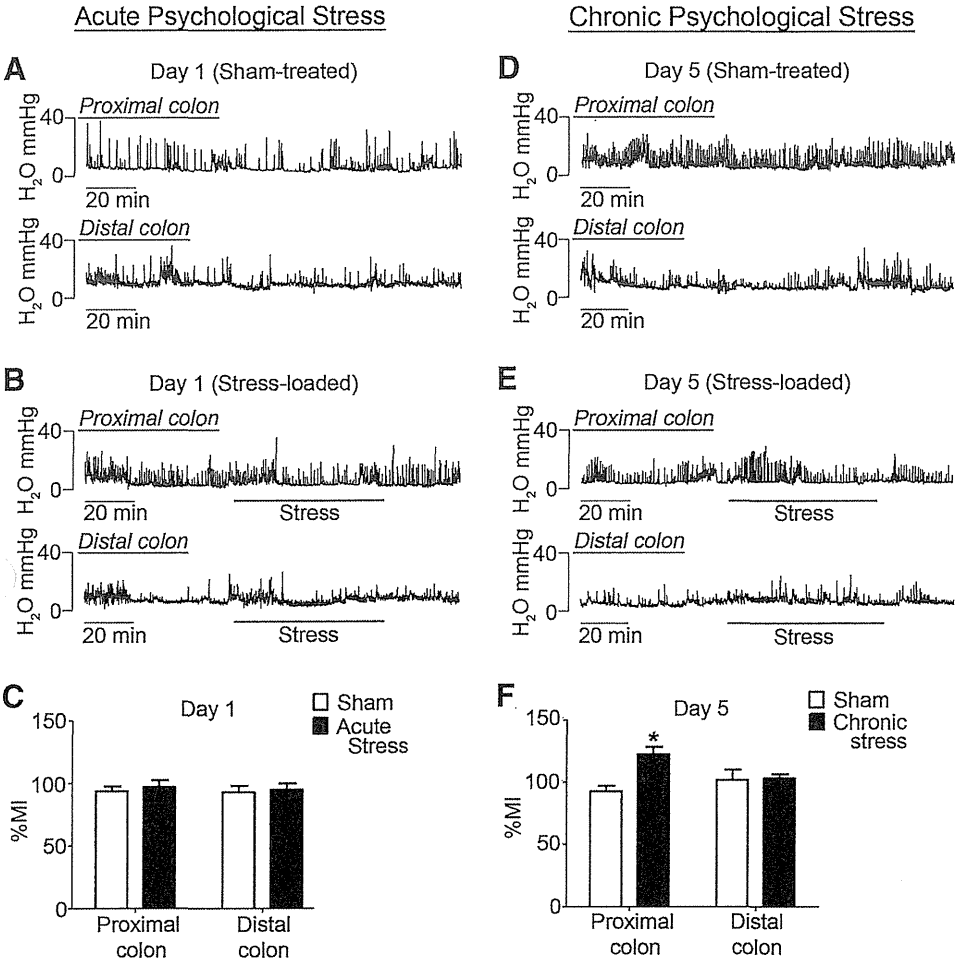

Fig. 3. Influences of acute (*day 1*) and chronic (*day 5*) psychological stress on the motor activity of the proximal and distal colon of rats. Traces of motility curve in paired sham-treated control on *day 1* (*A*) or *day 5* (*D*) and acute (*B*) or chronic (*E*) stress-loaded rats were recorded. The percentage motility index (%MI) of the proximal and distal colon in paired sham-treated controls and acute (*C*) or chronic (*F*) stress-loaded rats were calculated ($n = 4-12$). *$P < 0.05$ compared with paired sham-treated controls.

Because these changes in peripheral and central hormones and neurotransmitters are similar to those seen in humans under stress conditions, the CB experiment used in the present study seems to be appropriate for simulating a human psychological stress.

The results showed that repeated exposure to psychological stress for five successive days caused a decrease in the frequency of phase III-like contractions in the antrum and increased the %MI in the proximal colon. However, acute stress exposure did not alter upper and lower GI motility. Most of the previous studies that examined the effects of stress-induced abnormality in the GI motility applied physical stimuli, such as restraint stress or water immersion stress (13, 31, 32, 35). The effects of such physical stress on upper GI motility were quite different from those of the psychological stress stimuli of the present study. Acute restraint stress in rats decreased gastric motility; however, repeated restraint stress for five successive days reversed the decrease in gastric motility (45). Water immersion stress for 24 h delayed gastric emptying in rats; however, water immersion stress for five successive days accelerated gastric emptying (32). For colonic motility, on the other hand, repeated restraint stress for five successive days did not alter proximal or distal colonic motility (27); however, repeated restraint stress for 14 days increased distal colonic motility in mice (13).

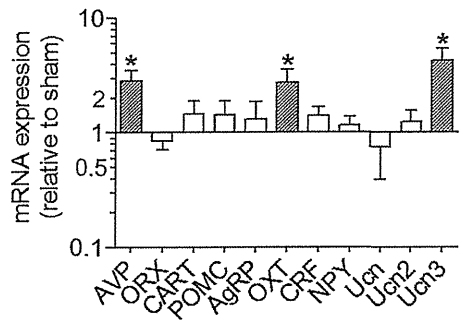

Fig. 4. Influence of 5-days successive chronic psychological stress on mRNA expression of AVP, orexin (ORX), amphetamine-regulated transcript (CART), proopiomelanocortin (POMC), agouti-related protein (AgRP), oxytocin (OXT), corticotoropin-releasing factor (CRF), neuropeptide Y (NPY), urocortin (Ucn), Ucn 2, and Ucn 3 in the hypothalamus ($n = 8-12$). *$P < 0.05$ compared with paired sham-treated controls.

Previous studies have examined the effects of psychological stress on colonic motility in which WA stress experiments have been used (5, 23, 42); however, only fecal pellet output, which does not always reflect the level of colonic motor activity (2), was used to assess the colonic motility measuring the motor activity in the proximal and distal colon (5, 23).

The present results showed that chronic psychological stress increased Ucn 3 mRNA expression in the hypothalamus. It has been shown that Ucn, Ucn 2, and Ucn 3, which are neuropeptides located in the hypothalamic paraventricular nucleus (9),

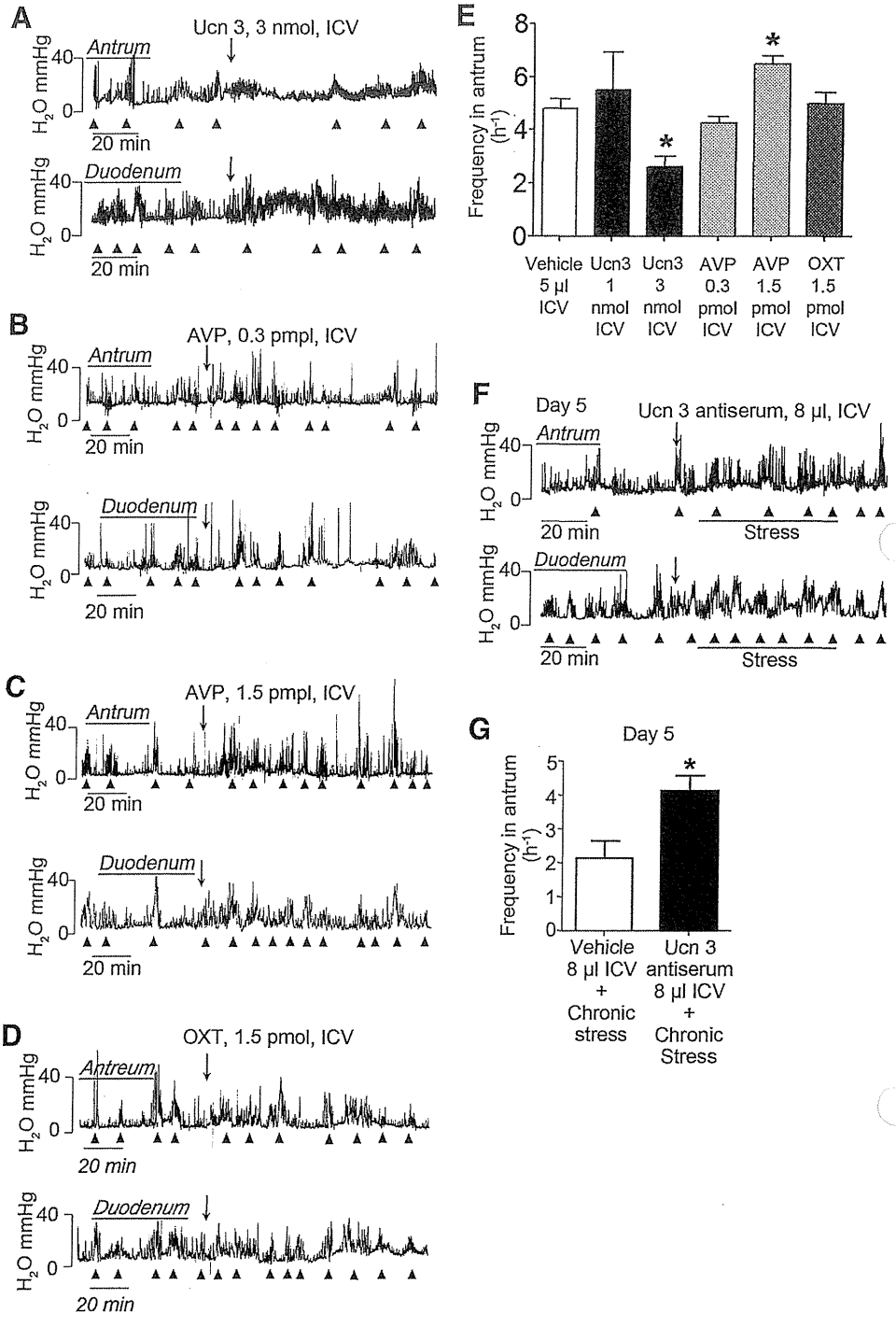

Fig. 5. Effects of Ucn 3, AVP, and OXT on gastroduodenal motility in normal rats and effects of ICV injection of Ucn 3 antiserum on the alteration of antrum motility induced by chronic psychological stress. Traces of gastroduodenal motility in normal rats treated with ICV injection of 3-nmol Ucn 3 (*A*), 0.3- (*B*) and 1.5-pmol AVP (*C*), 1.5-pmol OXT (*D*), and in chronic stress-loaded rats treated with ICV injection of Ucn 3 antiserum 10 min before psychological stress exposure on *day 5* (*F*). Frequency of phase III-like contractions (arrowheads) in the antrum from rats treated with ICV injection of Ucn 3, AVP, OXT (*E*) and chronic psychological stress-loaded rats treated with ICV injection of Ucn 3 antiserum (*G*) were counted ($n = 4-11$). $^*P < 0.05$ compared with vehicle-injected controls.

play roles that suppress food intake and induce anxiety-related behavior (10, 21, 41) via activation of CRF type 1 and/or type 2 receptors (34). The roles of Ucn or Ucn 2 on the GI motility have been well known; for example, hypothalamic Ucn reduces the gastric motility, while Ucn 2 prevents the increase in distal colonic motility induced by CRF (14, 19). Whereas the roles of Ucn 3 on the GI motility have not been fully examined. Because our results showed that psychological stress increased the mRNA expression of Ucn3 but not that of Ucn or Ucn 2, we examined the effects of ICV injection of Ucn 3 on GI motility. The results showed that Ucn 3 suppressed the phase III-like contractions in the antrum and duodenum, and further examination showed that ICV injection of Ucn 3 antiserum prevented alteration in antral motility induced by chronic psychological stress. These results suggest that Ucn 3 in the hypothalamus might be involved in chronic psychological stress-induced alteration in stomach motility.

Our results also showed that the mRNA expression of AVP in the hypothalamus was increased by chronic psychological stress. Previous studies have shown that mRNA expression of AVP is increased under conditions of both acute and repeated restraint stress in rats (15, 25) and have

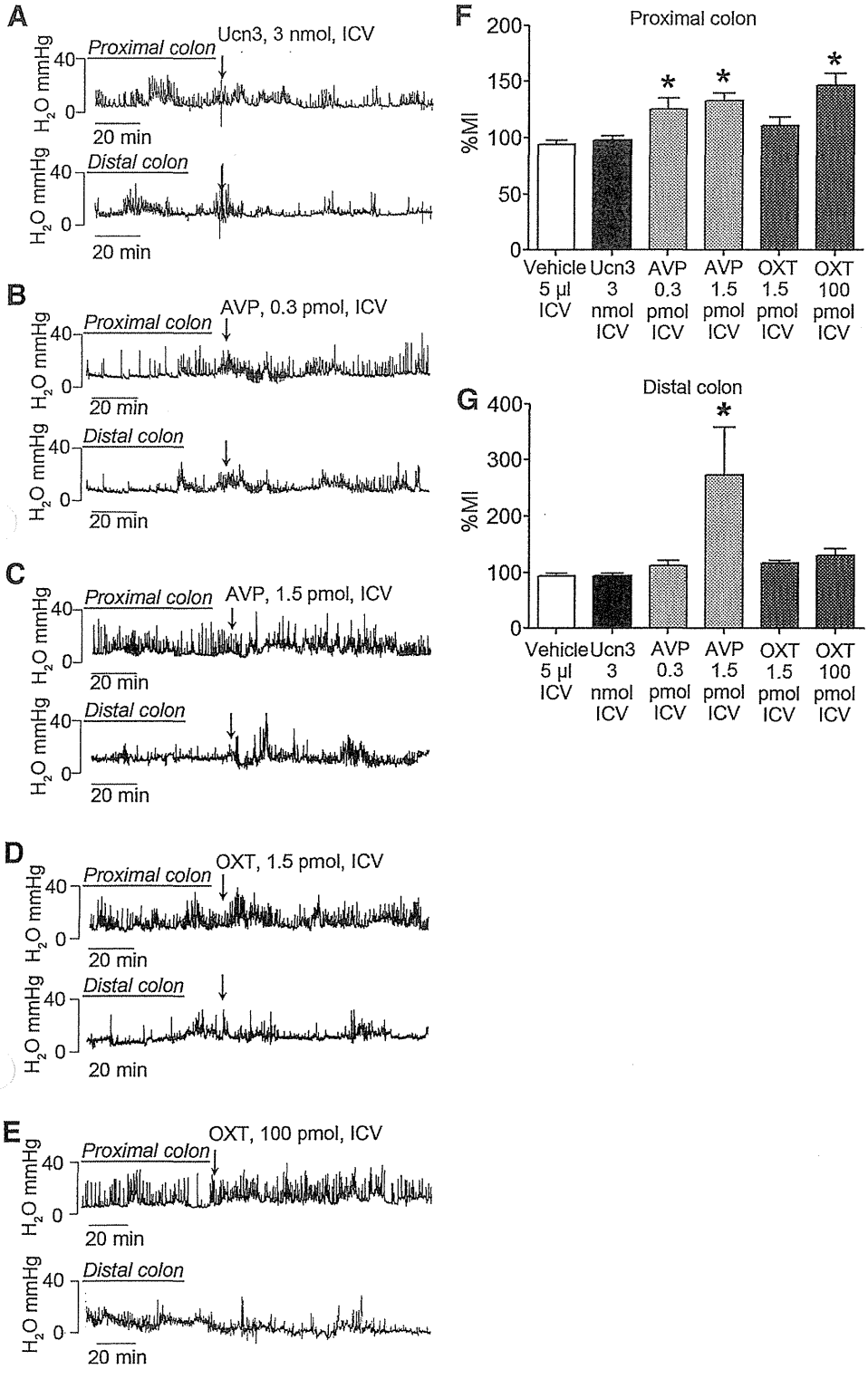

Fig. 6. Effects of Ucn 3, AVP, and OXT on the colonic motility. Traces of proximal and distal colonic motility in rats treated with ICV injection of 3 nmol Ucn 3 (*A*), 0.3 (*B*) and 1.5 pmol AVP (*C*), 1.5 (*D*) and 100 pmol OXT (*E*). %MI in the proximal (*F*) and distal colon (*G*) from rats treated with ICV injection of Ucn 3, AVP, and OXT ($n = 4-8$). *$P < 0.05$ compared with vehicle-injected controls.

also shown that the abnormality in colonic motility is induced by restraint stress (13, 26, 31). Bueno et al. (7) demonstrated that emotional stress, which was produced by placing animals from a home cage into a closed box in which they had previously received electrical foot shocks, increased proximal colonic motility, while ICV injection of AVP antibody prevented this reaction in rats. Our results showed that the acceleration of proximal colonic motility induced by chronic psychological stress was prevented by ICV injection of AVP V1b receptor antagonist. Therefore, the results obtained from emotional stress in the previous study are consistent with those obtained from psychological stress induced by the CB experiment in the present study. The present results show that the V1b receptor in the brain is involved in the alteration of colonic motility induced by chronic psychological stress.

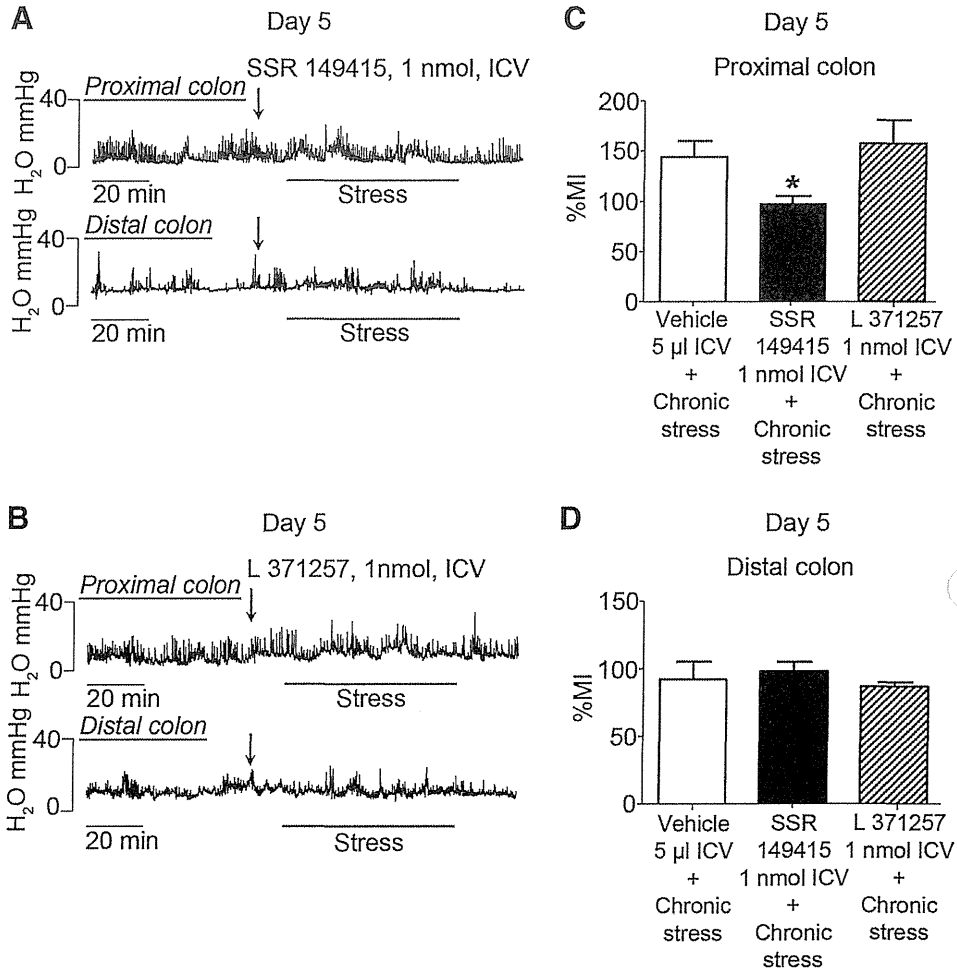

Fig. 7. Effects of SSR 149415 and L 371254 on the alteration of colonic motility induced by chronic psychological stress. Traces of proximal and distal colonic motility in rats treated with ICV injection of SSR 149415 (*A*) and L 371254 (*B*) 10 min before psychological stress exposure on *day 5*. The percentages of MI in the proximal (*C*) and distal colon (*D*) from chronic psychological stress-loaded rats treated with ICV injection of SSR 149415 or L 371254 were calculated (n = 4–8). *$P < 0.05$ compared with vehicle-injected controls.

Chronic psychological stress increased the mRNA expression of OXT in the hypothalamus. A recent study showed that OXT in the hypothalamus is associated with an adaptation to physical stress, such as restraint stress. For example, in wild-type mice, delayed gastric emptying caused by acute restraint stress was restored to the normal level in mice exposed to repeated restraint stress (3). However, OXT knockout mice failed to restore the delayed gastric emptying under repeated stress. The present study demonstrates that even when elevation of OXT mRNA levels occurred, gastric motility remains abnormal under chronic stress conditions. This finding suggests that adaptation might occur under physical stress by producing OXT, whereas it might not occur under psychological stress.

Previous studies have shown the effects of OXT on colonic motility in the psychological stress model in rats using a WA experiment (28). They showed that ICV injection of OXT at 50 or 100 pmol prevents the increase in distal colonic motility induced by acute WA stress but 5 pmol OXT had no effects (28). In the present study, ICV injection of 100 pmol OXT increased the proximal colonic motility; however, 1.5 pmol of OXT had no effects. These results suggest that OXT in the hypothalamus may have some effects on proximal colonic motility. However, this peptide might not be involved in the chronic psychological stress-induced alteration in colonic motility, because changes in proximal colonic motility induced by chronic psychological stress were not affected by ICV injection of OXT receptor antagonist as shown in the present study.

Most of previous studies that examined the effects of various stress conditions on GI motility showed that CRF type 1 and type 2 receptors in the brain are primarily involved (37, 38). However, the present study showed that the CRF type 2 receptor, mediated by Ucn 3 (26), is involved in the suppression of gastric motility, while the V1b receptor, mediated by AVP, is involved in the acceleration of colonic motility induced by chronic psychological stress. Previous study has shown that under stress conditions, expression of CRF type 2 receptor or V1b receptor is activated on neurons in the motor nucleus of vagus (24, 44), therefore pathways through motor nucleus of vagus and vagal efferent nerves may mediate the alteration of GI motility induced by chronic psychological stress. Because chronic psychological stress generated by the CB experiment caused the similar effects on GI motility to stress-related disorders, such as functional dyspepsia or irritable bowel syndrome, the models of the present study might be useful for examining the pathophysiology of these disorders (20, 30).

In conclusion, the present study demonstrated that chronic psychological stress generated by the CB technique disrupted fasted motility in the antrum and accelerated proximal colonic motility, while acute psychological stress had no effect. Ucn 3 and its CRF type2 receptor in the brain might mediate the